Effects of Phorbol Myristate Acetate and a Lymphokine on Cyclic 3',5'-Guanosine Monophosphate Levels and Proliferation of Macrophages

Elba M. Hadden, John R. Sadlik, Ronald G. Coffey, and John W. Hadden

ABSTRACT

The tumor promoter phorbol myristate acetate (PMA) was compared to a lymphokine macrophage mitogenic factor (MMF) for its ability to induce replication of guinea pig peritoneal and alveolar macrophages. Like MMF, PMA induces DNA synthesis of both cell populations with peak thymidine incorporation at 72 hr of culture. Optimal concentrations of PMA for the peritoneal and alveolar cells were 1.6 x 10^{-7} and 1.6 x 10^{-9} M, respectively. The magnitude of the effect is slightly less than MMF but greater than that of phytohemagglutinin or concanavalin A. Indomethacin added to inhibit prostaglandin synthesis potentiates the effects of MMF but has little effect on the actions of PMA and the other mitogens. Potentiation by indomethacin of the effects of PMA on the peritoneal cell was observed only at the suboptimal concentration of PMA (1.6 x 10^{-8} M). By adherence criteria and density gradient fractionation, the cell responding to PMA is confirmed to be the macrophage. Cell counts and nuclear radioautography confirm that replication in this system is reasonably well reflected by thymidine incorporation. The effects of PMA and its analogs as macrophage mitogens correlate with their tumor-promoting effects. Both PMA and MMF induce early increases in peritoneal macrophage levels of cyclic 3',5'-guanosine monophosphate without changes in the levels of cyclic 3',5'-adenosine monophosphate. These studies indicate that PMA offers a useful probe of macrophage function.

INTRODUCTION

PMA is generally known as a tumor promoter in the 2-stage murine carcinogenesis model. We and others have shown that in addition PMA is a mitogen for a variety of cell types including fibroblasts (11, 36) and lymphocytes (3, 11, 29, 39). We showed (10, 11) that PMA induces fibroblast proliferation in conjunction with early increases in cyclic GMP similar to those of lectin mitogens (17). The MMF utilized in the experiments reported here was vacuum dried and contained MMF active in inducing replication of macrophages in vitro. The MMF utilized in the experiments reported here was vacuum dried and partially purified by Sephadex chromatography as described previously (18). Supernatant controls were prepared by adding antigen at the end of the generating period and were processed in parallel.

Guinea pigs were given i.p. injections of 20 ml of paraffin oil from Fisher. After 3 days, the animals were killed by cervical dislocation, and the contents of the peritoneum were washed with 200 ml of HBSS from Grand Island Biological Co., (Grand Island, N. Y.). Before alveolar cells were obtained, the animals were bled by cardiac puncture and sacrificed. Lungs, heart, and trachea were removed in a block and connected through tubing to a 30-ml syringe with HBSS. Lungs were repeatedly filled and allowed to drain by gravity.

Subpopulations of oil-induced peritoneal macrophages were separated by centrifugation on discontinuous density gradients using bovine serum albumin (Pentex-bovine albumin; Pathocyte 5, Lot 35, from Miles Laboratories, Inc., Naperville, Ill.) according to the methods of Rice and Fishman (34).

Unfractionated cells were incubated at 2 x 10^6/ml HBSS from Microtest tissue culture plates (No. 3043; Falcon Products, Oxnard, Calif.). After 15 min at 37°C, about 50% of the cells attached and the monolayer was washed 3 times with HBSS and cultured in minimum essential medium (Grand Island Biological Co.) supplemented with penicillin, streptomycin, Fungizone and 20% heat-inactivated fetal calf lymphokine which induces macrophage proliferation. In this system, antigen-primed lymph node cells are challenged in vitro with antigen; a lymphokine, MMF, is produced which induces mature oil-induced peritoneal or alveolar macrophages of nonimmune animals to proliferate in culture (18). PMA is also shown to induce macrophage replication as measured by thymidine incorporation, increased cell counts, and nuclear autoradiographic labeling. Unlike MMF, the macrophage proliferation induced by PMA is mildly limited by endogenous prostaglandin production. The actions of both PMA and MMF are associated with early increases in cellular levels of cyclic GMP.

