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Induction of Chemotaxis in Mouse Peritoneal Macrophages by Phorbol Ester Tumor Promoters[1]

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ABSTRACT

The ability of the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), to induce chemotaxis in three different populations of mouse peritoneal macrophages was studied. TPA in the range of 10^{-9} to 10^{-7} M produced a dose- and time-related increase in chemotaxis in resident, thioglycollate-elicited, and divinyl ether maleic anhydride copolymer-activated macrophages. A maximal response was obtained after 4 hr incubation with 10^{-7} M TPA, and this concentration of TPA was as effective in inducing chemotaxis as was endotoxin-activated mouse serum. Orientation of macrophages towards TPA was also observed by microscopy. Within 2 hr, cells exposed to TPA sent out cytoplasmic processes along the TPA gradient. Parallel arrays of cells oriented towards the TPA were observed after 4 hr incubation. Two other diterpene tumor promoters, phorbol-12,13-didecanoate and mezerein, were also chemotactic for the macrophages, as was the peptide epidermal growth factor, which shares a number of effects with TPA on cells in culture. On the other hand, two phorbol esters inactive as tumor promoters, 4-α-phorbol-12,13-didecanoate and phorbol, were not chemotactic for macrophages. Retinoic acid, which inhibits tumor promotion, inhibited TPA-induced, but not endotoxin-activated mouse serum-induced chemotaxis. These findings, taken together with previous studies, indicate that phorbol ester tumor promoters are potent modulators of macrophage function.

INTRODUCTION

In addition to their tumor-promoting activity, the phorbol esters are potent irritants and inflammatory agents. In the 2-stage mouse skin carcinogenesis assay, application of as little as 17 nmol of the promoter TPA[4] to the skin of the mouse produces a rapid and dramatic inflammatory response characterized by erythema, edema, and diapedesis (6). The role of macrophages in inflammation has been extensively investigated. A number of factors have been characterized that stimulate the migration of macrophages to tissue sites following irritation or injury, including lymphocyte-derived chemotactic factors and complement factors (34). In addition, a number of synthetic peptides have also been found to enhance leukocyte migration *in vitro* (30). Previous studies have shown that tumor promoters have a number of effects on macrophages. These include enhancement of lymphocyte-activating factor production (24), increased *in vitro* tumor cytotoxicity (25, 26), and modulation of phagocytosis (19). It has also been reported that tumor promoters induce macrophage production of plasminogen activator (38), a serine protease which may be involved in macrophage chemotaxis *in vivo* (37). Chemotaxis, which is directed movement of cells along the concentration gradient of a specific chemoattractant, is thought to represent one of the mechanisms responsible for macrophage migration *in vivo* (34). We report here that tumor-promoting phorbol esters are by themselves potent chemoattractants for mouse peritoneal macrophages. Furthermore, EGF, which shares a number of effects with tumor promoters (10, 11, 20, 36, 42), is also a potent chemoattractant for macrophages. We have also found that retinoic acid, which is known to antagonize a number of effects of TPA including tumor promotion (39), specifically inhibited the phorbol ester-induced chemotaxis.

MATERIALS AND METHODS

Chemicals. TPA, PDD, 4-*O*-methyl-12-*O*-tetradecanoylphorbol-13-acetate, phorbol, and 4α-phorbol-12,13-didecanoate were obtained from Consolidated Midland, Brewster, N. Y. Mezerein was kindly provided by Dr. S. Kupchan's laboratory, University of Virginia, Charlottesville, Va. Each of these was dissolved in dimethylsulfoxide (1 mg/ml) and stored at −20°. EGF, obtained from Collaborative Research, Waltham, Mass., was dissolved in distilled water (0.1 mg/ml), and stored at −70°. Retinoic (all-*trans*) was purchased from Sigma Chemical Co., St. Louis, Mo. TG was obtained from Difco Laboratories, Inc., Detroit, Mich. MVE (MVE-2; M.W. 15,000) was kindly supplied by Dr. David Breslow, Hercules, Inc., Wilmington, Del. All drugs were made up fresh daily.

Cell Preparations. Three different populations of adherent mouse peritoneal exudate cells were studied: resident macrophages harvested from untreated male CD-1 mice, and macrophages recruited to the peritoneal cavity either by TG as "elicited" macrophages or by the pyran copolymer, MVE, as "activated" macrophages. The techniques for harvesting the macrophages have been described previously (19). Briefly, male CD-1 mice were given i.p. injections of either 1 ml of TG (10%) or 0.25 ml of MVE (25 mg/kg). Five days after TG or 7 days after MVE injection, the cells were harvested. This was accomplished by flushing the peritoneal cavity of the mouse with 10 ml of basal culture medium. The cells were then centrifuged at 500 × *g* and 4° for 10 min (Beckman TJ-6) and resuspended (2.2×10^6 macrophages/ml) in Dulbecco's minimum essential medium (Flow Laboratories, Rockville, Md.) supplemented with bovine serum albumin (10 mg/ml; Sigma).

