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Research Article

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RAPID

PUBLICATION

Tumor Promoter Phorbol Myristic Acetate Stimulates Immunoglobulin Secretion Correlated with Growth Cessation in Human B Lymphocyte Cell Lines

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ABSTRACT Immunoglobulin production by lymphoblast cell lines was studied using protein A-red blood cell plaque formation to detect individual secreting cells. Immunoglobulin (Ig) secretion by 6 of 12 human B-cell lines tested could be stimulated up to twentyfold by phorbol myristic acetate (PMA) at subtoxic concentrations of 10–1000 ng/ml depending on the line. Stimulation was found with both IgM and IgG cell lines. No switch of Ig class synthesis was found in the cell lines as a result of PMA incubation. Increase in Ig secretion was closely associated with cessation of growth resembling induction of terminal differentiation in the cells. PMA induction of Ig secretion in B lymphocytes from normal peripheral blood requires the cooperation of T cells. PMA stimulation of certain cell lines reported here suggests that the lines are late in the differentiation pathway to plasmacyte and can be easily triggered to secrete Ig by membrane-altering agents.

INTRODUCTION

The tumor-promoting properties of phorbol myristic acetate (PMA)[1] have been well correlated with in vitro growth stimulation and/or blocking of differentiated phenotypes in epidermal cells (1), myocytes (2), erythroleukemias (3, 4), preadipose fibroblasts (5), and melanoma cells (6). However, PMA induces maturation resembling terminal differentiation in tumor cell lines of the granulocyte-macrophage lineage (7–9). Induction of mature properties by PMA in myeloid cell lines requires gene activation (9) correlated with growth inhibition of the cultures (7, 9). PMA is mitogenic for human B lymphocytes (10) and a subset of human T cells (11). Evidence has also been presented for PMA stimulation of sheep erythrocyte (E)-rosette formation in a human T-cell line (12), induction of surface immunoglobulin (Ig) and Fc receptor expression in murine lymphomas (13), and induction of cytoplasmic Ig in chronic lymphocytic leukemia cells suggestive of partial maturation to plasmacytes (14). Human and murine B-lymphocyte cell lines differ over 500-fold in sensitivity to PMA toxicity suggesting their derivation from subsets of normal B cells differing in PMA responsiveness (15). The present study investigates the extent of terminal differentiation induced in human B-cell lines by PMA, measured as Ig secretion coupled with growth inhibition.

METHODS

Cell line cultures. Human B lymphoblastoid lines were described previously (16), except for CESS from a myelo-

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[1] *Abbreviations used in this paper*: Ig, immunoglobulin; PFC, plaque-forming cells; PMA, phorbol myristic acetate.

monocytic leukemia patient[2] and Epstein-Barr virus-induced lines from normal donors (17) and plasmacytoma ARH-77 (18). BM.A. was selected for azaguanine resistance from line BM-NH and ARH.A from ARH-77. BM.B was selected for bromodeoxyuridine resistance from BM-NH.[3] Cell lines were grown in RPMI 1640 medium plus 10% fetal calf serum.

Plaque-forming cell (PFC) assay for immunoglobulin secretion. The method of reverse PFC with protein A-coated sheep erythrocytes and polyvalent rabbit antihuman Ig (N. L. Cappel Laboratories Inc., Cochranville, Pa.) was used to detect all classes of Ig-secreting cells (19). IgG and IgM PFC were detected using IgG fraction of rabbit anti-IgG made monospecific by absorption on myeloma IgM columns (20) and Cappel rabbit anti-IgM, respectively. Polyvalent sera from several sources were screened to give maximum PFC on pokeweed mitogen-stimulated peripheral blood lymphocytes; class-specific antibody preparations were chosen to give maximum PFC with homologous cell lines and no PFC with the lines of the heterologous Ig class. Each culture was assayed in duplicate for PFC. No plaques were detected in the absence of complement, and the number of PFC without anti-Ig was <5% of that with inclusion of anti-Ig.

