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Antibody-induced cAMP accumulation in splenocytes from athymic nude mice

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Products from the hydrolysis of phosphatidylinositol 4,5-bisphosphate (IP_3) can increase and/or potentiate cAMP accumulation in a variety of cells. Antibody to surface immunoglobulins activates IP_3 hydrolysis in B-lymphocytes. In this study we have examined whether anti-Ig also stimulated and/or potentiated increases in the cAMP levels of splenocytes from athymic nude mice. Furthermore, since TPA potentiates anti-Ig-induced DNA synthesis and cAMP modulates DNA synthesis, the effects of TPA on any anti-Ig-induced changes in cAMP were also studied. Antibody (25 μg/ml) stimulated a rapid rise in cAMP which increased from 250 fmol/10^6 cells to 400 fmol/10^6 cells within 1 min and then subsided to 310 fmol/10^6 cells by 10 min. TPA (96 nM) suppressed the anti-Ig-induced cAMP accumulation at 1 min by 60%, but potentiated the forskolin (114 μM)-induced rise by 151%. Two other activators of protein kinase C, dioctanoylglycerol (5 μM), and anti-Ig (25 μg/ml), also potentiated the forskolin response by 198% and 52%, respectively. These results suggest that modulation of the adenylate cyclase system by anti-Ig may act in concert with cytokines and/or prostaglandins secreted by other lymphoid cells to define the state of proliferation or differentiation in B-cells.

Lymphocyte; Antibody; cyclic AMP; Protein kinase C; Phorbol ester; Forskolin

1. INTRODUCTION

Recently we reported that TPA potentiated anti-Ig-induced DNA synthesis in B-lymphocytes [1]. Two receptor-coupled effector systems whose products modulate DNA synthesis are the adenylate cyclase [2–4] and inositol phospholipid-dependent phospholipase C-coupled systems [5]. Adenylate cyclase-coupled systems generate cAMP, whereas phospholipase C-coupled systems produce IP_3 and diacylglycerol [6,7]. The products from each effector system can modulate the coupling or activity of the other system. For example, permeable analogs of cAMP or activators of adenylate cyclase can inhibit agonist-induced arachidonic acid release, and inositol phospholipid metabolism [8]. Alternatively, activation of phospholipase C modulates the adenylate cyclase system in different ways: production of IP_3, and mobilization of Ca^{2+} raise cAMP levels [9–12], whereas activation of protein kinase C can either enhance [13–16] or inhibit [17–20] agonist-induced increases in cAMP.

Since cross-talk between the phospholipase C and adenylate cyclase signaling systems is highly

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Abbreviations: cAMP, adenosine-3′,5′-cyclic phosphoric acid; anti-Ig, goat anti-mouse IgM μ-chain specific; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; ^{125}I-ScAMP, 2′-*O*-succinyl ([^{125}I]iodotyrosine methyl ester)-adenosine-3′,5′-cyclic phosphoric acid; BSA, bovine serum albumin; IBMX, isobutyl-3-methyl-1-xanthine; PEG-8000, polyethylene glycol-8000; PGE_1, prostaglandin E_1; diC_8, *sn*-1,2-dioctanoylglycerol; 4α-PDD, 4-α-phorbol-12,13-didecanoate

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cell type specific and anti-Ig activates the phospholipase C signaling system, we studied whether anti-Ig induced changes in the cAMP levels. Furthermore, since cAMP modulates DNA synthesis and TPA potentiates anti-Ig-induced DNA synthesis, we investigated whether TPA modulates any anti-Ig-induced cAMP accumulation in splenocytes from athymic nude mice. Splenocytes from young athymic nu/nu mice (< 12 weeks old) were chosen because they contain fewer Thy-1^+ cells than their nu/+ counterparts, do not express mature T-cell functions such as production of IL-2, cytotoxic T-lymphocyte responses or mitogenic responses to Con A, and therefore do not interact with or modulate B-lymphocyte responses [21]. We report here that anti-Ig induces an increase in cAMP and that TPA suppresses this stimulation while it enhances the forskolin-induced increase.

2. MATERIALS AND METHODS

Dupont-New England Nuclear supplied the ^{125}I-ScAMP. BSA, anti-Ig, IBMX, TPA, forskolin, ficol-paque, Hepes, PEG-8000, cAMP, c-globulin, triethylamine, acetic anhydride and PGE_1 were purchased from Sigma (St. Louis, MO). Dr Janice Douglas, Department of Medicine CWRU, kindly provided the antibody to ScAMP.