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[4] The abbreviations used are: TPA, 12-*O*-tetradecanoylphorbol-13-acetate; EGF, epidermal growth factor; PDD, phorbol-12,13-didecanoate; TG, thioglycollate; MVE, divinyl ether maleic anhydride copolymer; EAMS, endotoxin-activated mouse serum.

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Preparation of EAMS. Male CD-1 mice were anesthetized with chloroform, and blood was collected by cardiac puncture. The serum was separated from RBC by centrifugation at 800 × *g* and 4° for 15 min and then incubated with *Escherichia coli* lipopolysaccharide 0114:B4 (10 mg/ml; Difco) for 90 min at 37°. The endotoxin serum mixture was then heated for 30 min at 56° and centrifuged at 1000 × *g* and 4° for 10 min). A dose response for chemotactic activity was performed with the EAMS preparation. The maximal activity was obtained after 4 hr incubation with a 10-fold dilution of EAMS with culture medium. This concentration was used in all subsequent experiments.

Chemotaxis Assay. Chemotaxis was assayed in modified Boyden chambers using a 5.0-μm polycarbonate filter (Nucleopore Corp., Pleasanton, Calif.) (33, 34, 35). Chemotactic activity was measured after 1 to 4 hr by counting the number of macrophages migrating through the filter in 20 oil immersion microscopic fields (×1000). Data are presented as the number of cells migrating through 20 oil immersion fields ± S.E.

RESULTS AND DISCUSSION

Within 24 hr after the application of TPA to mouse skin, there is a marked accumulation of macrophages at this tissue site (6). To determine if this compound had a direct effect on the locomotion of macrophages, we measured the chemotactic response of macrophages towards TPA. The effect of 170 nM TPA on the chemotaxis of macrophages over a 4-hr time period is shown in Chart 1. EAMS, a well-characterized chemoattractant from mouse serum (34), was compared to TPA as a control. Chart 1 shows that 170 nM TPA was as effective in inducing chemotaxis in the macrophages as was a 10-fold dilution of EAMS. With both TPA and EAMS, there was a time-dependent increase in chemotaxis (up to 4 hr) in all 3 types of macrophages. After 4 hr incubation, MVE macrophages were 3 times more responsive than TG-recruited macrophages, while resident peritoneal macrophages were intermediate between TG and MVE cells. None of the macrophage cell types responded to the solvent control (0.01% dimethyl sulfoxide) or to media alone (Table 2). These findings are consistent with those reported by Meltzer *et al.* (23), who compared *Bacillus Calmette-Guérin*-activated macrophages to TG-recruited and resident macrophages using EAMS and a lymphocyte-derived chemotactic factor. The diverse responses obtained with the different types of macrophages in the present study suggest that, in the mouse skin carcinogenesis assay, TPA may selectively recruit specific subpopulations of macrophages. This is supported by preliminary observations in our laboratory and those reported by Schultz *et al.* (32) that i.p. injection of TPA selectively recruits activated macrophages.

It was possible that TPA did not induce directed cell movement (chemotaxis) but rather enhanced random movement (chemokinesis). In order to distinguish between these types of locomotion, equal concentrations of TPA were placed on both sides of the filter in the chemotactic chamber. As shown in Table 1, movement of the macrophages across the filter was reduced by more than 60% in all 3 macrophage cell types. Experiments were also performed in which the magnitude of the chemoattractant gradient across the filter was altered by placing various concentrations of TPA in the upper well of the chemotactic chamber, while keeping the concentration of TPA in the lower well constant (170 nM). Table 1 shows that as the magnitude of the chemotactic gradient was reduced, there was a corresponding decrease in cell movement in each of the 3 cell types. Since reduction and elimination of the concentration gradient in the chemotactic chamber significantly decreased cell movement, we concluded that TPA was a chemotactic stimulant for the macrophages. These results are consistent with previously described characteristics of chemotactic factors (12, 44). Since cells that respond to chemotactic factors will orient in a gradient of chemoattractant, we tested the ability of macrophages to orient in a gradient of TPA. Resident peritoneal macrophages were plated on glass coverslips and allowed to attach to the surface by incubation at 37° for 60 min. The coverslips were then placed perpendicular to the filter in the Boyden chambers with medium containing 170 nM TPA in the lower well and medium without TPA in the upper well of the chamber. After 2 and 4 hr, the coverslips were removed from

