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Adenosine 3′,5′-cyclic monophosphate (cAMP) inhibits phorbol ester-induced growth of an IL-2-dependent T cell line

Yuso Goto, Toshikazu Takeshita and Kazuo Sugamura

Department of Bacteriology, Tohoku University School of Medicine, Sendai 980, Japan

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We previously established a human T cell line, TPA-Mat, which can proliferate in response to not only interleukin-2 (IL-2), but also phorbol esters such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and phorbol-12,13-dibutyrate (PDBu). The present study demonstrated that the PDBu-dependent growth of TPA-Mat cells was inhibited up to 90% by adenosine 3′,5′-cyclic monophosphate (cAMP)) raising agents such as forskolin, cholera toxin and 1-methyl-3-isobutylxanthine, and cAMP analogues, whereas the IL-2-stimulated TPA-Mat growth was slightly inhibited. These findings suggest that the signal transduction pathway of PDBu-induced growth, which should involve activation of protein kinase C, is sensitive to cAMP, and that it cannot be exactly identical to the signal transduction pathway of Il-2-induced growth in TPA-Mat cells.

cyclic AMP; Protein kinase C; Interleukin-2

1. INTRODUCTION

The interaction of IL-2 with its high affinity receptor has been shown to transduce the growth signal, although the mechanism of intracellular growth signaling is still unknown. It has been suggested that IL-2 stimulates turnover of PI, which results in activation of PKC [1,2]. We have recently established a TPA-dependent cell line (TPA-Mat) from an IL-2-dependent human T cell line (ILT-Mat) [3]. TPA-Mat cells can proliferate dependent on IL-2 and various PKC activators such as TPA, PDBu, teleocidin and apliciatoxin. This suggests that PKC plays an important role in growth signaling. So far, two major classes of receptors transducing intracellular signals across the membrane have been identified. One class regulates cAMP and the other regulates turnover of PI, which generates diacylglycerol and inositol-1,4,5-trisphosphate, as second messengers (reviewed [4]). The diacylglycerol activates PKC, and inositol-1,4,5-trisphosphate increases intracellular free Ca^{2+} [5,6]. cAMP and PI turnover appear to antagonize each other in signal transduction in most tissues [5]. In lymphocytes, cAMP has been shown to inhibit PI turnover and thereby suppress the activation of PKC [7]. However, the effect of cAMP on the signal transduction following PKC activation is unknown. Therefore, we examined the effects of cAMP on cell growth signalings induced by IL-2 and PDBu, and demonstrated that cAMP significantly inhibits PDBu-induced signal transduction in TPA-Mat cells.

Correspondence address: K. Sugamura, Department of Bacteriology, Tohoku University School of Medicine, 2-1 Seiryo-machi, Sendai 980, Japan

Abbreviations: cAMP, adenosine 3′,5′-cyclic monophosphate; TPA, 12-*O*-tetradecanoylphorbol 13-acetate; PDBu, phorbol 12,13-dibutyrate; PKC, protein kinase C; IBMX, 1-methyl-3-isobutylxanthine; Bu-cAMP, dibutyryl-cAMP; 8-cAMP, 8-bromo-cAMP; PI, phosphatidylinositol; IL-2, interleukin 2; PKA, protein kinase A

2. MATERIALS AND METHODS

2.1. Cell culture

TPA-Mat cells were maintained in part in growth medium (RPMI 1640 medium supplemented with 10% fetal calf serum) containing 100 ng/ml of PDBu, and in part in growth medium

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containing 200 U/ml of human recombinant IL-2 (obtained from Shionogi Co., Osaka, Japan) at 37°C with 7% CO_2 in air.

2.2. *Incorporation of [^{3}H]thymidine (TdR)*

[^{3}H]TdR incorporation assays were carried out as described [8].

2.3. *Cyclic AMP measurement*

TPA-Mat cells were washed twice with growth medium, and plated into culture plates (Terumo, SH-T12FSW) at 1.0 × 10^6/ml. 100 μM of IBMX was added to each well, and the plates were incubated for 30 min at 37°C prior to addition of forskolin. After 5 min incubation with forskolin at various concentrations, an equal volume of chilled 12% trichloroacetic acid was added to each well. After standing overnight at 4°C, the trichloroacetic acid-extractant was centrifuged at 1500 × g at 4°C for 30 min. Trichloroacetic acid in the supernatant was extracted twice with 2 ml of diethyl ether saturated with distilled water, and the extracted solution was used for the cAMP assay. cAMP content was measured by the radioimmunoassay method with a Yamasa cAMP assay kit as reported by Honma et al. [9].

