Proc. Natl. Acad. Sci. USA
Vol. 78, No. 7, pp. 4402–4406, July 1981
Cell Biology

Tumor-promoting phorbol esters stimulate myelopoiesis and suppress erythropoiesis in cultures of mouse bone marrow cells

(erythropoietin/colony-stimulating factor/burst-promoting activity/hematopoietic progenitor cells)

FRITZ SIEBER*, ROBERT K. STUART†, AND JERRY L. SPIVAK*

*The Clayton Laboratories of the Department of Medicine, The Johns Hopkins University School of Medicine, 924 Traylor Research Building, Baltimore, Maryland 21205; and †The Experimental Hematology Laboratory of The Johns Hopkins Oncology Center, Baltimore, Maryland 21205

Communicated by Victor A. McKusick, April 6, 1981

ABSTRACT **Tumor-promoting phorbol esters affect the ability of mouse hematopoietic progenitor cells to form morphologically recognizable colonies in culture. They induce myeloid progenitor cells to form colonies of the monocyte/macrophage type in the absence of exogenous granulocyte/macrophage colony-stimulating factor. Conversely, similar concentrations of tumor-promoting phorbol esters inhibit the formation of colonies (bursts) by early erythroid progenitor cells, even when the culture medium contains saturating amounts of burst-promoting activity and erythropoietin. However, late erythroid progenitor cells, are not affected by phorbol esters. Only a temporary (45 min) exposure of marrow cells to phorbol esters is necessary to produce both stimulation of myeloid colony growth and inhibition of erythroid burst formation. Experiments with a radioactively labeled phorbol ester indicate a high affinity for cellular binding sites. The ability of various phorbol esters to stimulate myeloid colony growth and to inhibit erythroid burst formation correlates well with their ability to promote skin tumors in mice. The different responses of two developmentally closely related hematopoietic progenitor cells to the same phorbol esters indicate the usefulness of these substances in the further analysis of regulatory events affecting hematopoiesis.**

The ability of phorbol esters to promote the growth of tumors in tissues initiated by exposure to a subthreshold dose of a carcinogen has been extensively documented (reviews in refs. 1 and 2). More recent observations that the same compounds also affect the proliferation and differentiation of a wide variety of cultured cells (1, 2) suggest that growth control in normal and neoplastic cells share some mechanisms in common. It has been proposed that phorbol esters influence proliferation and differentiation by usurping the biological effects of endogenous hormones and growth regulators (3). Unlike many physiological regulators, phorbol esters are comparatively small and structurally well-defined molecules, and strikingly similar correlations between their structures and their biological activities have been demonstrated in different experimental systems (1, 2). Therefore, phorbol esters hold promise as useful model substances for the analysis of growth control at the cellular and molecular level.

The hematopoietic system of the adult organism provides an attractive example of precisely regulated cell growth because the proliferative and differentiative activities of pluripotent and committed stem cells are balanced to exactly meet the organism's requirements for mature members of a particular lineage. Three types of murine hematopoietic progenitor cells capable of forming morphologically recognizable colonies in culture are the subjects of this report: late erythroid progenitor cells [colony-forming unit(s)—erythroid, CFU-E], early erythroid progenitor cells [burst-forming unit(s)—erythroid, BFU-E] and granulocyte/macrophage (myeloid) progenitor cells [colony-forming unit(s)—granulocyte/macrophage, CFU-GM]. Proliferation of these three progenitor cells appears to be under the control of at least three macromolecular growth regulators: erythropoietin (4), burst-promoting activity (5), and granulocyte/macrophage colony-stimulating factor (GM-CSF) (6), respectively. Small quantities of these growth regulators have been obtained in highly purified form (5–7). However, because of the extreme scarcity of homogeneous material, very little is known about their mode of action.

Stuart and Hamilton (8) have shown that tumor-promoting phorbol esters cause mouse bone marrow cells to form monocyte/macrophage colonies even when no exogenous GM-CSF was added to the culture medium. A stimulation of terminal differentiation but not proliferation was observed also when phorbol esters were added to the culture medium of a human leukemia cell line (9). In the overwhelming majority of nonhematopoietic, nontransformed cells, however, comparable concentrations of tumor-promoting phorbol esters prevented or delayed terminal differentiation (2). Therefore, it was of obvious interest to determine whether the positive differentiative response to tumor-promoting phorbol ester is unique to progenitor cells of the myeloid pathway or whether it is a property of other types of hematopoietic progenitor cells as well. Thus, we studied the effects of phorbol esters on proliferation and differentiation of early and late erythroid progenitor cells. A preliminary report has been presented (10).

