

Polyamine Requirements for Induction of HL-60 Promyelocyte Differentiation by Leukocyte-conditioned Medium and Phorbol Ester¹

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ABSTRACT

The polyamines putrescine, spermidine, and spermine have been implicated in the regulation of both proliferation and differentiation. Spermidine is required for DNA replication, and the intracellular depletion of this polyamine can be used to distinguish cellular events related to proliferation. We have demonstrated previously that depletion of intracellular spermidine results in cytostasis of human HL-60 promyelocytes. The inhibition of HL-60 proliferation is associated with inhibition of differentiation induced by dimethyl sulfoxide, hexamethylene bisacetamide, butyric acid, and retinoic acid. The present studies extend these findings by monitoring the effects of spermidine depletion and inhibition of DNA replication on induction of HL-60 differentiation by leukocyte-conditioned medium and phorbol ester. The results demonstrate that both inducers enhance intracellular polyamine levels. However, depletion of intracellular spermidine inhibits induction of HL-60 differentiation by leukocyte-conditioned medium, but not by phorbol ester. Further, the addition of exogenous spermidine abrogates the inhibition of proliferation, and leukocyte-conditioned medium induced HL-60 differentiation, confirming the requirement of this polyamine for the expression of a differentiated phenotype. The present results and our previous findings suggest that spermidine is required for induction of HL-60 differentiation by a variety of agents, including leukocyte-conditioned medium, but that phorbol ester induces HL-60 differentiation in the absence of polyamine biosynthesis and DNA replication.

INTRODUCTION

The polyamines putrescine, spermidine, and spermine have been implicated in the regulation of cellular proliferation (10, 25) and differentiation (2, 6, 7, 26, 29). The activity of the rate-limiting enzyme in the polyamine synthetic pathway, ODCase,³ is increased prior to cellular proliferation, and inhibition of this enzyme by DFMO is associated with an arrest of cell growth (14-16). Spermidine is required for cell proliferation and reverses cytostasis induced by DFMO (19). Thus, the reversible depletion of intracellular spermidine by DFMO inhibits DNA replication, and this approach has been used to demonstrate the requirements

of spermidine and proliferation for the induction of MEL cell differentiation by DMSO, HMBA, and BA (23).

The human HL-60 promyelocytic cell line has also been studied as a model of myeloid differentiation (1). Previous studies have indicated that the induction of HL-60 differentiation by DMSO, RA, and BA is not inhibited by a block in cellular proliferation induced by hydroxyurea and 1- β -D-arabinofuranosylcytosine (4). It has also been demonstrated that phorbol ester-induced macrophage differentiation of HL-60 cells can occur in the absence of DNA synthesis (20, 27). In contrast, we have inhibited HL-60 proliferation by exposing cells to DFMO and have demonstrated that depleting intracellular spermidine blocks induction of HL-60 differentiation by DMSO, HMBA, BA, and RA (24). We have also demonstrated a highly significant relationship between inhibition of both proliferation and induction of HL-60 differentiation by these agents (24), thus suggesting that replication is required for expression of a differentiated HL-60 phenotype. Similarly, induction of HL-60 cells along the monocyte-macrophage lineage by lymphocyte-conditioned medium has been shown by DNA histogram analysis to require S-phase synthesis (31).

The relationship between proliferation and differentiation of HL-60 cells has therefore not been clearly established. In order to address this issue, we have examined HL-60 differentiation induced by either LCM or phorbol ester following complete inhibition of DNA synthesis and cellular proliferation by the depletion of intracellular spermidine. The results demonstrate that spermidine is required for induction of HL-60 differentiation by LCM but that phorbol ester induces HL-60 differentiation in the absence of this polyamine and DNA replication.