Athymic nude mice, 49–51 days old, were obtained from Charles River Labs. The spleens were isolated and single cell suspensions prepared in a balanced salt solution containing (in mM) 120 NaCl, 25 Hepes (pH 7.4), 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$ and 1 mg/ml BSA (BSS/BSA) by pressing the spleens through a 100 mesh stainless steel screen. The cells were washed 3× by centrifugation, and the red blood cells were separated on a discontinuous ficol-paque gradient ($d = 1.09$) by centrifugation at 800 × g for 30 min. The cells were washed 3× in BSS/BSA, resuspended at 1.25 × 10^6 cells/ml and stored at 0–4°C in BSS/BSA with 500 μM IBMX. Experiments performed without IBMX showed no qualitative differences.

cAMP was extracted in the following manner. The cells were sedimented for 30 s in an Eppendorf model 5412 microcentrifuge, chilled on ice for 30 s, the supernatant was aspirated off, and ice cold 0.1 N HCl was added to the pellet. The solutions were then stored overnight at 0–4°C before freezing at −70°C. The solutions were then assayed using a radioimmunoassay [22].

3. RESULTS AND DISCUSSION

A few recent reports have indicated that activating the PIP_2 cascade increases and/or potentiates cAMP accumulation in various cell types such as rat pinealocytes, vascular smooth muscle cells, and neutrophils [9–12]. Since cross linking surface immunoglobulins on B-lymphocytes with anti-Ig activates PIP_2 hydrolysis [23,24], it was interesting to investigate whether anti-Ig also stimulated and/or potentiated increased the levels of cAMP. Fig. 1 shows the results of experiments which investigated the effects of anti-Ig on cAMP levels in B-lymphocytes. Addition of anti-Ig stimulates a very rapid rise in the concentration of cAMP, which peaks within 2 min and then subsides to levels above the initial values. This rise in cAMP concentration increased with larger doses of anti-Ig beginning with 5 μg/ml and continuing through 25 μl/ml (not shown).

Many investigators have reported that cAMP modulates DNA synthesis and the cell type in B-lymphocytes [2,4,20], while others have reported that TPA potentiates anti-Ig-induced DNA synthesis [1,25]. Thus, the potentiation by TPA of the anti-Ig-induced DNA synthesis may result from a modulation of the anti-Ig-induced increase in cAMP. The experiments whose results are exhibited in fig. 1 also examined the effects of TPA on the anti-Ig-induced changes in cAMP. Although the kinetics remained unchanged, TPA decreased the anti-Ig-induced cAMP accumulation. Pretreating the cells with TPA for 3 min reduced the anti-Ig-induced increase in cAMP at 1 min by more than 60%.

The effect of TPA on the anti-Ig-induced changes in cAMP may result from a modification of the phospholipase C- or adenylate cyclase-coupled effector systems. Several reports indicate that TPA inhibits the PIP_2 cascade and Ca^{2+} mobilization in many systems including lymphocytes [26,27], can phosphorylate the adenylate cyclase of frog erythrocytes [16] and modify the guanine nucleotide-binding protein N_i of human platelets [28]. Studying the effects of TPA on forskolin-induced increases in cAMP can aid in elucidating the mechanism by which TPA