MATERIALS AND METHODS

Reagents. Phorbol and phorbol esters (P-L Laboratories) were stored as 0.01 M stock solutions in dimethyl sulfoxide in the dark at -15°C. Less concentrated working solutions were prepared by serial dilutions in α-medium (Flow Laboratories, McLean, VA). Tritiated 12-*O*-tetradeconoylphorbol 13-acetate ([20-^{3}H(N)]TPA; 17.2 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was obtained as a solution in toluene/ethanol (New England Nuclear), 9:1 (vol/vol), dried under nitrogen, and dissolved in dimethyl sulfoxide shortly before use. Methylcellulose (Methocel MC, 4000 cP; Tridom/Fluka, Buchs, Switzerland), fetal calf serum (Irvine Scientific, Irvine, CA), sheep plasma erythropoietin (step III; Connaught, Willowdale, Ontario, Canada) and α-medium were prescreened for their ability to optimally support hematopoietic colony growth in culture. GM-CSF was prepared from WEHI-3-conditioned medium (11) or L-cell-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CFU-E, colony-forming unit(s)—erythroid; BFU-E, burst-forming unit(s)—erythroid; CFU-GM, colony-forming unit(s)—granulocyte/macrophage; GM-CSF, granulocyte/macrophage (myeloid) colony-stimulating factor; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; PDD, phorbol 12,13-didecanoate; PDBz, phorbol 12,13-dibenzoate; PDA, phorbol 12,13-diacetate; 4α-PDD, 4α-phorbol 12,13-didecanoate.

conditioned medium (8). Catalase (34 units/mg) from bovine liver and superoxide dismutase (3000 units/mg) from bovine blood were purchased from Sigma, and mixed brain gangliosides were from Supelco (Bellefonte, PA).

Cells. Unless indicated otherwise, bone marrow cells were taken from the femurs of female B6D2F1 mice (8–12 weeks old; Jackson Laboratories or Cumberland View Farms). Some experiments were duplicated with cells from outbred female Swiss–Webster mice (Buckberg, Tomkins Cove, NY) or outbred male or female CD-1 mice (Charles River Breeding Laboratories) of the same age.

Bioassays. Erythroid and granulocyte/macrophage progenitors were detected by their capacity to form morphologically recognizable colonies in the methylcellulose culture system described by Iscove and Sieber (12). Colonies were scored *in situ* with an inverted microscope after 2 (CFU-E), 6 (CFU-GM), and 9 (BFU-E) days in culture according to established criteria (4, 12). All three types of colonies could be assayed independently in the same dish (4, 12). In selected experiments, the identity of the colonies was confirmed by transferring them to microscope slides and staining them with benzidine and hematoxylin. Some assays for CFU-GM were duplicated in agar cultures (13) with an *in situ* staining technique (8). Colonies containing more than 10% of a second cell type were classified as mixed colonies.

RESULTS

Effects on Myeloid Colony Formation. Bone marrow cells grown in methylcellulose cultures together with tumor-promoting phorbol esters formed myeloid colonies in the absence of exogenous GM-CSF (Fig. 1). The frequency and the morphological appearance of the colonies and the effective range of phorbol ester concentrations were similar to those reported by Stuart and Hamilton (8), who used agar instead of methylcellulose to immobilize the marrow cells. The colonies were predominantly of the monocyte/macrophage type, more densely packed, slightly smaller, and about 50% less numerous (when grown in 15% serum) than those formed in the presence of an optimum concentration of GM-CSF. Agar (Fig. 2 *A* and *B*) and methylcellulose (not illustrated) cultures of B6D2F1 cells gave rise to about 50% more myeloid colonies that cells from CD-1 animals when stimulated with phorbol esters. Control experiments, which used saturating concentrations of L-cell-derived GM-CSF instead of phorbol esters, indicated that this strain-specific difference was most likely due to an inherently lower frequency of CFU-GM (approximately 150–200 in CD-1 versus 250–350 in B6D2F1 per 10^5 nucleated bone marrow cells) in CD-1 bone marrow. The ability of phorbol esters to promote