MATERIALS AND METHODS

Cell Culture. HL-60 cells were grown in RPMI-1640 medium (Grand Island Biological Co., Grand Island, NY) containing 4 mM L-glutamine (Grand Island Biological Co.), 1% penicillin-streptomycin (Grand Island Biological Co.), 0.1 mM nonessential amino acids, and 1.0 mM sodium pyruvate in 20% heat-inactivated fetal bovine serum (Grand Island Biological Co.) at a density ranging from 1 to 2 $\times 10^5$ /ml in a 5% CO₂-humidified atmosphere at 37°. Viable cells were determined by trypan blue exclusion. DFMO (RF-71, 72A; Merrell Dow Pharmaceuticals, Cincinnati, OH) was added at a final concentration of 5 $\times 10^{-3}$ M with each passage 60, 36, and 12 hr prior to the addition of inducer. Phytohemagglutinin-stimulated LCM was prepared as described previously (28) and diluted to 20% in the HL-60 culture medium. TPA (Sigma Chemical Co., St. Louis, MO) was dissolved in acetone at 3.2 $\times 10^{-4}$ M and then further diluted to 3.2 $\times 10^{-9}$ M in the culture medium.

Histochemical Staining. Cytocentrifuge smears of culture cells were examined for the expression of α -naphthyl acetate esterase (nonspecific esterase) and NBT reduction (30). The percentage of positive cells was determined by counting 200 cells in duplicate.

Monoclonal Antibodies. The monoclonal antibody Mo1 was prepared

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³ The abbreviations used are: ODCase, ornithine decarboxylase; DFMO, α -difluoromethyl ornithine; MEL, murine erythroleukemia; DMSO, dimethyl sulfoxide; HMBA, hexamethylene bisacetamide; BA, butyric acid; RA, retinoic acid; LCM, leukocyte-conditioned medium; TPA, 12-O-tetradecanoylphorbol-13-acetate; NBT, nitroblue tetrazolium; NSE, nonspecific esterase.

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and used as a measure of HL-60 differentiation as described previously (28). Mo1 antigen is expressed by monocytes, granulocytes, null cells in the peripheral blood, and immature myeloid cells in the bone marrow. Monoclonal antibody MY4 reacts with the cell surface of monocytes (9). Antigen expression was detected by an indirect immunofluorescence assay using an Ortho Cytofluorograf FC200/4800A (Ortho Instruments, Westwood, MA). Background fluorescence was determined using a nonreactive IgG antibody. Ten thousand cells were analyzed per sample.

Measurement of Intracellular Polyamines. HL-60 cells (4×10^6) were harvested and washed twice with Dulbecco 'A' phosphate-buffered saline (Oxoid Limited, England). Polyamines were extracted in 4% sulfosalicylic acid and separated on a Beckman 121MB amino acid analyzer (8). The levels were quantitated using 3,3'-diaminodipropylamine as an internal standard. Putrescine, 3,3'-diaminodipropylamine, spermidine, and spermine are detectable at 22.5, 30.7, 43.7, and 44.8 min, respectively. The results (pmol/ 10^5 cells) are expressed as the mean \pm S.D. for 2 separate determinations, each performed in duplicate.

RESULTS

We have monitored the effects of LCM and TPA on intracellular polyamine levels in HL-60 cells (Chart 1). Putrescine increased approximately 2.5-fold at 6 hr after adding LCM, while TPA resulted in less pronounced increases in the level of this polyamine (Chart 1A). Similarly, intracellular spermidine (Chart 1B) increased following exposure to either LCM or TPA. In contrast, TPA had no detectable effect on spermine levels (Chart 1C), while increases in this polyamine were observed following exposure to LCM. These results suggest that exposure of HL-60 cells to either LCM or TPA is associated with increases in polyamine synthesis.

The effects of 5 mM DFMO on intracellular polyamine levels were also monitored in control and LCM- or TPA-treated HL-60 cells (Chart 2). The cells were treated with DFMO for 60 hr prior to the addition of inducer. Under these conditions, intracellular putrescine (Chart 2A) and spermidine (Chart 2B) were undetectable at the time of treatment with LCM or TPA. Further, these polyamines remained undetectable during the period monitored for induction of differentiation (LCM, 96 hr; TPA, 48 hr). Treatment with DFMO also resulted in decreased intracellular spermine levels. However, spermine remained detectable and was similar in the LCM- or TPA-treated cells (Chart 2C).