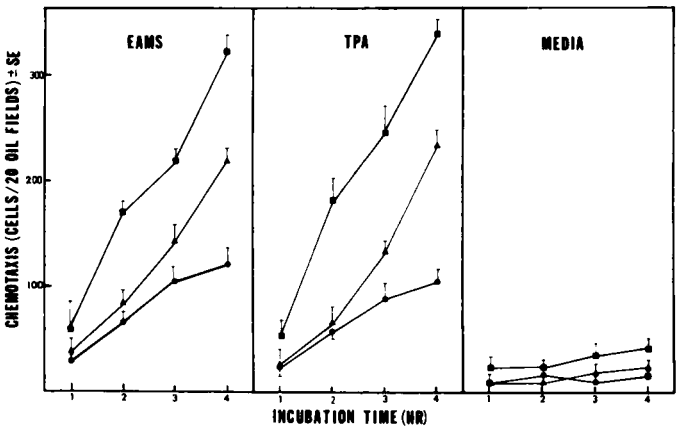

Chart 1. Time course of chemotaxis induced by TPA (170 nM), EAMS (10%), and media with MVE (■), resident (▲), and TG (●) macrophages. The chemoattractant to be tested was diluted to the appropriate concentration in Dulbecco's minimal essential medium containing bovine serum albumin (10 mg/ml) and placed in the lower well of the chemotactic chamber in 0.18-ml volumes. Polycarbonate membrane filters with 5-μm pores were placed over the lower wells. The upper well insert was screwed into place and filled with 0.3 ml of peritoneal exudate cells containing 2.2 × 10^6 macrophages/ml. After 4 hr incubation at 37° in a humidified CO_2 incubator, the filters containing the adhered, migrated macrophages were removed and stained with Camco Quick Stain. The number of migrated macrophages in 20 oil immersion fields (×1000) was counted microscopically. The data are presented as the number of cells migrating through 20 oil-immersion fields; *bars*, S.E. Each *column* represents the mean of at least 12 samples.

Table 1

Effects of TPA on the migration of mouse peritoneal macrophages

The cells were incubated in the upper well of the chemotactic chamber. Varying concentrations of TPA were added to the upper or lower wells. Following incubation for 4 hr at 37°, the 5-μm-pore filter separating the upper and lower wells was removed, stained, and counted as described in the legend to Chart 1. Each value represents the mean of 9 samples.

Concentration of TPA in chamber (M)		Chemotaxis (cells/20 oil immersion fields)		
Top	Bottom	Resident	TG	MVE
0	0	39.0 ± 8.5[a]	22.0 ± 3.7	46.9 ± 4.3
1.7×10^{-8}	1.7×10^{-8}	54.9 ± 8.4	23.4 ± 4.1	97.9 ± 6.8
0	1.7×10^{-8}	134.7 ± 9.1	62.1 ± 3.4	254.6 ± 7.9
5.0×10^{-8}	5.0×10^{-8}	49.3 ± 5.3	27.1 ± 6.2	89.8 ± 9.6
0	5.0×10^{-8}	162.5 ± 8.9	78.6 ± 5.9	278.4 ± 7.4
1.7×10^{-7}	1.7×10^{-7}	67.1 ± 6.4	34.8 ± 2.5	126.0 ± 12.0
1.7×10^{-8}	1.7×10^{-7}	171.6 ± 7.2	72.6 ± 4.9	278.4 ± 9.8
1.7×10^{-9}	1.7×10^{-7}	185.4 ± 6.8	81.3 ± 2.9	299.1 ± 9.2
0	1.7×10^{-7}	219.0 ± 6.6	96.0 ± 7.1	329.1 ± 12.4

[a] Mean ± S.E.

the chambers and stained for microscopic observation. We found that within 2 hr the macrophages began to line up in the gradient of TPA. Individual cells sent out large cytoplasmic processes which became elongated, in some cases extending several times the original length of the cells. After 4 hr incubation, parallel arrays of cells in the direction of the tumor promoter gradient were visible on the slides. Cells were also found to aggregate in chains in head-to-tail fashion along the chemotactic gradient. The effects of TPA on the orientation of the resident macrophages after 4 hr are shown in Fig. 1. Cells not exposed to the TPA gradient appeared to be randomly distributed on the slides (Fig. 1*A*). Thus, cell orientation in response to chemoattractants, which has been described previously for polymorphonuclear leukocytes (43), also occurs in macrophages in response to TPA.

Table 2

Effects of TPA and related macrocyclic diterpenes on chemotaxis by TG and MVE macrophages

The cells were incubated in the upper well of the chemotactic chamber for 4 hr at 37° with the chemotactic agents to be tested, media or dimethyl sulfoxide, in the lower well. The filters containing the adhered, migrated macrophages were then removed, stained, and counted as described in legend to Chart 1. Each value represents the mean of 6 samples.