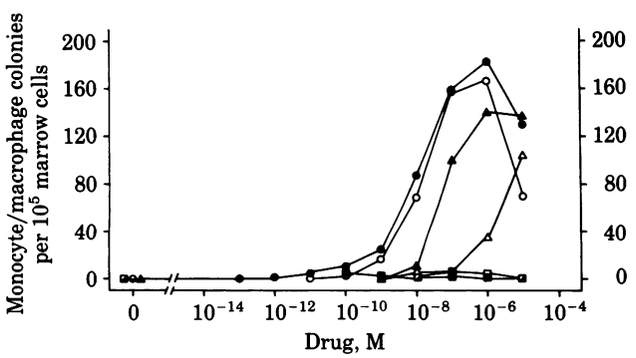

FIG. 1. Myeloid colony formation by bone marrow cells from B6D2F1 mice in the presence of 15% fetal calf serum and varying concentrations of phorbol esters and phorbol. The same results were obtained when cells were taken from outbred Swiss–Webster mice. Each point represents the mean colony count of at least two cultures. The solvent (dimethyl sulfoxide) had no effect. ●, TPA; ○, PDD; ▲, PDBz; △, PDA; ■, 4α-PDD; □, phorbol.

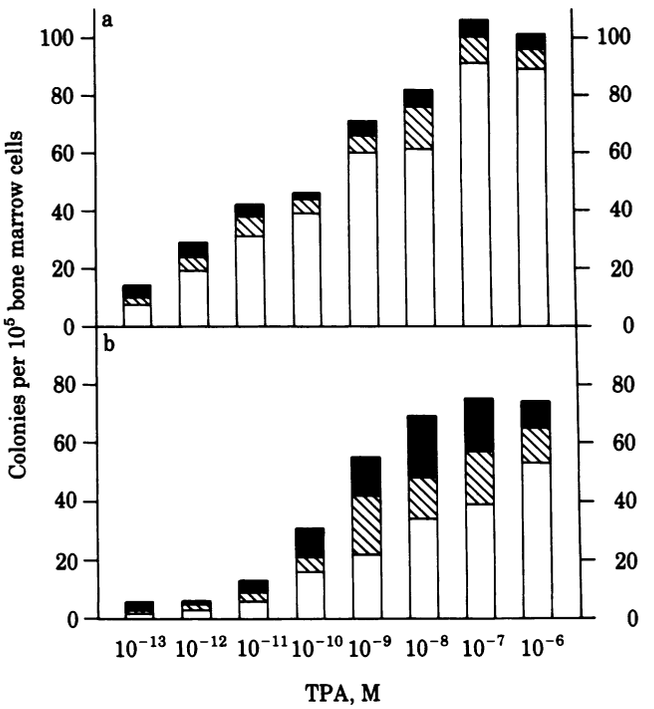

FIG. 2. Results of a morphological analysis of myeloid colonies formed in agar cultures in the presence of different concentrations of TPA. (*A*) Culture derived from a female B6D2F1 mouse. (*B*) Culture derived from a female CD-1 mouse. ■, Granulocyte colony; ▨, mixed colony; □, macrophage colony.

the formation of myeloid colonies *in vitro* correlated well with their ability to promote skin tumors in mice (Fig. 1): TPA and phorbol 12,13-didecanoate (PDD) were the most potent promotors of myeloid colony formation, phorbol 12,13-dibenzoate (PDBz) and phorbol 12,13-diacetate (PDA) were of intermediate potency, and 4α-phorbol 12,13-didecanoate (4α-PDD), phorbol and dimethyl sulfoxide were inactive.

Both phorbol ester-stimulated and GM-CSF-stimulated myeloid colony growth depended on the presence of fetal calf serum in the culture medium. However, they differed considerably with respect to the amount of serum they required for optimum growth: 15–20% (vol/vol) fetal calf serum was sufficient to optimally support myeloid colony formation in GM-CSF-containing cultures. By contrast, phorbol ester-induced colony formation increased with increasing serum content up to a concentration of about 40% (see also ref. 14). No myeloid colony growth was induced by phorbol esters when serum was omitted from the culture medium.

