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AGENTS THAT INCREASE CELLULAR cAMP INHIBIT PRODUCTION OF INTERLEUKIN-2, BUT NOT ITS ACTIVITY

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SUMMARY: Proliferation of lectin-treated mouse thymocytes induced by the tumor promoter, 12-O-tetradecanoyl-13-acetate, is markedly inhibited by cAMP analogues, prostaglandin E and methylisobutylxanthine. Proliferation induced by interleukin-2 is resistant to inhibition by these compounds. The tumor promoter induces interleukin-2 production in a subpopulation of lectin-treated thymocytes, and production of this lymphokine is inhibited by agents that increase cellular cAMP.

Lymphocyte activation is characterized by two major phases. The first phase is associated with production of the mitogenic lymphokine, interleukin-2 (IL-2)[1], and the second phase consists of the stimulation of mitogenesis by IL-2 in suitably primed cells (1). We studied the effect of cAMP analogues and agents that increase cellular cAMP on these two distinct phases of blastogenesis in a discrete population of mouse thymocytes. 12-O-Tetradecanoylphorbol-13-acetate (TPA) is co-mitogenic for a subpopulation of mouse thymocytes that lack receptors for peanut agglutinin (PNA-negative cells) (2). That is, while not mitogenic itself, TPA markedly potentiates proliferation in PNA-negative cells treated with the plant lectin, PHA. The co-mitogenic effect of TPA is mediated by the production of IL-2 (phase 1) and IL-2 can induce mitogenesis in PHA-treated cells (phase 2). The data indicate that cAMP analogues, prostaglandin E_1 and a cAMP phosphodiesterase inhibitor, methylisobutylxanthine (MIX), all markedly inhibit phase 1 of this reaction.

[1] Abbreviations used: IL-2, interleukin-2; TPA, 12-O-tetratecanoylphorbol-13-acetate; MIX, methylisobutylxanthine.

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They do not inhibit phase 2, but on the contrary, one of these agents, MIX, enhances proliferation mediated by IL-2.

MATERIALS AND METHODS

Thymocytes were removed from 4-6 week old Balb/c mice and cells lacking receptors for PNA (PNA negative cells) were isolated, essentially as described (3). PNA negative thymocytes ($2x10^6$/ml) were cultured in microtitre plates (200 µ/well) in RPMI 1640 medium containing 5% heat inactivated human serum and antibiotics. PHA (2 µg/ml) was added, along with either TPA (10 ng/ml) or IL-2 (1 u/ml). IL-2 containing medium was obtained from the Jurkat cell line (4) stimulated with TPA (10 ng/ml) and PHA (2 µg/ml). Cell free supernatants from these cultures were dialyzed against RPMI 1640, and IL-2 activity assayed on an IL-2-dependent mouse cytotoxic T cell line (CTLL) as described (5). The Jurkat and CTL lines were generous gifts of Dr. Kendall Smith. The medium was diluted 1:30-40 to provide 1 u/ml IL-2 in the experimental cultures. A unit of IL-2 activity is defined as the amount that results in 50% of the maximal response of a murine IL-2-dependent CTL line at a dilution of 1/16-1/32. Thymocytes, with the various additions, were incubated for 72 hr at 37° in a 5% CO_2-95% air, humidified atmosphere, and ^{3}H-thymidine incorporation (1 µCi/well) during the terminal 20 hr of culture determined.

RESULTS

Dibutyryl cAMP and 8-bromo cAMP inhibit mitogenesis in PHA-treated PNA-negative thymocytes stimulated with TPA (Figure 1, A&B). We have recently shown that TPA mediates its co-mitogenic effect via production of IL-2 (2). When PHA-treated PNA-negative cells were provided with an external source of IL-2, the cAMP analogues did not inhibit mitogenesis. We used supernates from a cloned human leukemic cell line, Jurkat, stimulated with PHA (2 µg/ml) and TPA (10 µg/ml) as a source of IL-2 (4). The supernatants were diluted 1:30-40 to provide a concentration of IL-2 of 1 u/ml as assayed on an IL-2-dependent murine cytotoxic T-cell line. In selected experiments TPA was removed from serum-free Jurkat supernatants by dialysis (6) and the mitogenic effect of the dialyzed preparations was also found to be resistant to inhibition by the cAMP analogues. The inhibitory effect of the cAMP analogues appears to be specific for cAMP, since 8-bromo and dibutyryl cGMP did not inhibit the co-mitogenic action of TPA. IL-2-mediated thymocyte proliferation was also resistant to the cGMP analogues.