Reagents. Phorbol myristic acetate (PMA, tetradecanoylphorbol acetate) (Consolidated Midland, Corp., Brewster, N. Y.) was dissolved 5 mg/ml in dimethylsulfoxide and then diluted in saline. Dimethylsulfoxide alone, at the highest concentration used in combination with PMA (0.1%), or at fivefold higher amounts, had no effect on cell line growth or Ig secretion.

TABLE I

Induction of PFC in Human B Lines by PMA

Line	PMA	IgM-PFC	IgG-PFC
	μg/ml		
BM	—	150±11	0
	1	435±26	0
BM.A	—	5±0.7	0
	0.2	22±2.0	0
1788.2	—	0.05±0.01	0
	0.2	0.45±0.10	0
HIRS	—	0	2±2
	0.1	0	187±7
RMcG	—	0	6±0
	0.1	0	45±5
ARH-77	—	0	0.3±0.1
	1	0	5±0.4

Cells cultured at 2 × 10^5/ml for 2 d and PFC per 10^3 recovered cells determined. Cultures showed >90% viability in all cases although PMA inhibited growth 50–95%.

RESULTS

Culture of some human B lines with PMA caused three- to ninetyfold increases in Ig-secreting cells detected by the protein A-PFC assay (Table I). The plaque size for PMA-treated and control cultures was similar, suggesting that cells with undetectable Ig secretion were induced to high rate production. Cell lines differed in optimal dose of PMA to augment PFC, which generally correlated with severe growth inhibition. The doses used in Table I were not toxic at 2 d incubation, but usually caused some cell death at five- to tenfold higher concentrations or longer time intervals. No line was found to switch to a new Ig class or produce two classes of Ig when incubated with PMA.

Fig. 1 shows that 0.2 μg/ml PMA was optimal for 2-d PFC response by line RPMI 1788 and 0.05 μg/ml for 3 d incubation. Both concentrations allowed less than a doubling of cell numbers over 3 d, while control cells increased 4.8-fold. PMA did not enhance PFC in strains of a number of cell lines, usually those with undetectable PFC in control cultures, including DAUDI, RAMOS, ARH.A, CESS, BM.B, and RPMI 8866.

DISCUSSION

Our previous studies (16) showed that normal human T cells could stimulate IgM secretion and induce IgG production in certain B-lymphoblastoid lines. Stimulation of Ig secretion in cell lines by cocultivation with normal T cells has been confirmed by Kempner et al. (21). Our present results show that cell lines with ongoing Ig secretion can be stimulated directly by PMA. Ig secretion is also induced in human peripheral blood cells by PMA, but this reaction requires the cooperation of T cells (manuscript in preparation) similarly to induction by pokeweed mitogen (22). Therefore, certain dividing B cells (cell lines) may be at a differentiation state close to the Ig-producing plasmacyte and be easily triggered to mature by differentiation-altering agents.

PMA induces or stimulates expression of mature markers and functions in human and murine granulocyte (7, 8), macrophage (9, 23) and lymphocyte (12–14) tumor lines, and stimulates progenitor cells in normal bone marrow to form myeloid colonies in agar (24). PMA binds to cell membranes (25) and alters surface receptor properties (26, 27). The potent effect of phorbol ester on lymphocyte function suggests that it will be a valuable tool in further study of lymphocyte differentiation states and subsets of the corresponding leukemias. The small amounts of PMA required (0.01–1 μg/ml) for induction of Ig secretion rules out other known mitogens as possible contaminants responsible for the activity, and the chemically defined nature of PMA should allow biochemical studies of binding effects and immediate events in lymphocyte stimulation.

[2] Bradley, R., and G. Pilkington. Unpublished observations.
[3] Ralph, P., and I. Nakoinz. Unpublished observations.