Effects on Colony Formation by Late Erythroid Progenitors. In marked contrast to their profound effect on myeloid progenitor cells (CFU-GM), tumor-promoting phorbol esters had no obvious effects on late erythroid progenitor cells (CFU-E). They failed to induce the formation of erythroid colonies in the absence of erythropoietin when tested at concentrations ranging from 10 fM to 100 μM. They also failed to modify the response of CFU-E to saturating (Fig. 3) or nonsaturating (not illustrated) doses of erythropoietin. At very high concentrations (10 μM), phorbol esters appeared to inhibit proliferation by all types of progenitor cells regardless of their potency as tumor promotors.

Effects on Colony Formation by Early Erythroid Progenitors. Unlike CFU-GM, which were stimulated, or CFU-E, which were not affected, early erythroid progenitor cells (BFU-E) were inhibited by phorbol esters (Fig. 4). Again, there was an obvious correlation between the phorbol esters' ability to inhibit erythroid burst formation *in vitro* and their ability to

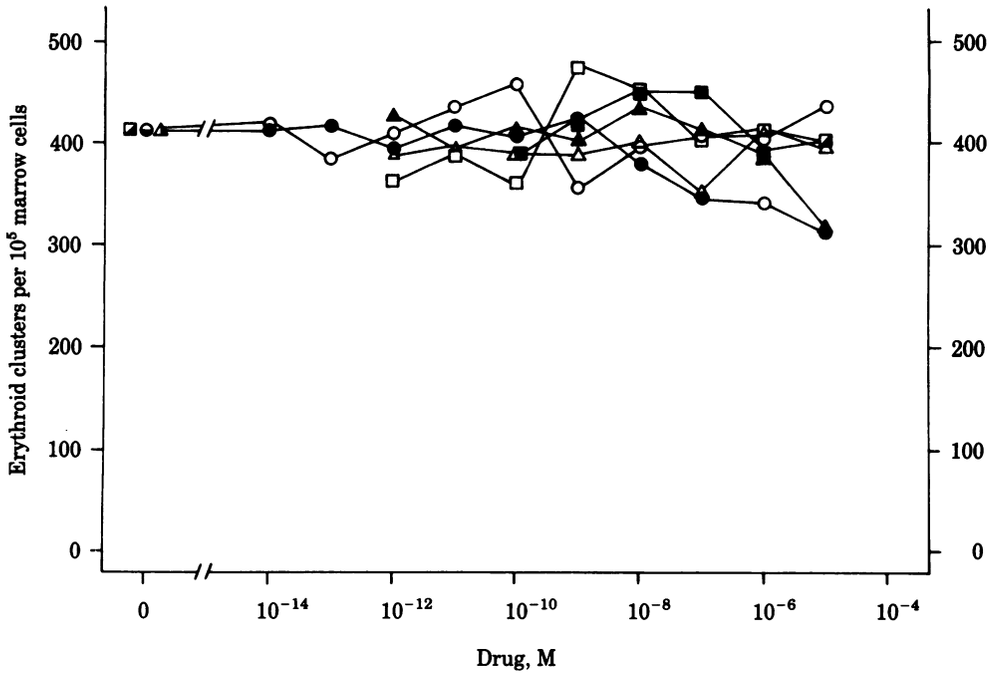

FIG. 3. Erythroid cluster formation by marrow cells from B6D2F1 mice in the presence of a saturating concentration of erythropoietin (2 units/ml) and varying concentrations of phorbol esters and phorbol. The same results were obtained with outbred Swiss–Webster mice. Each point represents the mean colony count of at least two cultures. Colonies grown in the presence of 10^{-5} M phorbol ester were smaller than those grown at lower concentrations. ●, TPA; ○, PDD; ▲, PDBz; △, PDA; ■, 4α-PDD; □, phorbol.