The effects of DFMO, LCM, and TPA on HL-60 proliferation are illustrated in Chart 3. Proliferation of cells treated with LCM was similar to that of untreated cells over a 72-hr period. The DFMO-treated cells achieved cytotostasis at the time of LCM addition, and there was no subsequent proliferation. In contrast,

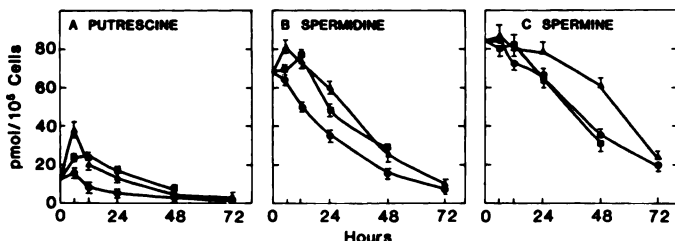


Chart 1. Effects of LCM and TPA on HL-60 intracellular polyamine levels. HL-60 cells were maintained in logarithmic growth phase at a density of 5×10^5 cells/ml by serial passage at 60, 36, and 12 hr prior to the addition of inducer. LCM (20%) or TPA (3.2×10^{-8} M) was added at 0 hr. Untreated (●), LCM (▲), and TPA (■)-treated cells (4×10^6) were monitored for intracellular putrescine, spermidine, and spermine at the indicated times. Points, mean pmol/ 10^5 cells of 2 separate determinations performed in duplicate; bars, S.D.

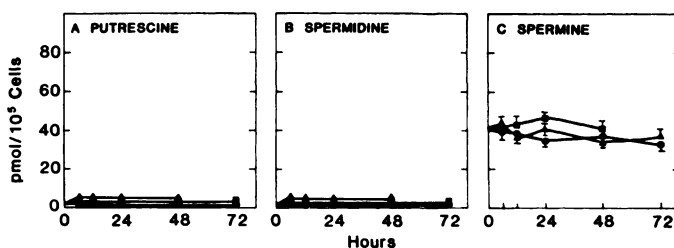


Chart 2. Effects of DFMO on polyamine levels in HL-60 cells treated with LCM and TPA. HL-60 cells were grown in logarithmic phase and seeded at a density of 5×10^5 cells/ml. DFMO (5 mM) was added at each passage 60, 36, and 12 hr prior to addition of LCM or TPA (0 hr). The DFMO (●), DFMO-LCM (▲), and DFMO-TPA (■)-treated cells were monitored for intracellular putrescine, spermidine, and spermine at the indicated times. Points, mean pmol/ 10^5 cells of 2 separate determinations performed in duplicate; bars, S.D.

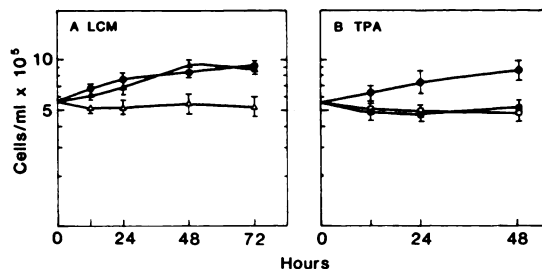


Chart 3. Effects of DFMO, LCM, and TPA on HL-60 cell growth. HL-60 cells were either maintained in logarithmic growth phase as described in the legend to Chart 1 or treated with 5 mM DFMO as described in the legend to Chart 2. LCM or TPA was added at 0 hr. A, ●, untreated cells; ▲, LCM; and △, DFMO-LCM-treated cells. B, ●, untreated cells; ■, TPA; and □, DFMO-TPA-treated cells. Points, mean of 2 determinations performed in duplicate; bars, S.D. Viability was over 90% in each group.