MATERIALS AND METHODS

Male Hartley guinea pigs, 250 to 300 g, were obtained from Cumm Research (Wayne, N. J.) and were kept at least 1 week before use. PMA, phorbol dibenzoate, phorbol dibutyrate, phorbol diacetate, and phorbol dioleate were obtained from Consolidated Midland Corp. (Brewster, N. Y.). They were all dissolved in dimethyl sulfoxide (Fisher Scientific, Fairlawn, N. J.). The maximum concentration of the solvent in culture was 0.1%, and at this concentration it had no effect on control cultures. Con A (Sigma Chemical Co., St. Louis, Mo.) and PHA (Burroughs Wellcome, Greenville, N. C.) were used at predetermined optimal mitogenic concentrations (5 and 1.5 µg/ml, respectively).

MMF was prepared as described previously (18). Briefly, guinea pig lymph node lymphocytes previously sensitized in vivo to antigen were exposed in vitro to antigen for 24 hr, and the culture supernatant fluids were collected. The supernatant fluids previously have been shown to contain MMF active in inducing replication of macrophages in vitro. The MMF utilized in the experiments reported here was vacuum dried and partially purified by Sephadex chromatography as described previously (18). Supernatant controls were prepared by adding antigen at the end of the generating period and were processed in parallel.

ABSTRACT

Laboratory of Immunopharmacology, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Received December 21, 1981; accepted May 12, 1982.
serum (Sterile Systems, Logan, Utah). The monolayers were confirmed to be >96% pure macrophages by visual criteria and by uptake of latex beads and cultured at a final cell concentration of 5 x 10^5/ml. Gradient-fractionated cells were not attached prior to culture and were incubated at a final concentration of 5 x 10^6 cells/ml. After the first 24 hr of culture in an atmosphere of 5% CO2, plates were transferred to 3% CO2.

Cultures were labeled with tritiated thymidine (specific activity, 20 Ci/mol; New England Nuclear, Boston, Mass.) at a concentration of 2.5 μCi/ml for the last 24 hr of culture. Microplates were frozen and thawed twice, and the cells collected on glass fiber filter pads and processed with a multiple automatic sample harvester (Otto Hillel, Madison, Wis.) for liquid scintillation counting.

Cultures concomitant with those described above were performed in Lab-Tek tissue culture chamber slide (Lab-Tek Products, Miles Laboratories, Inc.). Lab-Tek cultures were terminated by washing 3 times with warm HBSS containing 5% fetal calf serum. The slide partitions were removed, and all fluid was rapidly drained. The dried monolayer was fixed in absolute methanol for 3 min, rinsed in distilled water, and air-dried. Autoradiographs were done using Kodak nuclear track emulsion (19). The slides were kept in contact with the emulsion in the cold for 18 to 24 hr before development. Using an ocular grid and 430 x magnification, labeled and nonlabeled cells were scored from 20 different areas in each chamber. The number of cells was determined.

Dialyzed PMA supernatant fluids were prepared by incubating macrophages for 72 hr with 1.6 x 10^-7 M PMA and then pooling the supernatant fluids. These were dialyzed in Spectroprobe 2 filters (Spectrum Medical, Los Angeles, Calif.) twice against 1000 volumes of water and once against 500 volumes of minimal essential media. The method has been confirmed to remove PMA.

For cyclic nucleotide measurements, peritoneal macrophages (5 x 10^6) were adhered to Petri dishes (60 x 15 mm; Falcon Products) for 20 min at 37°. Nonadherent cells were removed by 3 washes in HBSS. The adherent cells (approximately 50%) were incubated in 1.8 ml HBSS for 1 hr at 37° prior to beginning the experiment. PMA or control dimethyl sulfoxide was added in 0.2 ml for varying periods up to 20 min, and the cultures were terminated with 1 ml 1.5 M perchloric acid. The plates were frozen overnight and thawed, and the cells were transferred with the aid of a rubber policeman to glass tubes. In some experiments, the attachment and incubation were performed in glass tubes thereby obviating the transfer. Cyclic nucleotides were extracted, purified, and assayed as previously described (7, 8).

RESULTS

PMA induced guinea pig peritoneal exudate and alveolar macrophages to replicate in culture as measured by tritiated thymidine incorporation. Two representative experiments are depicted in Chart 1. In 8 experiments, alveolar macrophages which have a higher basal thymidine incorporation were stimulated optimally by 1.6 x 10^-9 M PMA and were markedly inhibited by concentrations above 1.6 x 10^-8 M. Peritoneal macrophages (12 experiments) were optimally stimulated by 1.6 x 10^-7 M PMA.