promote skin tumors *in vivo*. The genetic background of the donor animal determined, in part, the extent to which erythroid burst formation was inhibited. In cultures derived from B6D2F1 or Swiss–Webster mice, as little as 10 nM TPA was usually sufficient to completely suppress erythroid burst formation. However, marrow cells from both male and female CD-1 mice contained a sizeable subpopulation of BFU-E capable of forming colonies in the presence of up to 1 μM TPA. Typically about 40% of all CD-1 BFU-E were TPA–resistant, with extreme values ranging from 9% to 75% in a sample of 11 animals taken from the same shipment. However, the total number of BFU-E in CD-1 mice did not fluctuate and was not significantly different from the number of BFU-E detected in B6D2F1 animals. The same strain-specific differential sensitivity of BFU-E to TPA was found whether colonies were scored *in situ* (unstained) or after transfer to microscope slides and staining with benzidine and hematoxylin. Size and morphological appearance of the reduced number of bursts formed in the presence of a partially inhibitory concentration of phorbol esters were normal. When CD-1 and B6D2F1 cells were cocultured in the presence of 0.1 or 1 μM TPA, the number of erythroid bursts produced was equal to the sum of bursts produced by the same cells in separate cultures. This suggested that the strain-specific response to TPA was more likely attributable to a differential sensitivity of the colony-forming cells than to an influence on a subpopulation of regulatory accessory cells.

Complete inhibition of burst formation by B6D2F1 cells was seen only when TPA was added during the first 3 days of culture; there was no inhibition when TPA addition was delayed for 6 or more days, indicating that phorbol ester sensitivity is limited to the earliest phases of erythroid development.

A temporary exposure (45–60 min) of marrow cells to TPA followed by repeated centrifugations and resuspensions in fresh culture medium was sufficient to produce both the stimulation

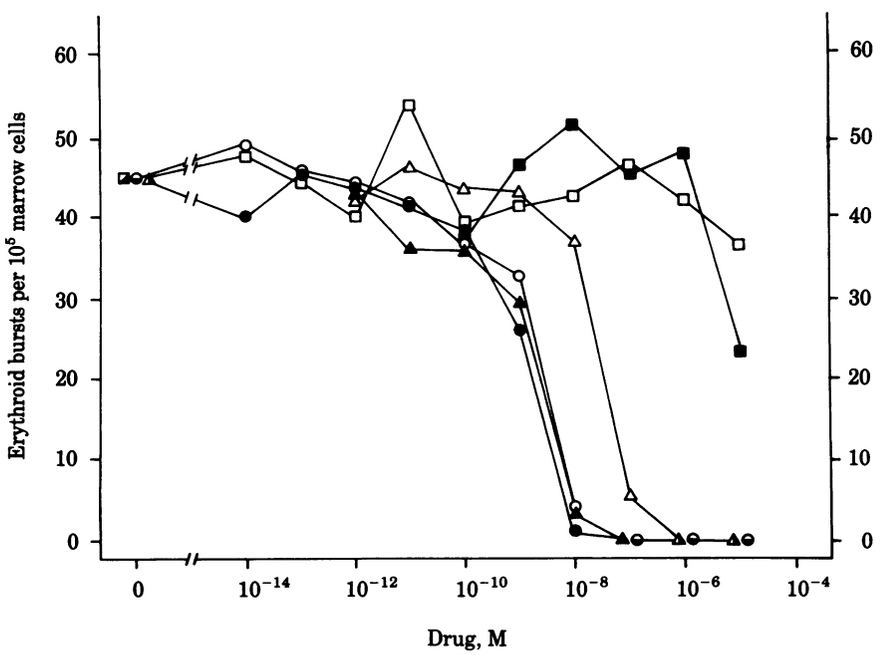

FIG. 4. Erythroid burst formation by marrow cells from B6D2F1 mice in the presence of erythropoietin (2 units/ml) and varying concentrations of phorbol esters and phorbol. The same results were obtained when cells were taken from outbred Swiss–Webster mice. Each point represents the mean colony count of at least two cultures. Bursts grown in the presence of partially inhibitory concentrations of phorbol esters reached normal dimensions and had the usual morphological appearance. The inhibitory potency of commercial preparations of PDBz varied considerably; one lot was about two orders of magnitude more potent than the one used for the experiments depicted above. Dimethyl sulfoxide did not affect burst formation. ●, TPA; ○, PDD; ▲, PDBz; △, PDA; ■, 4α-pDD; □, phorbol.