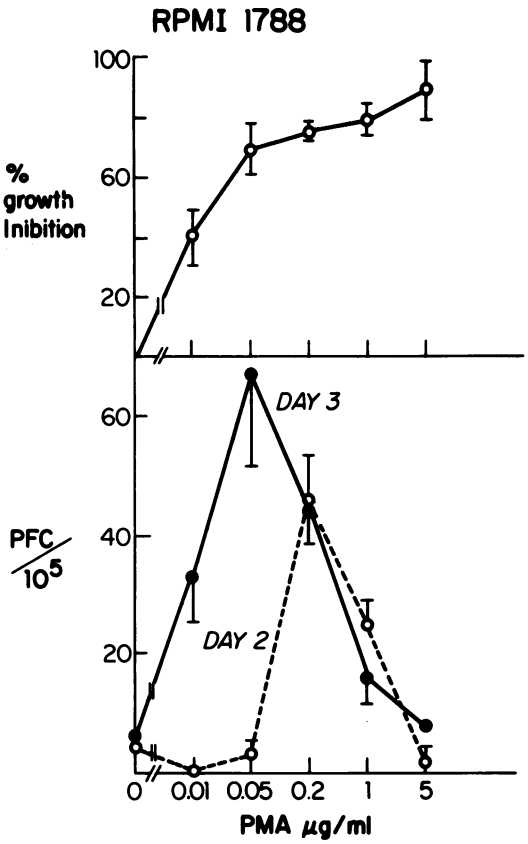

FIGURE 1 PMA stimulation of Ig secretion by human B cell line RPMI 1788. Top panel: Cells were cultured 2 or 3 d with PMA, viable cells counted and average growth inhibition by PMA calculated. Bottom panel: PFC per 10^5 RPMI 1788 cells recovered at day 2 (○) or day 3 (●). Cell numbers in control cultures increased 4.8-fold at day 3. Cell viability was >90% at day 3 for PMA concentrations of 1 μg/ml or less.