Assayed over 4 days of culture of peritoneal macrophages, the effects of the optimal PMA concentrations (1.6 x 10^-7 M) was most apparent at 72 hr (Table 1). The lymphokine MMF showed parallel but somewhat greater effects also peaking at 72 hr of culture. At 3 days of culture, the magnitude of the effects of PMA (1.6 x 10^-7 M) was less than that of the lymphokine MMF but greater than those of optimal concentrations of PHA or Con A (Table 2). Indomethacin, added to inhibit prostaglandin production, potentiated the effect of MMF on thymidine incorporation at 72 hr of culture. Unlike MMF, the effect of optimal concentrations of PMA on thymidine incorporation is not significantly potentiated by the addition of indomethacin (10^-5 M) at the onset of culture. Only the effect of a suboptimal concentration of PMA (1.6 x 10^-8 M) was significantly augmented by 43% (p < 0.01 by paired Student’s t test; data not shown). Also, the suppressive effects of supr-optimal concentrations of PMA on thymidine incorporation of peritoneal and alveolar macrophages were not reversed by indomethacin (data not shown). Neither the effect of PHA nor that of Con A was significantly potentiated by indomethacin, and alone indomethacin had no effect on thymidine incorporation.

In order to demonstrate that thymidine incorporation accurately reflects PMA-induced cell replication in this monolayer culture system, counts of peritoneal cells were performed, and radioautography for thymidine nuclear labeling was carried out (Table 3). In each of 3 experiments, PMA at a concentration of 1.6 x 10^-7 M induced optimal increases in cell number and labeling indices (over 40-fold) as is the case for thymidine incorporation. The increase in cells with labeled nuclei following PMA stimulation occurred exclusively in typical macrophages by appearance. It is apparent that higher concentrations of PMA (1.6 x 10^-8 and 10^-6 M) induce a high degree of labeling but do not allow the same degree of cell replication and therefore total thymidine incorporation as do the optimal concentrations. The cell counts and the nuclear labeling indices

---

4 P. Ralph, personal communication.
under the various conditions correlate moderately well with thymidine incorporation; however, cells stimulated with low concentrations of PMA show greater than expected numbers compared to the control. This observation derives apparently from effects of PMA to increase cell adherence compared to the control. Also, cells stimulated by supraoptimal concentrations of PMA show a greater percentage of nuclear labeling than would be expected on the basis of cell number and thymidine incorporation. This observation derives in part from toxic effects of these concentrations of PMA on the cells. A single experiment confirmed that alveolar macrophages also increase in number when stimulated by PMA at optimal concentrations.

The contrast between the dose-response profile of PMA effects on alveolar and peritoneal macrophages (Chart 1) suggested the possibility of differential actions of PMA on macrophage subpopulations. For this reason and in order to further confirm that macrophages are the direct target of PMA action, we separated the peritoneal exudate cells on discontinuous albumin gradients according to the method used by Rice and Fishman (34). PMA over a broad concentration range in the absence of indomethacin induced proliferation equivalently in all gradient fractions (Chart 2). According to the report of Rice and Fishman, Fractions B and C approach 98% pure macrophages. It is apparent that PMA is fully active on virtually pure macrophages prepared in this manner as well as prepared by adherence methods, indicating that PMA action is directly on the macrophage and not mediated by lymphocytes. The lack of effect of the readdition of lymphocytes pulsed with PMA for 24 hr to macrophage monolayers at concentrations of up to 2% supports this conclusion (data not shown). In addition, we prepared dialyzed supernatants from PMA-stimulated macrophages and were unable to demonstrate the presence of a soluble mediator like MMF or colony-stimulating factor which would induce macrophage replication using this assay.

PMA as a peritoneal macrophage mitogen was compared with a series of its analogs in 3 experiments; Table 4 shows the results of a representative experiment. PMA and the dicosenoate were most active, while phorbol itself and the diacetate, bibutyrate, and dibenzoate derivatives showed little or no activity.