3. RESULTS

The growth of TPA-Mat cells was stimulated by PDBu and by IL-2 in a dose-dependent manner (fig.1). PDBu-stimulated TPA-Mat growth was inhibited up to 90% in the presence of 10 μM forskolin. In contrast, IL-2-stimulated TPA-Mat growth was slightly inhibited by forskolin (fig.1). Similar results to the above were obtained in three experiments. The inhibitory effect of forskolin on TPA-Mat growth is quantitatively shown in fig.2. IL-2-stimulated TPA-Mat growth was weakly affected by forskolin even at a concentration of

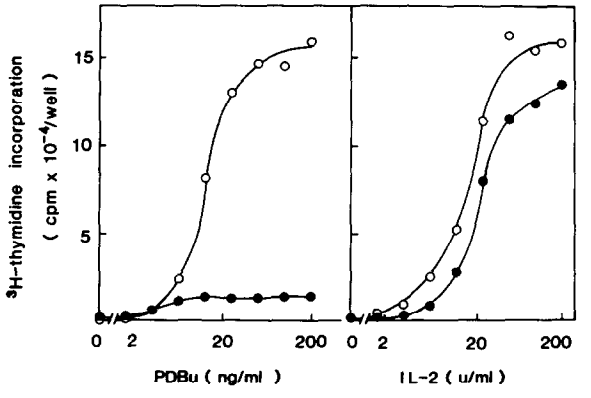

Fig.1. Effects of forskolin on PDBu- or IL-2-dependent growth of TPA-Mat cells. TPA-Mat cells were incubated for 48 h in a growth medium containing the indicated doses of PDBu or IL-2 in the presence (●) or absence (○) of 10 μM forskolin. During the last 4 h of incubation, [^{3}H]TdR was added, and the [^{3}H]TdR incorporated was measured.

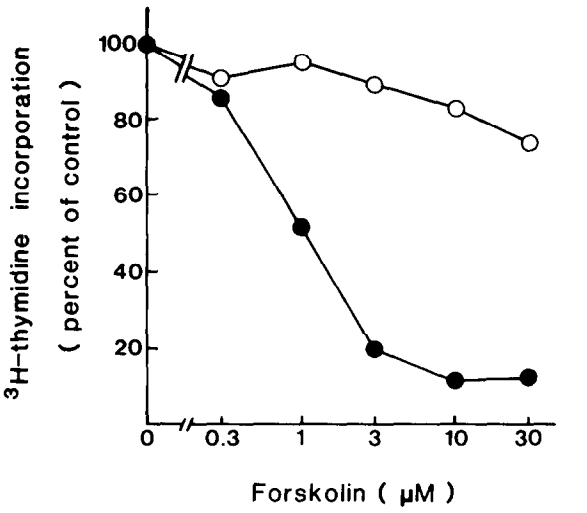

Fig.2. Dose response of forskolin for inhibition of TPA-Mat cell growth. TPA-Mat cells were incubated for 48 h in a growth medium containing 100 ng/ml of PDBu (●) or 200 U/ml of IL-2 (○) in the presence of the indicated doses of forskolin. During the last 4 h of incubation, [^{3}H]TdR was added, and the [^{3}H]TdR incorporated was measured.

30 μM, whereas PDBu-stimulated TPA-Mat growth was remarkably inhibited by forskolin in a dose-dependent manner. Inhibitions of PDBu-

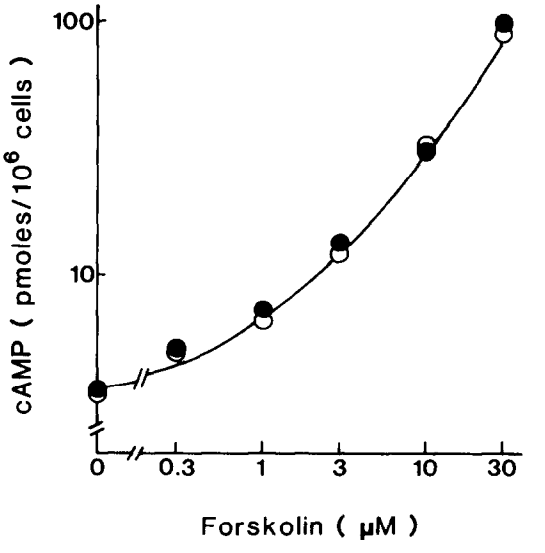

Fig.3. Intracellular cAMP levels of TPA-Mat cells cultured with PDBu or IL-2. TPA-Mat cells cultured with PDBu (●) or IL-2 (○) were treated with IBMX for 30 min and then with forskolin for 5 min. They were extracted with trichloroacetic acid, and used for the cAMP assay as described in section 2.