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REFERENCES

1. Yuspa, S. H., U. Lichti, T. Ben, E. Patterson, H. Hennings, T. J. Slage, N. Colburn, and W. Kelsey. 1976. Phorbol esters stimulate DNA synthesis and ornithine decarboxylase activity in mouse epidermal cell cultures. *Nature (Lond.).* **262:** 402–404.
2. Cohen, R., M. Pacifici, N. Rubenstein, J. Biehl, and H. Holtzer. 1977. Effect of tumor promoter on myogenesis. *Nature (Lond.).* **266:** 538–540.
3. Rovera, G., T. O'Brien, and L. Diamond. 1977. Tumor promoters inhibit spontaneous differentiation of Friend erythroleukemia cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* **74:** 2894–2898.
4. Yamasaki, H., E. Fibach, U. Nudel, I. B. Weinstein, R. A. Rifkind, and P. A. Marks. 1977. Tumor promoters inhibit spontaneous and induced differentiation of murine erythroleukemia cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* **74:** 3451–3455.
5. Diamond, L., T. O'Brien, and G. Rovera. 1977. Inhibition of adipose conversion of 3T3 fibroblasts by tumor promoters. *Nature (Lond.).* **269:** 247–248.
6. Mufson, R. A., P. B. Fisher, and I. B. Weinstein. 1979. Effect of phorbol ester tumor promoters on the expression of melanogenesis in B-16 melanoma cells. *Cancer Res.* **39:** 3915–3919.
7. Rovera, G., D. Santoli, and C. Damsky. 1979. Human promyelocyte leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 2779–2783.
8. Lotem, J., and L. Sachs. 1979. Regulation of normal differentiation in mouse and human myeloid leukemic cells by phorbol esters and the mechanism of tumor promotion. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 5158–5162.
9. Ralph, P., H. E. Broxmeyer, and I. Nakoinz. 1977. Immunostimulators induce granulocyte-macrophage colony-stimulating activity and block proliferation in a monocyte tumor cell line. *J. Exp. Med.* **146:** 611–616.
10. Abb, J., G. J. Bayless, and F. Deinhardt. 1979. Lymphocyte activation by the tumor-promoting agent 12-0-tetradecanoyl-phorbol-13-acetate (TPA). *J. Immunol.* **122:** 1639–1642.
11. Tourlaine, J.-L., J. W. Hadden, F. Touraine, E. M. Hadden, R. Estensen, and R. A. Good. 1977. Phorbol myristate acetate: a mitogen selective for a T-lymphocyte subpopulation. *J. Exp. Med.* **145:** 460–465.
12. Nagasawa, K., and T. W. Mak. 1980. Phorbol esters induce differentiation in human malignant T lymphoblasts. *Proc. Natl. Acad. Sci. U. S. A.* **77:** 2964–2968.
13. Ralph, P., I. Nakoinz, and W. C. Raschke. Induction of differentiation functions coupled with growth inhibition in lymphocyte and macrophage tumor cell lines. *In* Maturation Factors in Cancer. M. A. S. Moore, editor. Raven Press, New York. In press.
14. Tötterman, T. H., K. Nilsson, and C. Sundström. Phorbol ester-induced differentiation of chronic lymphocytic leukemia cells. *Nature (Lond.).* **288:** 176–178.
15. Ralph, P. 1979. Functional subsets of murine and human B lymphocyte cell lines. *Immunol. Rev.* **48:** 107–121.
16. Kishimoto, T., T. Hirano, T. Kuritani, Y. Yamamura, P. Ralph, and R. A. Good. 1978. Induction of IgG production in human B lymphoblast cell lines with normal human T lymphocytes. *Nature (Lond.).* **271:** 756–758.
17. Hansen, J. A., S. M. Fu, P. Antonelli, M. Kamoun, J. N. Hurley, R. J. Winchester, B. Du Pont, and H. G. Kunkel. 1979. B-lymphoid cell lines derived from HLA-D homozygous donors. *Immunogenetics.* **8:** 51–56.
18. Burk, K. H., B. Drewinko, J. M. Trujillo, and M. J. Ahearn. 1979. Establishment of a human plasma cell line *in vitro*. *Cancer Res.* **38:** 2508–2511.
19. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting immunoglobulin of a given type of class. *Eur. J. Immunol.* **6:** 558–563.
20. Hirano, T., T. Kishimoto, T. Kuritani, A. Muraguchi, Y. Yamamura, P. Ralph, and R. A. Good. 1979. *In vitro* immune response of human peripheral blood lymphocytes. IV. Specific induction of human suppressor T cells by an antiserum to the T leukemic cell line HSB. *J. Immunol.* **123:** 1133–1140.

21. Kempner, P. H., W. Leibold, and R. A. Gatti. 1980. T-cell regulation of B-lymphoblastoid cell line function. *Cell. Immunol.* **55:** 32–41.

22. Janossy, G., and M. Greaves. 1975. Functional analysis of murine and human B-lymphocyte subsets. *Transplant. Rev.* **24:** 177–236.

23. Ralph, P., and I. Nakoinz. 1981. Differences in antibody-dependent and nonspecific killing of tumor cells by macrophage cell lines. *Cancer Res.* In press.

24. Lotem, J., and L. Sachs. 1979. Regulation of normal differentiation in mouse and human myeloid leukemic cells by phorbol esters and the mechanism of tumor promotion. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 5158–5161.

25. Driedger, P. E., and P. M. Blumberg. 1980. Specific binding of phorbol ester tumor promoters. *Proc. Natl. Acad. Sci. U. S. A.* **77:** 567–571.

26. Lee, L.-S., and I. B. Weinstein. 1978. Tumor promoting phorbol esters inhibit binding of epidermal growth factors to cellular receptors. *Science (Wash. D. C.).* **202:** 313–315.

27. Shoyab, M., J. E. DeLarco, and G. D. Todaro. 1979. Biologically active phorbol esters specifically alter affinity of epidermal growth factor membrane receptors. *Nature (Lond.).* **279:** 387–391.