Table 1. Temporary exposure of bone marrow cells to tumor-promoting phorbol ester

	Colonies per 10^5 nucleated bone marrow cells*			
			CFU-GM	
Treatment	BFU-E	CFU-E	Methylcellulose	Agar
None	48.0 ± 4	447 ± 36	0	0
TPA, 1 μM	5.0 ± 0.9	249 ± 16	2 ± 1	6 ± 1
TPA, 10 μM	0.5 ± 0.3	171 ± 26	89 ± 14	109 ± 14

Nucleated bone marrow cells (7×10^6) from B6D2F1 mice were incubated for 45 min at 37°C in 1 ml of α-medium supplemented with 10% fetal calf serum and TPA as indicated. At the end of the incubation period, cells were washed four times by centrifugation and resuspension in 10 ml of fresh medium supplemented with 5% fetal calf serum. Incubations at 25°C or 0°C gave the same results. The incomplete recovery of CFU-E after the TPA treatment may have been due to increased fragility of TPA-treated CFU-E or their improved adhesion to the centrifuge tube because continuous exposure to the same concentration of TPA resulted only in a slight reduction of erythroid cluster growth.

* Means of quadruplicate cultures ± SEM.

of myeloid colonies and the inhibition of erythroid bursts (Table 1). The effects of a temporary exposure were comparable to those of a continuous exposure to an ≈100- to 1000-fold lower concentration of the same phorbol ester. When cells that had been temporarily exposed to TPA were cocultured with untreated cells, the mixture adopted the growth pattern of the treated subpopulation (Table 2). Thus, in a mixed culture, the number of myeloid colonies was always higher than expected, whereas the number of erythroid bursts was lower than expected.

In order to determine the extent of binding of phorbol esters to cells, short term exposures of marrow cells were repeated with radioactively labeled TPA. These experiments showed that a substantial amount of TPA remained associated with the cell pellet and was not removed by the standard washing procedure (Fig. 5). If such labeled cells had been diluted and cultured according to the usual protocol, each culture would have contained TPA at a concentration about 1000 times lower than the one used for the short-term incubation of marrow cells.

Several substances were tested for their ability to antagonize the tumor promotor TPA: prostaglandin $=E_1$ (1 μM) reduced the number (by about 60–70%) and size of myeloid colonies grown in response to TPA. However, it did not reverse the inhibitory action of TPA on erythroid burst formation. Mixed gangliosides interfered with TPA-induced growth of myeloid colonies when their concentration in culture exceeded 0.1–0.2 mg/ml. They failed to reduce the inhibitory effect of TPA on BFU-E. The same concentrations of mixed gangliosides and prostaglandin E_1 also inhibit GM-CSF-induced colony growth (15, 16). Retinoic acid (10 μM and 1 μM; ref 17) was ineffective. Simultaneous additions of up to 1000-fold higher doses of phorbol or 4α-PDD reduced neither the stimulation of myeloid colony growth nor the inhibition of erythroid colony growth by TPA. Supplementation of the culture medium with superoxide dismutase (up to 108 units/ml) or catalase (up to 0.6 unit/ml), or both, did not restore burst formation in the presence of TPA, suggesting that inhibition of erythroid burst formation was not primarily a consequence of excessive superoxide production (18) by TPA-stimulated polymorphonuclear leukocytes.

DISCUSSION

In the present study, we observed that tumor-promoting phorbol esters had a dual effect on proliferation or differentiation, or both, of normal mouse hematopoietic progenitor cells. They stimulated the formation of monocyte/macrophage colonies by CFU-GM but inhibited the formation of erythroid bursts by BFU-E. Although the exact mechanism by which phorbol esters achieve these effects is unclear, our data allow a distinction between several possible mechanisms. It appears highly unlikely that inhibition of erythroid burst formation was due to nutrient depletion caused by excessive myeloid colony growth. First, when the culture medium was supplemented with a saturating dose of GM-CSF in the absence of phorbol ester, myeloid colonies were larger and more numerous, yet erythroid bursts continued to grow in the same dish. Second, suppression of myeloid colony growth by prostaglandin E_1 also did not restore erythroid colony formation. Third, a temporary exposure to 1 μM TPA reduced erythroid burst formation by 90%, although it induced only a negligible number of myeloid colonies (Table 1).

Table 2. Colony formation by mixed cultures of TPA-treated and untreated marrow cells

	TPA treatment		Colonies*		
No. of cells	μM	min	BFU-E	CFU-E	CFU-GM
1×10^5	None	—	53.0 ± 3.9	507 ± 36	15.3 ± 2.7
1×10^5	10	45	0	99 ± 9	74.7 ± 2.2
1×10^5	1	45	3.5 ± 0.5	ND	ND
1×10^5	0.1	45	52.3 ± 2.1	ND	ND
5×10^4	None	—	0	305 ± 18	100.3 ± 4.8
with 5×10^4	10	45			
(Expected)			(26.5)	(303)	(45)
5×10^4	None	—	10.8 ± 1.7	ND	ND
with 5×10^4	1	45			
(Expected)			(28.3)		
5×10^4	None	—	51.8 ± 2.9	ND	ND
with 5×10^4	0.1	45			
(Expected)			(52.7)		

B6D2F1 marrow cells were incubated in TPA as outlined in Table 1, washed, and cultured either separately or mixed with untreated cells. ND, not done.