		Chemotaxis (cells/20 oil fields)	
Chemoattractant	Concentration	TG	MVE
Media		11.5 ± 1.4[a]	38.8 ± 3.0
Dimethyl sulfoxide	0.01%	12.9 ± 2.2	40.0 ± 5.7
EAMS	10%	98.6 ± 6.6	305.0 ± 12.6
TPA	1.7 nM	41.1 ± 2.9	107.5 ± 12.0
	17.0 nM	58.7 ± 4.8	238.0 ± 5.7
	170.0 nM	101.5 ± 5.8	302.8 ± 17.8
PDD	1.7 nM	30.1 ± 5.4	101.0 ± 5.6
	17.0 nM	56.7 ± 3.2	150.8 ± 10.2
	170.0 nM	70.2 ± 3.2	179.0 ± 8.4
Mezerein	1.7 nM	40.0 ± 5.9	96.7 ± 7.7
	17.0 nM	52.2 ± 5.8	150.2 ± 9.1
	170.0 nM	86.6 ± 5.9	259.5 ± 10.6
4-*O*-Methyl-12-*O*-tetradecanoylphorbol-13-acetate	170.0 nM	16.4 ± 3.6	98.8 ± 2.3
4α-Phorbol-12,13-didecanoate	170.0 nM	12.3 ± 0.8	11.0 ± 2.8
Phorbol	170.0 nM	12.1 ± 3.2	22.5 ± 0.7

[a] Mean ± S.E.

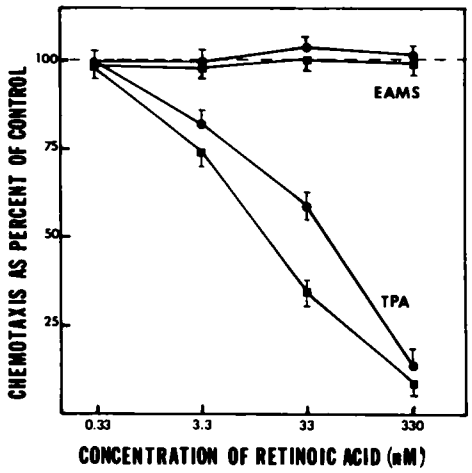

Chart 2. Effects of all-*trans*-retinoic acid on TPA- and EAMS-induced chemotaxis by TG (■) and MVE (●) macrophages. The cells were incubated in the upper well of the chemotactic chamber together with varying concentrations of retinoic acid or the solvent control (0.01% dimethyl sulfoxide) for 4 hr at 37°. TPA (170 nM) or EAMS (10%) was placed in the lower well and separated from the cells by a 5-μm pore filter. The filters containing the adhered, migrated macrophages were then removed, stained, and counted as described in legend of Chart 1. The data indicate the number of cells migrating through 20 oil immersion fields; *bars*, S.E. Each *column* represents the mean of 8 samples.

Two additional biologically active tumor-promoting agents, PDD and mezerein (7), were also found to be chemoattractants for the macrophages (Table 2). TPA was the most potent of the 3 agents, producing a dose-related increase in chemotaxis of both TG and MVE cells in the concentration range of 1.7 to 170 nM. On the other hand, chemotaxis was not induced by 170 nM phorbol, the biologically inactive parent alcohol of TPA, or by 4α-phorbol-12,13-didecanoate, the biologically inactive epimer of PDD (7), in either TG or MVE cells. 4-*O*-methyl-12-*O*-tetradecanoylphorbol-13-acetate, which has been reported to be a weak tumor promoter (19, 41, 42) produced a small but reproducible increase in cell movement at 170 nM.

Fischer *et al.* (5) have found that the acquisition of chemotactic responsiveness to the synthetic peptide, formylmethionylleucylphenylalanine, in a human monocyte cell line was associated with the binding of the peptide to the cell surface. In our studies, the facts that extremely low concentrations of the promoters were effective in inducing a chemotactic response in a dose-related manner and that the responses demonstrated stereospecificity suggest that a specific receptor on the macrophages may mediate tumor promoter-induced chemotaxis. In this regard, specific saturable receptors for TPA have recently been characterized in chick embryo fibroblasts (4) and in mouse epidermal cells (3).