The effects of PMA (10^-7 M) and the lymphokine on peritoneal macrophage levels of cyclic AMP and cyclic GMP were analyzed over 20 min of culture in preliminary experiments (Chart 3A). PMA induced greater than 2-fold increases (p < 0.01 by paired Student’s t test) in macrophage levels of cyclic GMP apparent at 3 min of culture and persisting up to 20 min (4 experiments). In contrast to its effect on cyclic GMP levels, PMA had no significant effect on cyclic AMP levels. MMF (9 experiments) induced increases in cyclic GMP levels of 2-fold, peaking at 5 min of incubation (p < 0.01) without change in cyclic AMP levels (Chart 3B).

**DISCUSSION**

In the present experiments, PMA is shown to induce replication of guinea pig oil-induced peritoneal and alveolar macrophages in monolayer culture. By criteria of morphology, adherence, and buoyant density, the responding cell is the macrophage. While the lymphocyte is known to be a target for PMA action and that when activated it could produce a lym-

### Table 1

*Kinetics of the response of guinea pig peritoneal macrophages to MMF and PMA studied in the presence and absence of indomethacin (10^-5 M)*

The mitogens were used at optimal concentrations for cell proliferation: PMA, 1 x 10^-7 M, and MMF, 5 μl/well. Cultures were terminated at 48, 72, and 96 hr after a terminal 24-hr pulse of [3H]thymidine.

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>48 hr cpm</th>
<th>72 hr cpm</th>
<th>96 hr cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,516 ± 311a</td>
<td>1,167 ± 138</td>
<td>3,235 ± 340</td>
</tr>
<tr>
<td>Control + indomethacin</td>
<td>1,595 ± 197</td>
<td>1,899 ± 227</td>
<td>3,739 ± 543</td>
</tr>
<tr>
<td>PMA</td>
<td>27,879 ± 1,943</td>
<td>35,664 ± 2,113</td>
<td>50,433 ± 5,418</td>
</tr>
<tr>
<td>PMA + indomethacin</td>
<td>27,644 ± 1,943</td>
<td>38,565 ± 1,015</td>
<td>25,107 ± 111</td>
</tr>
<tr>
<td>MMF</td>
<td>46,897 ± 6,270</td>
<td>67,801 ± 3,562</td>
<td>39,469 ± 12,281</td>
</tr>
<tr>
<td>MMF + indomethacin</td>
<td>56,991 ± 2,292</td>
<td>88,887 ± 7,475</td>
<td>51,114 ± 17,618</td>
</tr>
</tbody>
</table>
|a Mean ± S.E. of one of 3 experiments.

### Table 2

*Comparison of the optimal dose of 4 mitogens tested on the [3H]thymidine incorporation of oil-induced guinea pig peritoneal macrophages at 3 days of culture*

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>No. of experiments</th>
<th>cpm Mitogen</th>
<th>cpm Mitogen + indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>4,028 ± 392</td>
<td>2,708 ± 175</td>
</tr>
<tr>
<td>MMF</td>
<td>4</td>
<td>68,796 ± 3,636</td>
<td>83,765 ± 4,067</td>
</tr>
<tr>
<td>PMA</td>
<td>4</td>
<td>35,824 ± 1,968</td>
<td>38,040 ± 2,745</td>
</tr>
<tr>
<td>Con A</td>
<td>3</td>
<td>26,203 ± 1,629</td>
<td>29,678 ± 5,153</td>
</tr>
<tr>
<td>PHA</td>
<td>3</td>
<td>18,890 ± 1,387</td>
<td>22,907 ± 2,083</td>
</tr>
<tr>
<td></td>
<td>Mean ± S.E. of the indicated number of experiments.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a Indomethacin effect significantly different (p &lt; 0.01 by paired Student’s t test).</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3

*Effect of PMA on cell counts and the incorporation of [3H]thymidine by guinea pig peritoneal macrophages as measured by autoradiography and scintillation counting at 3 days of culture*

Using autoradiography, the percentage of [3H]thymidine-labeled macrophages was determined. Twenty different fixed units of area were surveyed in each Lab-Tek well, and the numbers of labeled and nonlabeled cells were scored. Simultaneously, a separate experiment using similar conditions was done for the determination of [3H]thymidine incorporation.