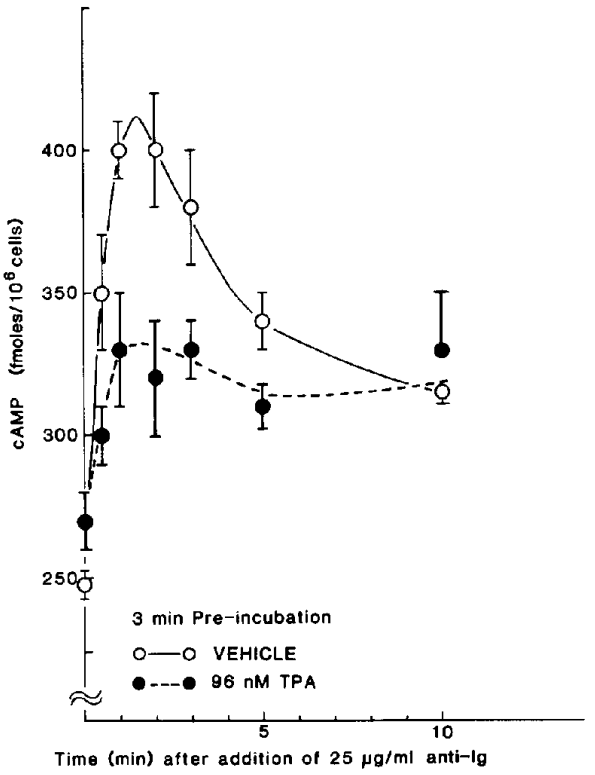

Fig.1. Temporal dependence of the anti-Ig-induced cAMP accumulation: modulation by TPA. Cells (1.25×10^6 cells/ml) were incubated at 37°C for 10 min with IBMX prior to an incubation for the indicated time with 25 μg/ml anti-IgM in the presence of vehicle (○) or 96 nM TPA (●) added 3 min prior to antibody. Values are the means ± SE of a single representative experiment performed in triplicate.

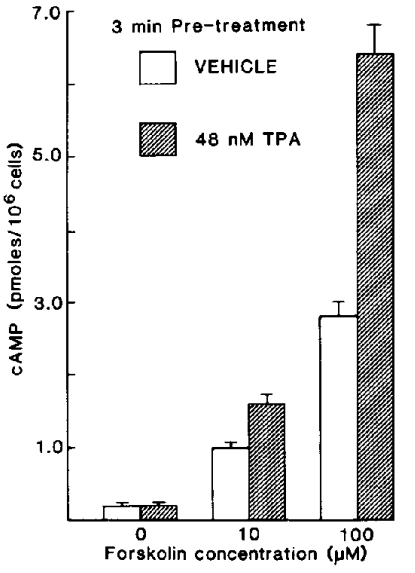

Fig.2. Potentiation of the forskolin-induced cAMP accumulation by TPA. Cells were treated as in fig.1 except that a 10 min incubation with the indicated amount of forskolin replaced anti-IgM and 48 nM TPA was used. Values are the means ± SD of a single representative experiment performed in triplicate.

decreases the anti-Ig-induced increase in cAMP. Since forskolin interacts with adenylate cyclase, any interaction of TPA with adenylate cyclase should express itself by modifying the forskolin-induced increase in cAMP [29]. Figs 2 and 3 show the results of experiments that studied whether modification of adenylate cyclase by TPA could account for the observed decrease discussed above. In splenocytes from athymic nude mice, TPA potentiated the forskolin-induced increase in cAMP. This potentiation increased with forskolin concentration. TPA-treated cells, in the presence of 500 μM IBMX, increased the forskolin-induced cAMP increase by 60%, whereas treating the cells with 114 μM forskolin following exposure to TPA increased the cAMP concentration 128% compared with forskolin alone (fig.2). Fig.3 shows that the magnitude of the TPA-induced potentiation also increased with the duration of forskolin exposure. Thus, while TPA certainly modifies the adenylate cyclase system, this modification cannot explain the TPA-induced suppression of the anti-Ig-induced increase in cAMP.

TPA activates protein kinase C [6], and this activation may explain the observed potentiation of the forskolin-induced response.

This implies that other activators of protein kinase C, such as diacylglycerols or agonists which generate diacylglycerol, should also potentiate the forskolin response. Experiments that compared the effects of TPA with those of diC_8 and anti-Ig on forskolin-induced cAMP accumulation are depicted in fig.4. A 3 min preincubation with either 5 μM diC_8 or 25 μg/ml anti-Ig potentiated the forskolin response.

There are no published experiments on the effects of anti-Ig on B-cell cAMP levels. However, Cambier et al. [30] reported under 'data not shown' that monoclonal antibodies against im-