It was also of interest to examine the effects of retinoic acid on macrophages. Retinoic acid has been found to inhibit a number of effects of the phorbol esters on mouse skin (39) and in certain cell culture systems (17). We therefore tested the ability of retinoic acid to modulate TPA-induced chemotactic activity. We found that all-*trans*-retinoic acid specifically inhibited TPA-induced chemotaxis in both MVE and TG macrophages but had no effect on EAMS-induced chemotaxis (Chart 2). This effect was dose dependent in the concentration range of 3.3 to 330 nM. Retinoic acid alone was not chemotactic for macrophages. Furthermore, incubation of the cells with 330 nM retinoic acid for 4 hr had no effect on cell viability as determined by trypan blue dye exclusion. This result, together with the lack of an effect of retinoic acid on EAMS-induced chemotaxis, indicated that the retinoic acid inhibition of TPA-induced chemotaxis was not due to toxicity. The selective inhibition of TPA-induced chemotaxis by retinoic acid suggests that TPA and EAMS may induce chemotaxis in macrophages by different mechanisms. It has also been reported that retinoic acid suppresses tumor promotion by TPA; however, it is not known whether retinoic acid interferes with macrophage functions in mouse skin (39).

We also tested the ability of EGF to induce macrophage chemotaxis since it shares a number of effects with phorbol ester tumor promoters on cells in culture. These include the ability to induce ornithine decarboxylase (11, 36, 42), initiate RNA and DNA synthesis (10, 11), enhance deoxyglucose uptake (11, 36), stimulate the release of plasminogen activator (20), and enhance phagocytosis in macrophages (19). In addition, TPA has been found to inhibit the binding of ^{125}I-EGF to its cell surface receptors (21, 31). We found that EGF, like TPA, induced chemotaxis in both TG and MVE macrophages (Chart 3). In both types of cells, EGF produced a dose-dependent effect within the concentration range of 0.16 to 16 nM.

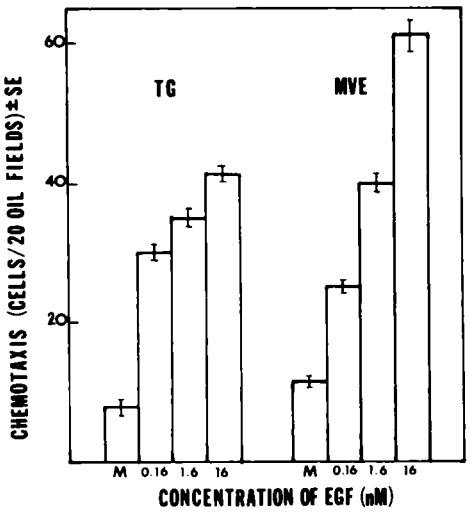

Chart 3. Effects of EGF on chemotaxis by TG and MVE macrophages. The cells were incubated for 4 hr at 37° in the upper well of the chemotactic chamber with varying concentrations of EGF or the media control (*M*) in the lower well, separated by a 5-μm pore filter. The filters containing the adhered, migrated macrophages were then removed, stained, and counted as described in the legend of Chart 1. The data indicate the number of cells migrating through 20 oil immersion fields; *bars*, S.E. Each *column* represents the mean of 8 samples.

In the present studies, we have shown that tumor promoters and EGF are potent chemotactic agents. The fact that EGF has been found to be a normal constituent of animal and human sera (18) suggests that it may normally play a role in chemotaxis *in vivo*, either alone or in concert with other factors involved in host-defense mechanisms. Since TPA is not a natural mammalian cell product, it may act by usurping the action of some endogenous substance which plays a regulatory role in the normal activity of macrophages (*e.g.*, EGF). In this regard, Hibbs *et al.* (9) have previously found that macrophage activity could be regulated by normal components of the serum. It remains to be determined whether any of these factors are similar to the action of EGF or TPA.

The role of macrophages in tumor promotion is not now clear. Although most tumor promoters are potent inflammatory agents (1, 2, 6, 8, 27), inflammation alone is not sufficient for tumor promotion (1, 8, 28, 29). It is possible that, in TPA-treated mouse skin, macrophages may actually help promote tumor development from initiated cells. For example, Nathan *et al.* (25, 26) have reported that TPA enhances the release of hydrogen peroxide from macrophages. This compound may react chemically with cellular macromolecules and interfere with their normal functions. Alternatively, it has been found that macrophages can enhance the proliferation of both normal (13, 15, 16, 22, 40) and tumor cells (13–15) *in vitro*, perhaps through the release of certain growth factors. It is possible that macrophages exert a similar effect on initiated cells. Further studies are necessary to explore these possibilities.