Prostaglandins of the E series increase intracellular levels of cAMP in many cell types (7). TPA-induced proliferation of PHA-treated PNA-negative cells was exquisitely sensitive to inhibition by nanogram quantities of

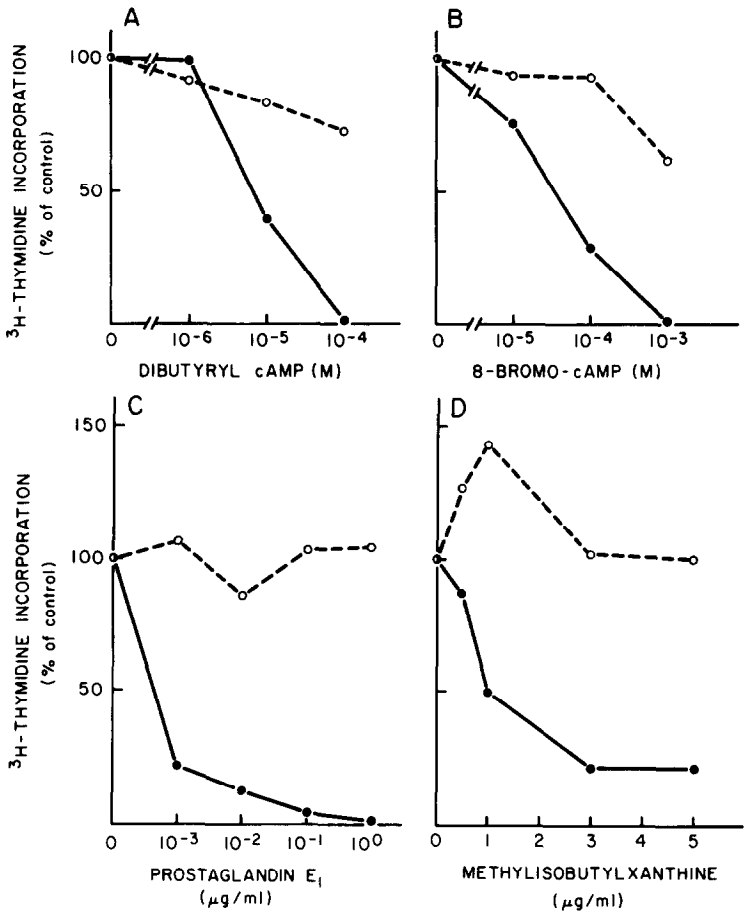

Figure 1. Effect of dibutyryl cAMP (A) and 8-bromo-cAMP (B) PGE_1 (C) and MIX (D) on ^{3}H-thymidine incorporation, stimulated by either TPA (●) or IL-2 containing medium (o), in PHA-treated, PNA-negative mouse thymocytes.

prostaglandin E_1 (Figure 1C). Prostaglandin E_2 gave similar results. IL-2-mediated proliferation was resistant to inhibition by the prostaglandin compounds used.

MIX, an inhibitor of cAMP phosphodiesterase, also inhibited TPA-induced proliferation of PHA-treated PNA-negative thymocytes, but did not inhibit IL-2-mediated proliferation (Figure 1D). In constrast to the other agents used, however, MIX potentiated IL-2-mediated proliferation at concentrations that inhibited TPA-mediated proliferation.

We next directly measured the effect of 8-bromo cAMP, PGE_1, and MIX on IL-2 production by PNA-negative cells stimulated with PHA (2 μg/ml) and TPA (10 ng/ml). Cell-free supernatants from PNA-negative cells, incubated for

Table 1
INHIBITION OF THYMOCYTE PROLIFERATION AND PRODUCTION OF IL-2 BY PGE_1, cAMP AND METHYLISOBUTYLXANTHINE

Additions	PNA-Negative Murine Thymocytes Activated with PHA + TPA[a]		IL-2 Dependent Murine Cytotoxic T Cell Line Activated with IL-2[c]
	^{3}H-Thymidine Incorporation (cpm/culture)	IL-2 Production[b] (arbitrary units)	^{3}H-Thymidine Incorporation (cpm/culture)
None	327,722	100	15,883
+PGE_1 (10 ng/ml)	66,135	53	15,041
+PGE_1 (100 ng/ml)	34,352	16	16,481
+PGE_1 (1000 ng/ml)	15,055	<5	12,528
+8-Bromo cAMP (10^{-4}M)	227,524	56	18,482
+MIX (5 µg/ml)	77,871	37	16,625