Table 1

Effects of cAMP analogues or cAMP-raising reagents on the growth of TPA-Mat cells

Treated with	Growth of control (%)	
	Maintained with: PDBu	IL-2
PDBu or IL-2 alone	100	100
+ db-cAMP (300 μM)	22.1	88.1
+ 8b-cAMP (300 μM)	47.2	70.1
+ IBMX (100 μM)	33.7	76.6
+ cholera toxin (100 ng/ml)	11.6	81.6
+ forskolin (10 μM)	15.4	71.5
Untreated	0.6	0.6

TPM-Mat cells were maintained in a growth medium containing PDBu or IL-2. They were then examined for [^{3}H]TdR incorporation under the indicated treatment

dependent growth of about 90% and 50% were obtained with 10–30 μM and 1 μM of forskolin, respectively. To determine whether the striking difference in sensitivity to forskolin was due to the degree of cAMP production in TPA-Mat cells, we measured intracellular cAMP levels after treatment with forskolin in both PDBu- and IL-2-stimulated TPA-Mat cells. Fig.3 shows that intracellular cAMP levels were significantly and dose-dependently elevated by forskolin under both culture conditions, and the elevated cAMP levels did not change during at least 48 h cultivation after forskolin treatment.

We then investigated the effects of other cAMP raising agents or cAMP analogues on PDBu- or IL-2-stimulated TPA-Mat growth. db-cAMP and 8b-cAMP are cAMP analogues, IBMX is a cAMP phosphodiesterase inhibitor, and cholera toxin is an adenylate cyclase activator. All the reagents had similar effects on TPA-Mat growth (table 1).

4. DISCUSSION

We showed that cAMP did not inhibit IL-2-dependent growth of a TPA-(or PDBu)-dependent human T cell line (TPA-Mat), but did inhibit PDBu-dependent growth of it. The TPA-Mat, which was established from an IL-2-dependent human T cell line, has the ability to proliferate dependent on various PKC activators such as TPA, PDBu, teleocidin, mezerein and apliciatoxin [3]. All the PKC activators are known to bind to PKC directly and to activate it [5,10,11]. Thus the growth signaling induced by PDBu in TPA-Mat cells could be due to PKC activation. The present study indicated that cAMP blocked the transduction pathway of the growth signal induced by PDBu in TPA-Mat cells. The antagonistic interaction between PKC and cAMP-dependent protein kinase, PKA, has been reported in various cells in relation to some signal transduction systems (review [12]). In peripheral lymphocytes, cAMP is known to block PI turnover, which suppresses the activation of PKC [7]. The present data may provide a new insight into the antagonistic interaction between PKA and PKC in the signal transduction pathway of cell growth. In contrast to PDBu-stimulated growth, IL-2-stimulated growth of TPA-Mat cells was slightly inhibited by cAMP. These different effects of cAMP are not due to the difference in the rate of generation or degradation of cAMP between PDBu- and IL-2-stimulated TPA-Mat cells, since there are no significant differences in intracellular cAMP levels between them at various times of treatment with forskolin. Therefore, the following explanation for the different effects of cAMP on growth of TPA-Mat cells can be proposed: the growth signal induced by IL-2 may be transmitted not only along the cAMP-sensitive pathway, where the PKC activation may be involved, but also along the cAMP-insensitive pathway, where PKC activation may not be involved.

We recently established a unique cell line, CT-Mat, derived from ILT-Mat. The growth of CT-Mat cells is sustained by cholera toxin instead of by IL-2, and the cholera toxin-mediated growth signal does not seem to be transmitted through PKC activation (Takeshita, T. et al., in preparation). Furthermore, it has been reported that IL-2 induces generation of cAMP but not of inositol phosphates in activated human T cells [13]. Together with the present study, these data suggest that the growth signaling from the IL-2 receptor may be mediated by a pathway which does not involve PKC.

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