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REFERENCES

1. Berenblum, I. Irritation and cocarcinogenesis. Arch. Pathol., *38:* 233–244, 1944.
2. Berenblum, I. A speculative review: the probable nature of promoting action and its significance in the understanding of the mechanism of carcinogenesis. Cancer Res., *14:* 471–477, 1954.
3. Delclos, D. B., Nagle, D. S., and Blumberg, P. M. Specific binding of phorbol ester tumor promoters to mouse skin. Cell, *19:* 1025–1032, 1980.
4. Driedger, P. E., and Blumberg, P. M. Specific binding of phorbol ester tumor promoters. Proc. Natl. Acad. Sci. U. S. A., *77:* 567–571, 1980.
5. Fischer, D. G., Pike, M. G., Koren, H. S., and Snyderman, R. Chemotactically responsive and nonresponsive forms of a continuous human monocyte cell line. J. Immunol., *125:* 463–465, 1980.
6. Frei, J. V., and Stephens, P. The correlation of promotion and of tumor growth and induction of hyperplasia in epidermal two-stage carcinogenesis. Br. J. Cancer, *22:* 83–92, 1968.
7. Hecker, E. Structure-activity relationships in diterpene esters irritant and cocarcinogenic to mouse skin. *In*: T. J. Slaga, A. Sivak, and R. K. Boutwell (eds.), Mechanisms of Tumor Promotion and Cocarcinogenesis, Vol. 2, pp. 11–48. New York: Raven Press, 1978.
8. Hennings, H., and Boutwell, R. K. Studies on the mechanism of skin tumor promotion. Cancer Res., *30:* 312–332, 1970.
9. Hibbs, J. B., Taintor, R. R., Chapman, H. A., and Weinberg, J. B. Macrophage tumor killing: influence of the local environment. Science (Wash. D. C.), *197:* 279–282, 1977.
10. Hollenberg, M. D., and Cuatrecassas, P. Epidermal growth factor: Receptors in human fibroblasts and modulation of action by cholera toxin. Proc. Natl. Acad. Sci. U. S. A., *70:* 2964–2968, 1977.
11. Hoober, J. K., and Cohen, S. Epidermal growth factor. The stimulation of protein and ribonucleic acid synthesis in chick embryo fibroblasts. Biochim. Biophys. Acta., *138:* 347–356, 1967.
12. Keller, H. U., and Sorkin, E. Studies on chemotaxis. IV. The influence of serum factor on granulocyte locomotion. Immunology, *10:* 409–416, 1966.
13. Keller, R. Modulation of cell proliferation by macrophages. A possible function apart from cytotoxic tumour rejection. Br. J. Cancer, *30:* 401–415, 1974.
14. Keller, R. Susceptibility of normal and transformed cell lines to cytostatic and cytocidal effects exhibited by macrophages. J. Natl. Cancer Inst., *56:* 369–374, 1976.
15. Keller, R. Macrophage-mediated natural cytotoxicity against various target cells *in vitro*. I. Macrophages from diverse anatomical sites and different strains of rats and mice. Br. J. Cancer, *37:* 732–741, 1978.
16. Keller, R., Bregnard, A., Gehring, W. J., and Schroeder, H. E. Morphologic and molecular changes in target cells during *in vitro* interaction with macrophages. Exp. Cell Biol., *44:* 108–124, 1976.
17. Kensler, T. W., and Mueller, G. C. Retinoic acid inhibition of the comitogenic action of mezerein and phorbol esters in bovine lymphocytes. Cancer Res., *38:* 771–775, 1978.
18. Ladda, R. L., Bullock, L. D., Gianopoulos, R., and McCormick, L. Radioreceptor assay for epidermal growth factor. Anal. Biochem., *93:* 236–241, 1979.
19. Laskin, D. L., Laskin, J. D., Weinstein, I. B., and Carchman, R. A. Modulation of phagocytosis by tumor promoters and epidermal growth factor in normal and transformed macrophages. Cancer Res., *40:* 1028–1035, 1980.
20. Lee, L. S., and Weinstein, I. B. Epidermal growth factor, like phorbol esters induces plasminogen activator in HeLa cells. Nature (Lond.), *274:* 696–697, 1978.
21. Lee, L. S., and Weinstein, I. B. Tumor promoting phorbol esters inhibit binding of epidermal growth factor to cellular receptors. Science (Wash. D.C.), *202:* 313–315, 1978.
22. Leibovich, S. J. Production of macrophage-dependent fibroblast-stimulating activity (M-FSA) by murine macrophages. Effects on BALBc 3T3 fibroblasts. Exp. Cell Res., *113:* 47–78, 1956.
23. Meltzer, M., Stevenson, M., Tucker, R., and Leonard, E. Peritoneal macrophages from BCG-infected mice: tumor cytotoxicity and chemotactic responses *in vitro*. *In*: M. Fink (ed.), Macrophages in Neoplasm, pp. 211–225. New York: Academic Press, Inc., 1976.
24. Mizel, S. B., Rosenstreich, D. L., and Oppenheim, J. J. Effects of phorbol myristic acetate on LAF production by P388D$_1$ cells. Fed. Proc., *37:* 1589, 1978.
25. Nathan, C. F., Bruckner, L. H., Silverstein, S. C., and Cohn, Z. A. Extracellular cytolysis by activated macrophages and granulocytes. I. Pharmacologic triggering of effector cells and the release of hydrogen peroxide. J. Exp. Med., *149:* 84–99, 1979.
26. Nathan, C. F., Silverstein, S. C., Bruckner, L. H., and Cohn, Z. A. Extracellular cytolysis by activated macrophages and granulocytes. II. Hydrogen peroxide as a mediator of cytotoxicity. J. Exp. Med., *149:* 100–113, 1979.
27. Raick, A. N. Ultrastructural, histological, and biochemical alterations produced by 12-*O*-tetradecanoyl-phorbol-13-acetate on mouse epidermis and their relevance to skin tumor promotion. Cancer Res., *33:* 269–286, 1973.
28. Raick, A. N., and Brudzy, K. Ultrastructural and biochemical changes induced in mouse epidermis by a hyperplastic agent, ethylphenylpropriolate. Cancer Res., *33:* 2221–2230, 1973.
29. Saffiotti, U., and Shubik, P. Studies on promoting action of skin carcinogenesis. Natl. Cancer Inst. Monogr., *10:* 489–507, 1963.
30. Schiffman, E., Corcoran, B. A., and Wahl, S. M. *N*-Formylmethionyl peptides as chemoattractants for leukocytes. Proc. Natl. Acad. Sci. U. S. A., *72:* 1059–1062, 1975.