<table>
<thead>
<tr>
<th>Mitogen</th>
<th>No. of cells counted/unit area</th>
<th>% of cells labeled</th>
<th>cpm [3H]thymidine incorporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>153</td>
<td>9</td>
<td>2,608 ± 317a</td>
</tr>
<tr>
<td>PMA (1.6 x 10^-7 M)</td>
<td>589</td>
<td>14</td>
<td>7,759 ± 645</td>
</tr>
<tr>
<td>PMA (1.6 x 10^-7 M)</td>
<td>700</td>
<td>60</td>
<td>33,694 ± 1,888</td>
</tr>
<tr>
<td>PMA (1.6 x 10^-7 M)</td>
<td>418</td>
<td>62</td>
<td>26,962 ± 3,917</td>
</tr>
<tr>
<td>PMA (1.6 x 10^-7 M)</td>
<td>317</td>
<td>71</td>
<td>14,496 ± 3,366</td>
</tr>
<tr>
<td>MMF</td>
<td>895</td>
<td>43</td>
<td>55,564 ± 7,039</td>
</tr>
</tbody>
</table>
|a Mean ± S.E. of 3 experiments performed.
phokine which makes macrophages replicate, the populations of responding macrophages are sufficiently pure to discount a role for activated lymphocytes and their products in the response. The effects of PMA are optimally obtained at 72 hr of culture in the system used and compare in timing to those of the lymphokine MMF and to PHA and Con A. The optimal concentration of PMA was 1.6 x 10^-7 M (100 ng/ml) on peritoneal macrophages and 1.6 x 10^-9 M (1 ng/ml) on alveolar macrophages. The alveolar cells show a higher basal replication rate; therefore, the inhibitory effects of concentrations in excess of 1.6 x 10^-8 M may result from the known effects of mitogens at optimal mitogenic concentrations to suppress proliferation of endogenously activated cells (33). Alternatively, alveolar macrophages may as a subpopulation manifest intrinsic differences in sensitivity to or binding of PMA.

The separation of peritoneal macrophages into subpopulations using discontinuous albumin gradients showed no significant differences in sensitivity to or binding of PMA. The response of macrophages to a broad concentration range of PMA shows relatively little intrinsic regulation by prostaglandin production since the addition of indomethacin potentiated the proliferative response only at a suboptimal concentration of PMA (1.6 x 10^-7 M). The effects of MMF, on the other hand, are potentiated by indomethacin over the first 4 days of culture at an optimal concentration of MMF and over the entire MMF dose-response curve, particularly at higher concentrations of MMF (19). The effect of PMA, therefore, differs from that of MMF in a way which suggests that MMF or

---

Table 4
Relative activity of phorbol and its derivatives on the incorporation of [3H]-thymidine by guinea pig peritoneal macrophages.

<table>
<thead>
<tr>
<th>Substance</th>
<th>cpm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2,237 ± 227a</td>
</tr>
<tr>
<td>Phorbol</td>
<td>1,740 ± 36</td>
</tr>
<tr>
<td>Phorbol didecanoate</td>
<td>13,662 ± 1,806</td>
</tr>
<tr>
<td>PMA</td>
<td>11,285 ± 567</td>
</tr>
<tr>
<td>Phorbol diacetate</td>
<td>1,594 ± 401</td>
</tr>
<tr>
<td>Phorbol dibutyrate</td>
<td>2,653 ± 403</td>
</tr>
<tr>
<td>Phorbol dibenzoate</td>
<td>1,875 ± 502</td>
</tr>
</tbody>
</table>

*a Mean ± S.E.

---

Chart 2. Response of subpopulations of guinea pig peritoneal macrophages to PMA. Macrophages from oil-induced exudates were separated on bovine albumin gradients. The different layers (B, C, D, E, and P) correspond to the interfaces of bovine serum albumin of 8 to 11, 11 to 15, 15 to 20, and 20 to 30% and the pellet below 30%, respectively. Dotted columns, effects of 1.6 x 10^-8 M PMA; open columns, 1.6 x 10^-7 M PMA. Uptake of [3H]thymidine was assayed at 72 hr. The results represent the mean of triplicate samples of 2 different experiments; bars, S.E.