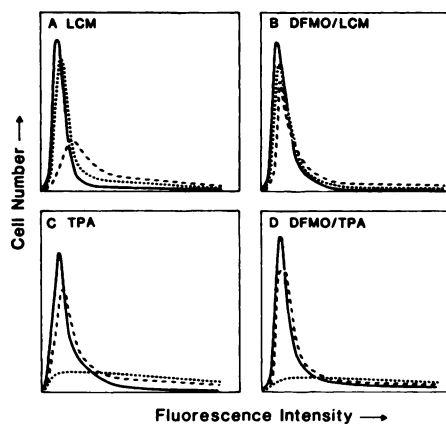


Chart 4. Expression of cell surface Mo1 and MY4 antigens on HL-60 cells treated with DFMO, LCM, and TPA. HL-60 cells were maintained in logarithmic growth phase (Chart 1 legend) or treated with 5 mM DFMO (Chart 2 legend). LCM or TPA was added at 0 hr. Reactivity of anti-Mo1 (—) and anti-MY4 (---) was monitored after 96 hr for LCM-treated cells (A and B) and after 48 hr for TPA-treated cells (C and D). —, fluorescence pattern with an isotype identical to control monoclonal antibody.

TPA inhibited HL-60 proliferation (Chart 3B), and similar results were obtained with HL-60 cells treated with both DFMO and TPA. Further, the results obtained with DFMO-treated cells were similar to those obtained with DFMO-LCM- or DFMO-TPA-treated cells.

The induction of HL-60 differentiation by LCM and TPA was monitored using monoclonal antibodies anti-MY4 and anti-Mo1. Chart 4A illustrates the pattern of reactivity obtained after 96 hr of exposure to LCM. The cell surface expression of MY4 was

increased significantly, while reactivity with anti-Mo1 increased only slightly. These increases in reactivity were completely blocked by treatment with DFMO (Chart 4B). In contrast, exposure of HL-60 cells to TPA was associated with little, if any, enhancement of MY4 cell surface antigen expression, while there was a marked increase in reactivity with anti-Mo1. This increase in Mo1 antigen expression, however, was not inhibited by DFMO. These results are listed in Tables 1 and 2.

The HL-60 cells were also monitored for NSE and NBT staining. DFMO alone had no significant effect on histochemical staining. The exposure of HL-60 cells to LCM for 96 hr resulted in significant increases in both NSE and NBT staining, consistent with maturation along both the monocyte-macrophage and myeloid lineages (Table 1). The expression of each of these parameters was inhibited by DFMO treatment. Further, the inhibition of cell surface antigen expression and histochemical staining by DFMO was abrogated by the addition of exogenous spermidine, thus confirming the involvement of this polyamine in the expression of the differentiated phenotype.

Table 2 lists the results obtained following exposure of HL-60 cells to TPA for 48 hr. Staining for NBT reduction after TPA exposure was similar to control levels. The percentage of cells adhering to the tissue culture flask was also monitored as another parameter of monocyte-macrophage maturation. In these experiments, the expression of Mo1 antigen, NSE staining, and the percentage of adherent cells increased with TPA exposure. More importantly, DFMO treatment had no effect on the expression of these markers of monocyte-macrophage differentiation.

DISCUSSION

We have monitored previously the relationship of intracellular polyamine levels with proliferation and differentiation of MEL cells (23). The results demonstrated a significant relationship between intracellular spermidine levels and MEL proliferation. The results also demonstrated that spermidine was required for induction of MEL differentiation by DMSO, HMBA, and BA. A significant relationship between MEL proliferation and differentiation suggested that spermidine was involved primarily in proliferation and, thereby, differentiation. It was not possible to deter-

mine a direct requirement for spermidine in differentiation. Other studies have suggested that polyamines play a role in proliferation, but not differentiation, of erythroid precursor cells *in vivo* (17, 18). Further, a requirement of polyamine biosynthesis has been shown for accessory cell regulation of hematopoiesis (22). Although polyamine depletion also inhibits the differentiation of the myoblast cells (3), inhibition of ODCase activity by DFMO induces murine embryonal carcinoma cell differentiation (21). It is thus of interest to determine whether induction of differentiation not linked to a DNA replicative process would require polyamine biosynthesis for expression of the differentiated phenotype.