31. Schoyab, M., DeLarco, J. E., and Todaro, G. J. Biologically active phorbol esters specifically alter the affinity of EGF membrane receptors. Nature (Lond.), *279:* 387–391, 1979.
32. Schultz, R. M., Chirigos, M. A., and Olkowski, Z. L. Stimulation and inhibition of neoplastic cell growth by tumor promoter-treated macrophages. Cell Immunol., *54:* 98–106, 1980.
33. Snyderman, R. L., Altman, C., Hausman, M. S., and Mergenhagen, S. E. Human mononuclear leukocyte chemotaxis: a quantitative assay for humoral and cellular chemotactic factor. J. Immunol., *108:* 857–860, 1972.
34. Snyderman, R., and Mergenhagen, S. Chemotaxis of macrophages. *In*: D. S. Nelson (ed.), Immunobiology of the Macrophage, pp. 323–348. New York: Academic Press, Inc., 1976.
35. Snyderman, R., and Pike, M. C. Chemotaxis of mononuclear cells. *In*: B. R. Bloom and J. F. David (eds.), *In Vitro* Methods in Cell Mediated and Tumor Immunity, pp. 651–682. New York: Academic Press, Inc., 1976.
36. Stastny, M., and Cohen, S. Epidermal growth factor. IV. The induction of ornithine decarboxylase. Biochim. Biophys. Acta, *204:* 578–589, 1970.
37. Vassalli, J. D., Hamilton, J., and Reich, E. Macrophage plasminogen activator: Modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors and cyclic nucleotides. Cell, *8:* 271–281, 1976.
38. Vassalli, J. D., Hamilton, J., and Reich, E. Macrophage plasminogen activator: induction by concavalalin A and phorbol myristate acetate. Cell, *11:* 696–705, 1977.
39. Verma, A., and Boutwell, R. K. Vitamin A acid (retinoic acid), a potent inhibitor of 12-*O*-tetradecanoyl-phorbol-13-acetate-induced ornithine decarboxylase activity in mouse epidermis. Cancer Res., *37:* 2196–2201, 1977.
40. Wahl, S. M., Wahl, L. M., McCarthy, J. B., Chedid, L., and Mergenhagen, S. E. Macrophage activation by mycobacterial soluble compounds and synthetic muramyl dipeptide. J. Immunol., *22:* 2226–2231, 1979.
41. Wigler, M. D., DeFeo, D., and Weinstein, I. B. Induction of plasminogen activator in cultured cells by macrocyclic plant diterpene esters and other agents related to tumor promotion. Cancer Res., *38:* 1434–1437, 1978.
42. Yuspa, S. H., Lichti, U., Ben, T., Patterson, E., Hennings, H., Slaga, T. J., Colburn, N., and Kelsey, W. Phorbol esters stimulate DNA synthesis and ornithine decarboxylase activity in mouse epidermal cell cultures. Nature (Lond.), *262:* 402–403, 1976.
43. Zigmond, S. H. Ability of polymorphonuclear leukocytes to orient in gradients of chemotactic factors. J. Cell Biol., *75:* 606–616, 1977.
44. Zigmond, S. H., and Hirsch, J. G. Leukocyte locomotion and chemotaxis: new methods for evaluation and demonstration of a cell derived chemotactic factor. J. Exp. Med., *137:* 387–410, 1973.