* Mean of quadruplicate cultures ± SEM.

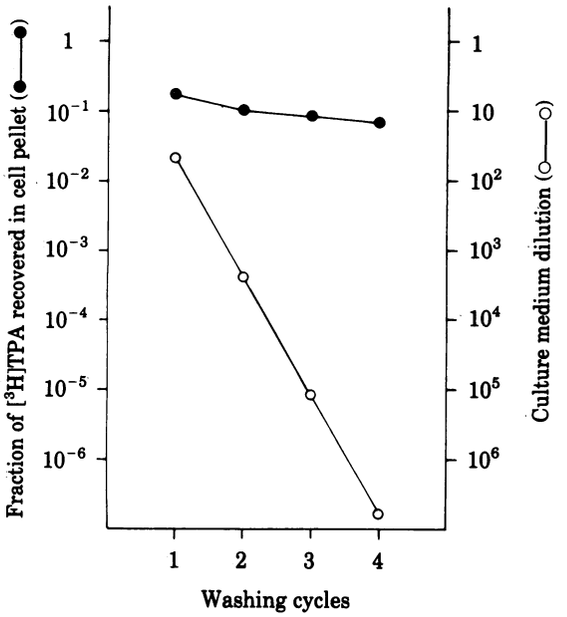

FIG. 5. Binding of [^{3}H]TPA to mouse bone marrow cells. B6D2F1 bone marrow cells (7×10^6) were incubated in 1 ml of α-medium supplemented with 10% fetal calf serum and 1 μM [^{3}H]TPA for 45 min at 37°C. The incubation was stopped by diluting the suspension 1:10 by the addition of α-medium supplemented with 5% fetal calf serum, followed by repeated centrifugations and resuspensions in 10 ml of fresh α-medium (plus 5% fetal calf serum). NCS tissue solubilizer (Nuclear Chicago) was used to solubilize cell-associated radioactivity.

The data also indicate that phorbol esters did not affect the interaction of erythropoietin with erythroid progenitor cells. CFU-E, the primary target cell of erythropoietin, was refractory to all but the highest phorbol ester concentrations (Fig. 3) and erythroid burst formation by BFU-E was only affected when phorbol esters were added during the early (erythropoietin-independent) phase of development.

In addition to direct effects of phorbol esters on colony-forming cells, effects on cells regulating hematopoiesis (19) must be considered also. The data summarized in Table 2 (coculture of TPA-treated and untreated cells) offer some support to a model that postulates the involvement of a regulatory cell. However, small amounts of phorbol ester released by treated cells during the culture period could also account for the overall growth pattern of the mixed cultures.

The *in vitro* colony assay used in this study was designed to detect committed progenitor cells. Therefore, our data provide no evidence that phorbol esters affect the commitment of the pluripotent stem cell to a particular pathway (stem cell competition; ref. 20), thus favoring myeloid development at the expense of erythroid development.

The findings of a marked inhibition of erythroid burst formation by tumor-promoting phorbol esters is in contrast to recent observations by Fibach *et al.* (21). These authors found that TPA enhanced both size and number of erythroid bursts in cultures of adult marrow and fetal liver cells derived from CD-1 mice. When we repeated our experiments with CD-1 mice we consistently found that TPA suppressed erythroid burst formation in CD-1 cultures as well. Our assays of erythroid burst formation were based both on a visual examination of unstained cultures under an inverted microscope and a microscopic examination of the contents of entire culture dishes, which had been transferred to slides and stained with a neutral benzidine stain. We used a neutral benzidine stain because acidified stains like the one used by Fibach *et al.* (21) react with nonerythroid cells as well and may lead to overestimates of the number of erythroid bursts present in a culture (22).