Polyamine biosynthesis has been shown to be essential for HL-60 cell growth (13), and we have shown previously that intracellular spermidine levels correlate significantly with HL-60 proliferation (24). ODCase activity and putrescine levels (12) increase during treatment of HL-60 cells with phorbol ester and RA. The increases in ODCase activity, rather than absolute polyamine levels, have been reported to be essential only for proliferation and not differentiation of HL-60 cells (11, 13). In contrast, we have monitored intracellular polyamine levels and have demonstrated a requirement of spermidine in the induction of HL-60 differentiation by DMSO, HMBA, RA, and BA (24). Further, the inhibition of HL-60 differentiation by DFMO is partially reversed by exogenous spermidine. Since proliferation is dependent upon the presence of intracellular spermidine, one explanation for these findings is that DNA replication is required for HL-60 differentiation with these inducers.

The arrest of HL-60 cellular proliferation by depletion of intracellular polyamines has been used in the present studies to determine the requirements of spermidine and proliferation in the induction of HL-60 differentiation by LCM and TPA. The results demonstrate that depletion of intracellular spermidine blocks the induction of a differentiated phenotype by LCM. Further, repletion of intracellular spermidine reverses cytostasis and results in the expression of cell surface and histochemical markers of both monocyte-macrophage and granulocyte differentiation. These findings suggest that induction of HL-60 differentiation by LCM requires spermidine and proliferation. Similarly, polyamines have been shown to play an essential role in the induction of both proliferation and granulocytic differentiation of normal human bone marrow progenitor cells by placenta-conditioned medium (29).

In contrast, depletion of intracellular polyamines had no effect on the induction of a differentiated phenotype by TPA, thus suggesting that induction of differentiation by this agent does not require spermidine and cell replication. This finding confirms previous observations that polyamines (13) and cell proliferation (20, 27) are not required for TPA induction along the monocyte-macrophage lineage. Thus, the induction of HL-60 differentiation by TPA probably differs considerably from the mechanisms of the other inducers that require spermidine and proliferation. It is possible that TPA activates a late step in the pathway of events leading to differentiation by bypassing early events involving DNA replication. The recent demonstration that TPA activates a cell membrane protein kinase in certain cells (5) is of interest in this regard, and the possible role of such protein kinases in myeloid differentiation requires further investigation.

Finally, previous reports have supported the dissociation of HL-60 proliferation and differentiation (4, 11, 13). Thus, the arrest of HL-60 proliferation by hydroxyurea and 1- β -D-arabinofurano-

Table 1
Effect of DFMO on induction of HL-60 differentiation by LCM

	Mo1	MY4	NSE	NBT
Control	8.3 \pm 0.5 ^a	22.6 \pm 1.9	3.6 \pm 0.8	5.2 \pm 1.9
DFMO	7.3 \pm 3.0	20.4 \pm 8.2	2.9 \pm 0.8	4.1 \pm 1.1
LCM	17.1 \pm 1.7	58.9 \pm 5.2	22.8 \pm 6.0	40.0 \pm 2.0
DFMO-LCM	7.9 \pm 2.7	15.1 \pm 4.5	3.7 \pm 1.0	4.7 \pm 1.9
DFMO-LCM-spermidine ^c	26.2 \pm 3.0	46.7 \pm 0.3	ND ^b	42.0 \pm 3.0

^a Mean \pm S.D. of the percentage of positive cells for 2 determinations performed in duplicate after 96 hr of LCM exposure.

^b ND, not determined.

^c Spermidine (5×10^{-6} M) was added with inducer.

Table 2
Effect of DFMO on induction of HL-60 differentiation by TPA

	Mo1	MY4	NSE	Adherence
Control	2.9 \pm 1.8 ^a	6.0 \pm 1.0	2.2 \pm 0.5	3.2 \pm 1.0
DFMO	4.5 \pm 2.0	6.2 \pm 0.8	2.8 \pm 0.6	0.3 \pm 0
TPA	62.8 \pm 1.1	11.7 \pm 1.5	65.8 \pm 5.1	61.9 \pm 0.6
DFMO-TPA	77.1 \pm 1.3	4.2 \pm 0.4	80.3 \pm 2.5	62.0 \pm 1.1