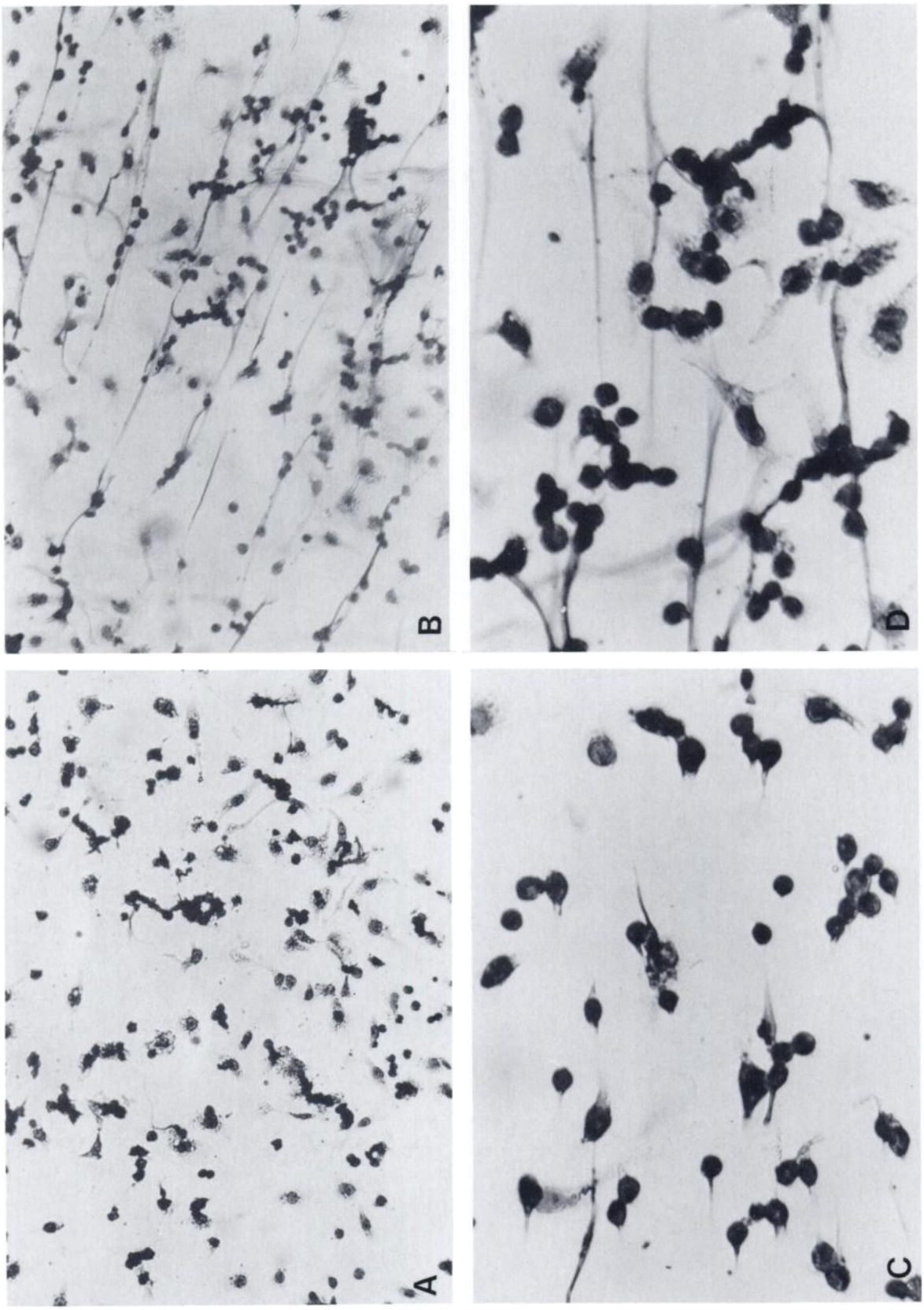

Fig. 1. Cellular orientation of resident peritoneal macrophages in a gradient of TPA. Cells were allowed to adhere to glass coverslips for 60 min and were then exposed to a TPA concentration gradient for 4 hr, as described in the text. The cells were then fixed and stained with Camco Quick Stain (Scientific Products, McGaw Park, Ill.). *A*, cells incubated in the presence of media alone. *B*, cells incubated in the presence of TPA. ×100. *C* and *D*, cells incubated in the presence of TPA. ×400.