Chart 3. Effect of PMA (A) and MMF (B) on macrophage levels of cyclic GMP (cGMP) and cyclic AMP (cAMP). Oil-induced peritoneal macrophages were incubated with PMA (4 experiments) or MMF (9 experiments) and cyclic nucleotide levels of cells and supernatant fluids were assayed as described (6). Data are expressed as ratio to control since basal levels of cyclic nucleotides varied considerably from one animal to another; bars, S.E. Basal levels of cyclic GMP and cyclic AMP in these experiments averaged 39 fmol and 9 pmol/mg protein, respectively.
other factors in the MMF preparation induce prostaglandin production by oil-induced guinea pig peritoneal macrophages but that PMA does not or that PMA-stimulated macrophages are less sensitive to inhibition by prostaglandins. Based on other evidence (4, 23, 31), the latter explanation seems the most likely. PHA and Con A were also unaffected by indomethacin, indicating that prostaglandin induction is not a general characteristic of macrophage mitogens.

Experiments with cell counts and nuclear labeling indicate that thymidine incorporation accurately reflects PMA-induced macrophage replication in this system at low concentrations of PMA. The disproportionately high nuclear labeling index observed with PMA at higher concentrations indicates that either cells are being triggered and enter DNA synthesis but cannot complete the replication cycle and/or that cells not triggered are being destroyed and removed from the monolayer.

The action of PMA and its analogs to induce macrophage proliferation parallels their effects to promote tumors and skin irritation in mice. While this work was in progress, similar observations have been made showing that PMA and its analogs stimulate mouse macrophage proliferation in a way which correlates with tumor promotion (21). Two reports also have appeared to show that PMA is mitogenic for mouse bone marrow precursor cells for granulocytes and macrophages (12, 38). The effect of potency for this action also parallels tumor promotion. In addition, the effect appears to be direct since no evidence of colony-stimulating factor induction was obtained to explain an indirect effect (38). That the macrophage can be a direct target of PMA action is exemplified by the fact that a totally homogeneous, established myeloid leukemic cell line responds to PMA with differentiation (26).

Quite a number of macrophage changes following PMA stimulation have been reported, including migration inhibition with radial microtubular aggregation (4, 31); increased pinocytosis and spreading (30); increased phagocytosis (27); lysosomal enzyme release including elastase, neutral protease, and plasminogen activator (2, 5, 9, 20, 23); and increased oxidative burst with superoxide ion and H₂O₂ production (31, 40).

The relation of the above processes to the induction of proliferation is not completely evident; however, a number of the processes can be considered to be linked and a variety of evidences would indicate that signals which trigger phagocytosis, enzyme release, and the oxidative burst have a common mechanism in macrophages with those signals which induce proliferation (15). A variety of mechanisms have been attributed to the action of PMA in cells other than macrophages, including increased phospholipase activity leading to the deacylation of membrane lipids (22, 27); arachidonic acid release; prostaglandin and thromboxane production (22, 28); increased ATPase activity with potassium ion transport (32, 41) decreases in cyclic AMP levels (13); increases in guanylate cyclase activity and cyclic GMP generation (7, 11, 13, 35).

Our observations in the guinea pig macrophage indicate that PMA, like MMF, induces increases in cyclic GMP without significant increases in cyclic AMP. This observation is shared by mitogens for a variety of other cells including the lymphocyte (see Ref. 16 for review). Pick et al. (4, 31) have failed to observe increases in macrophage levels of cyclic GMP with PMA but did note a decrease in cyclic AMP levels and a decrease in sensitivity of adenylate cyclase to stimulation by prostaglandin. The disparity in the observations of Pick and our own observations presumably rests on technical grounds, since Pick was unable to observe increased levels of cyclic GMP with a number of agents like zymosan and the calcium ionophore reported by others to be active to increase cyclic GMP (37). The increased levels of cyclic GMP induced in macrophages by PMA and MMF, if comparable in mechanism to those in lymphocytes (7, 8), probably involve a release of arachidonic acid from membrane phospholipids through the action of phospholipase A or C and the generation of hydroxy- and hydroperoxyeicosatetraenoic acids via the lipoxygenase pathway. These products in turn activate guanylate cyclase. The significance of the increased cyclic GMP in the induction of macrophage proliferation may reside in actions of cyclic GMP related to superoxide formation (37), nuclear protein phosphorylation (24), and RNA synthesis (1, 25).

REFERENCES


AUGUST 1982

3069
Effects of Phorbol Myristate Acetate and a Lymphokine on Cyclic 3′:5′-Guanosine Monophosphate Levels and Proliferation of Macrophages

Elba M. Hadden, John R. Sadlik, Ronald G. Coffey, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/42/8/3064