^a Mean \pm S.D. of the percentage of positive cells for 2 determinations performed in duplicate after 48 hr of TPA exposure.

sylyctosine did not block induction of differentiation by DMSO, RA, and BA (4). Although increases in ODCase activity have been shown to be essential for proliferation and not differentiation (11, 13), polyamine levels were either not monitored (11) or only partially depleted upon addition of the inducer (13). In contrast, we have completely depleted intracellular polyamines to demonstrate the requirements of spermidine for both proliferation and induction of HL-60 differentiation with DMSO, HMBA, BA, RA, and LCM. The differentiated HL-60 phenotype, however, can clearly be expressed in the absence of spermidine when using TPA as inducer. These findings might therefore suggest that spermidine and initiation of DNA replication are required for induction of HL-60 differentiation with certain inducers other than TPA, while disruption of ongoing DNA synthesis with S-phase-specific agents (4) is not sufficient to inhibit appearance of the differentiated phenotype.

REFERENCES

- Collins, S., Gallo, R., and Gallagher, R. Continuous growth and differentiation of human leukemic cells in suspension culture. *Nature (Lond.)*, 270: 347-349, 1977.
- Emmanuelsson, H., and Heby, O. Inhibition of putrescine synthesis blocks development of the polychaete *Ophryotrocha labronica* at gastrulation. *Proc. Natl. Acad. Sci. USA*, 75: 1039-1042, 1978.
- Erwin, G., Ewton, D., Fiorini, J., and Pegg, A. Polyamine depletion inhibits the differentiation of L6 myoblast cells. *Biochem. Biophys. Res. Commun.*, 114: 944-949, 1983.
- Ferrero, D., Tarella, C., Gallo, E., Ruscetti, F., and Breitman, T. Terminal differentiation of the human promyelocytic leukemia cell line, HL-60, in the absence of cell proliferation. *Cancer Res.*, 42: 4421-4426, 1982.
- Feuerstein, N., and Cooper, H. Rapid protein phosphorylation induced by phorbol ester in HL-60 cells. *J. Biol. Chem.*, 258: 10786-10793, 1983.
- Fozard, J., Part, M., Prakash, N., Grove, P., Schechter, A., and Koch-Weser, J. L-Ornithine decarboxylase: an essential role in early mammalian embryogenesis. *Science (Wash. DC)*, 208: 505-508, 1980.
- Gazit, Y., and Friend, C. Polyamine biosynthesis enzymes in the induction and inhibition of differentiation in Friend erythroleukemia cells. *Cancer Res.*, 40: 1727-1732, 1980.
- Gehrke, C., Juo, K., and Ellis, R. An improved automated ion-exchange method. *J. Chromatogr.*, 143: 345-361, 1977.
- Griffin, J., Ritz, J., Nadler, L., and Schlossman, S. Expression of myeloid differentiation antigens on normal and malignant myeloid cells. *J. Clin. Invest.*, 68: 932-941, 1981.
- Heby, O. Role of polyamines in the control of cell proliferation and differentiation. *Differentiation*, 19: 1-20, 1981.
- Heby, O., Oredsson, S., Olsson, I., and Marton, L. A role of polyamines in mouse embryonal carcinoma (F9 and PCC3) cell differentiation but not in human promyelocytic leukemia (HL-60) cell differentiation. *Adv. Polyamine Res.*, 4: 727-742, 1983.
- Huberman, E., Weeks, C., Herrmann, A., Callihan, M., and Stage, T. Alterations in polyamine levels induced by phorbol diesters and other agents that promote differentiation in human promyelocytic leukemia cells. *Proc. Natl. Acad. Sci. USA*, 78: 1062-1066, 1981.
- Luk, G., Civin, D., Weissman, R., and Baylin, S. Ornithine decarboxylase: essential in proliferation of human promyelocytic leukemia cells. *Science (Wash. DC)*, 216: 75-77, 1982.