[a]PNA-negative thymocytes were treated with PHA (2 µg/ml) and TPA (100 ng/ml) and effects of PGE_1, 8-Bromo cAMP and MIX determined on the PHA + TPA-activated cells. In the absence of PHA + TPA, ^{3}H-thymidine incorporation was 1,674 cpm and <5 u IL-2 were produced.

[b]Twenty-four hour supernatants of PNA-negative thymocytes, incubated under the various experimental conditions described, were collected and dialyzed. IL-2 activity was determined by measuring the mitogenic effects of serial dilutions (1/4-1/512) on a murine CTLL as described (5). The amount of IL-2 produced by PNA-negative cells in the absence of cAMP, PGE_1 and MIX was arbitrarily defined as 100 u/ml.

[c]IL-2-dependent CTLL cells were treated with IL-2 at a dilution that resulted in 50% of maximal response and effects of PGE_1, 8-Bromo cAMP and MIX determined on the IL-2 activated CTLL cells. In the absence of IL-2, ^{3}H-thymidine incorporation was 1,090 cpm.

24 hr under the experimental conditions detailed above, were exhaustively dialyzed and assayed for IL-2 content in two assays. In the first assay, we determined their activity in promoting proliferation in PNA-positive thymocytes in the presence of PHA. PHA-treated PNA-positive thymocytes do not respond to IL-1 (8) or to TPA (2), but only to IL-2 (2,8). In the second assay we determined their activity in promoting proliferation in an IL-2-dependent murine cytotoxic T cell line (5). Data from both assays indicate that inhibition of IL-2 production induced by the cAMP analogues PGE_1, and MIX paralleled the effect of these compounds in inhibiting proliferation of PHA-treated PNA-negative thymocytes stimulated with TPA. Table I indicates data obtained utilizing the standard CTLL assay for IL-2 production (5). The direct effect of PGE_1, 8-bromo cAMP and MIX on the proliferative response of the CTLL to a standard IL-2 preparation was determined. This response was refractory to inhibition by these agents (Table I).

DISCUSSION

Conflicting reports have appeared concerning the role of cAMP in mediating mitogenic signals for lymphocytes (9). There is, however, general

agreement that treatment of lymphocytes with cAMP analogues, or with agents known to increase cellular cAMP, inhibit blastogenic responses to mitogens and antigens (10). Agents that increase cellular cAMP have differential inhibitory effects on lymphocyte proliferation induced by different mitogens (11). We have found that responses of human PBM to PHA and Con A, for instance, are resistant to these agents whereas responses to the galactosyl binding lectins, soybean agglutinin and peanut agglutinin are much more sensitive to them. It should be noted that responses of human PBM to PHA are much more resistant to inhibition by cAMP than are responses of murine PNA-negative cells activated with PHA plus TPA. Mitogenic responses of human PBM activated with optimal concentrations of PHA or Con A continue to respond to approximately 55-75% of control levels in the presence of concentrations of PGE_1, up to 10 μg/ml (12). Preliminary results in our laboratory indicate that the direct mitogenic response of human PBM to TPA is also resistant to cAMP. Rappaport and Dodge (13) have, however, recently reported that IL-2 production by human PBM stimulated with PHA was inhibited by prostaglandins of the E series at physiologic concentrations of PGE (10 ng/ml). Thus, it appears that there exists a much more strict correlation between cell proliferation and IL-2 production in mouse thymocytes activated with PHA and TPA than in human lymphocytes activated with PHA.

The studies reported here indicate that the inhibitory effect of cAMP on murine thymocyte proliferation depends on the stage of the proliferative pathway at which cells are exposed to increased levels of cAMP. The inhibitory effect of cAMP on lymphocyte proliferation is not the result of interference with the DNA synthetic machinery itself but rather on the induced synthesis of the mitogenic lymphokine, IL-2.

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