Some discrepancies in the findings of the two laboratories also may be attributable to the use of an outbred and, thus, not strictly defined mouse strain. Our report of a variable percentage of TPA-resistant BFU-E in CD-1 mice shows how much genetically nonidentical subjects can differ with respect to their response to identical doses of the same tumor promotor.

In conclusion, the opposing effects of tumor-promoting phorbol esters on two hematopoietic progenitor cells (CFU-GM and BFU-E) which are thought to be developmentally closely related (23) suggest that sensitivity to phorbol esters should provide a useful marker in the further analysis of the earliest events in blood cell development.

We gratefully acknowledge the expert technical assistance of Mrs. E. Connor and Mrs. M. Zicha. This work was supported by Grants AM 16702 and AM 27157 from the National Institute of Arthritis, Metabolism, and Digestive Diseases; a Hubert E. and Anne E. Rogers Scholarship (to F.S.); and Research Career Development Award HL 004801 from the National Heart, Lung, and Blood Institute (to J.L.S.). R.K.S. is a Special Fellow of the Leukemia Society of America.

1. Slaga, T. J., Sivak, A. & Boutwell, R. K., eds. (1978) *Mechanisms of Tumor Promotion and Cocarcinogenesis* (Raven, New York), Vol. 2.
2. Diamond, L., O'Brien, T. G. & Baird, W. M. (1980) *Adv. Cancer Res.* **32**, 1–74.
3. Weinstein, I. B., Wigler, M. & Pietropaolo, C. (1977) in *The Origins of Human Cancer*, eds. Hiatt, H. H., Watson, J. D. & Winsten, J. A. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), Vol. 4, pp. 751–772.
4. Iscove, N. N., Sieber, F. & Winterhalter, K. H. (1974) *J. Cell. Physiol.* **83**, 309–329.
5. Iscove, N. N. (1978) in *Hemopoietic Cell Differentiation*, eds. Cline, M. J., Golde, D., Metcalf, D. & Fox, C. F. (Academic, New York), p. 37.
6. Metcalf, D. (1977) *Hemopoietic Colonies* (Springer, Berlin), pp. 75–79.
7. Miyake, T., Kung, C. K.-H. & Goldwasser, E. (1977) *J. Biol. Chem.* **252**, 5558–5564.
8. Stuart, R. K. & Hamilton, J. A. (1980) *Science* **208**, 402–404.
9. Rovera, G., O'Brien, T. G. & Diamond, L. (1979) *Science* **204**, 868–870.
10. Sieber, F., Stuart, R. K. & Spivak, J. L. (1980) *J. Cell Biol.* **87**, 18a (abstr.).
11. Williams, N., Eger, R. R., Moore, A. M. S. & Mendelsohn, N. (1978) *Differentiation* **11**, 59–63.
12. Iscove, N. N. & Sieber, F. (1975) *Exp. Hematol.* **3**, 32–43.
13. Bradley, T. R. & Metcalf, D. (1966) *Aust. J. Exp. Biol. Med. Sci.* **44**, 287–299.
14. Stuart, R. K., Hamilton, J. A., Sensenbrenner, L. L. & Moore, M. A. S. (1981) *Blood*, in press.
15. Lenz, R. & Pluznik, D. H. (1980) *Exp. Hematol.* **8**, Suppl. 7, 18 (abstr.).
16. Williams, N. (1979) *Blood* **53**, 1089–1094.
17. Belman, S. & Troll, W. (1978) in *Mechanisms of Tumor Promotion and Cocarcinogenesis*, eds. Slaga, T. J., Sivak, A. & Boutwell, R. K. (Raven, New York), pp. 117–134.
18. Tsan, M.-F. (1980) *Clin. Res.* **28**, A326 (abstr.).
19. Sharkis, S. J., Spivak, J. L., Ahmed, A., Misiti, J., Stuart, R. K., Wiktor-Jedrejzak, W., Sell, K. W. & Sensenbrenner, L. L. (1980) *Blood* **55**, 524–527.
20. Van Zant, G., Goldwasser, E. & Pech, N. (1979) *Blood* **53**, 946–965.
21. Fibach, E., Marks, P. A. & Rifkind, R. A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5273–5277.
22. Gallicchio, V. S. & Murphy, M. J., Jr. (1979) *Exp. Hematol.* **7**, 219–224.
23. Gregory, C. J. (1976) *J. Cell. Physiol.* **89**, 289–302.