- Luk, G., Goodwin, G., Marton, L., and Baylin, S. Polyamines are necessary for the survival of human small-cell lung carcinoma in culture. *Proc. Natl. Acad. Sci. USA*, 78: 2355-2358, 1981.
- Mamont, P., Duchesne, M., Joder-Ohlenbusch, A., and Grove, J. Effects of ornithine decarboxylase inhibitors on cultured cells. In: N. Sella, M. Jung, and J. Koch-Weser (eds.), *Enzyme-activated Irreversible Inhibitors*, pp. 43-54. Amsterdam: Elsevier-North Holland Biomedical Press, 1978.
- Metcalf, B., Bey, P., Danzin, C., Jung, M., Casara, P., and Vevert, J. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (EC 4.1.1.17) by substrate and product analogues. *J. Am. Chem. Soc.*, 100: 2551-2553, 1978.
- Niskanen, E., Kallio, A., McCann, P., and Baker, D. The role of polyamine biosynthesis in hematopoietic precursor cell proliferation in mice. *Blood*, 61: 740-745, 1983.
- Niskanen, E., Kallio, A., McCann, P., Pou, G., Lyda, S., and Thornhill, A. Divergent effects of ornithine decarboxylase inhibition on murine erythropoietic precursor cell proliferation and differentiation. *Cancer Res.*, 43: 1536-1540, 1983.
- Porter, C. Spermidine requirement for cell proliferation in eukaryotic cells: structural specificity and quantitation. *Science (Wash. DC)*, 219: 1083-1085, 1983.
- Rovera, G., Olashaw, N., and Meo, P. Terminal differentiation in human promyelocytic leukaemic cells in the absence of DNA synthesis. *Nature (Lond.)*, 284: 69-70, 1980.
- Schindler, J., Kelly, M., and McCann, P. Inhibition of ornithine decarboxylase induces embryonal carcinoma cell differentiation. *Biochem. Biophys. Res. Commun.*, 114: 410-417, 1983.
- Sharkis, S., Luk, G., Collector, M., McCann, P., Baylin, S., and Sensenbrenner, L. Regulation of hematopoiesis. II. The role of polyamine inhibition on helper or suppressor influences of the thymus. *Blood*, 67: 604-607, 1983.
- Sugiura, M., Shafman, T., and Kufe, D. Effects of polyamine depletion on proliferation and differentiation of murine erythroleukemia cells. *Cancer Res.*, 44: 1440-1444, 1984.
- Sugiura, M., Shafman, T., Mitchell, T., Griffin, J., and Kufe, D. Involvement of spermidine in proliferation and differentiation of human promyelocytic leukemia cells. *Blood*, 63: 1153-1158, 1984.
- Tabor, C., and Tabor, H. 1,4-Diaminobutane (putrescine), spermidine, and spermine. *Annu. Rev. Biochem.*, 45: 285-306, 1976.
- Takigawa, M., Ishida, H., Takano, T., and Suzuki, F. Polyamines and differentiation: induction of ornithine decarboxylase by parathyroid hormone is a good marker of differentiated chondrocytes. *Proc. Natl. Acad. Sci. USA*, 77: 1481-1485, 1980.
- Territo, M., and Koeffler, H. Induction by phorbol esters of macrophage differentiation in human leukemia cell lines does not require cell division. *Br. J. Haematol.*, 47: 479-483, 1981.
- Todd, R., Griffin, J., Ritz, J., Nadler, L., Abrams, T., and Schlossman, S. Expression of normal monocyte-macrophage differentiation induced by leukocyte-conditioned medium or phorbol diester. *Leukemia Res.*, 5: 491-495, 1981.
- Verma, D., and Sunkara, P. An essential role for polyamine biosynthesis during human granulopoietic differentiation. *Cancer Res.*, 41: 3046-3049, 1982.
- Yam, L., Li, C., and Crosby, W. Cytochemical identification of monocytes and granulocytes. *Am. J. Clin. Pathol.*, 55: 283-290, 1971.
- Yen, A., and Chiao, J. Control of cell differentiation during proliferation. 1. Monocytic differentiation of HL-60 promyelocytes. *Exp. Cell Res.*, 146: 87-93, 1983.