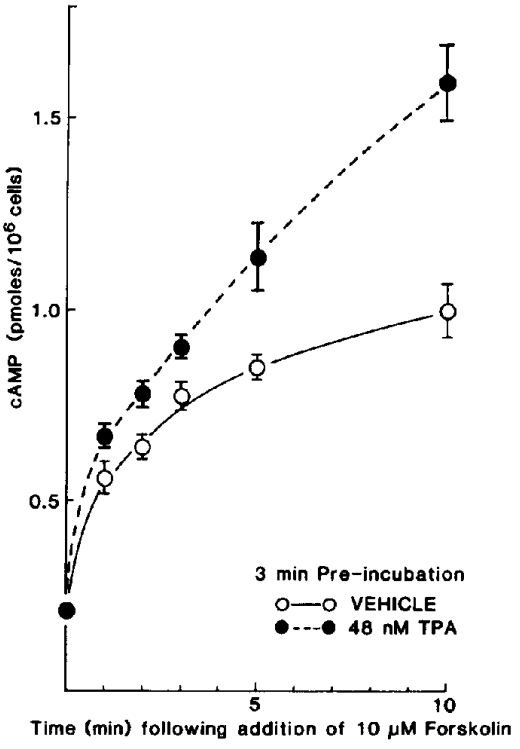

Fig.3. Temporal response of the forskolin-induced cAMP accumulation: modulation by TPA. Cells were treated as in fig.2 and incubated in 10 μM forskolin for the indicated time in the presence of vehicle (○) or 48 nM TPA (●). Values are the means ± SD of a single representative experiment performed in triplicate.

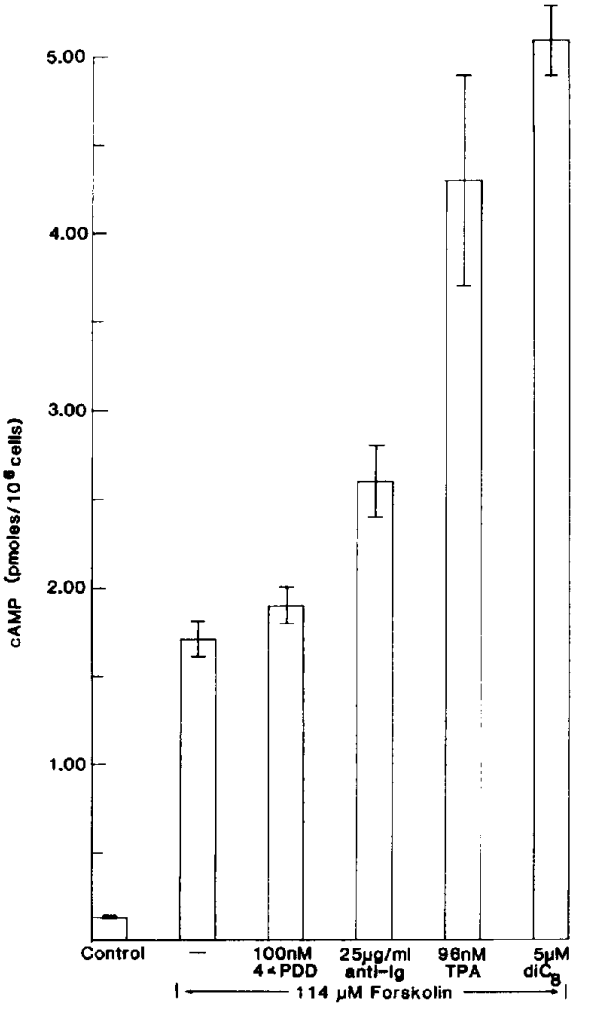

Fig.4. Potentiation of forskolin-induced cAMP accumulation by activators of protein kinase C or generators of diacylglycerols. Cells were treated as in fig.2. using 114 μM forskolin and the agents indicated in the figure.

munoglobulins had no effect on the cAMP levels in highly purified small B-lymphocytes, whereas Muraguchi et al. [29] speculated from 'data not shown' that stimulation with anti-IgM induced a steady increase in adenylate cyclase activity in highly purified small B-cells within the first 24 h. The results reported in this paper support the comment made by Muraguchi and are consistent with reports that other activators of PIP_2 hydrolysis also induce increases in cAMP levels and/or potentiate agonist-induced cAMP accumulation in other cells [9–12].

The experiments studying the mechanism by which TPA suppresses the anti-Ig-induced increase in cAMP do not examine events upstream of adenylate cyclase. Thus, the suppression might result from an alteration of the adenylate cyclase-coupled G proteins, a modification in the antigen-receptor interaction, or an alteration in the mechanism that mediates the cross-talk between the phospholipase C- and adenylate cyclase-coupled effector systems. Alternatively, the anti-Ig-induced rise in cAMP may indirectly result from the hydrolysis of PIP_2, and the TPA-induced suppression might reflect inhibition of that hydrolysis.

In conclusion, anti-Ig induces a biphasic increase in the levels of cAMP. Whether this increase results from a direct activation of adenylate cyclase, an indirect activation by secondary or ter-

tiary messengers generated by PIP_2 hydrolysis, or by inhibition of a cAMP-dependent phosphodiesterase remains unclear. In addition, TPA suppresses the anti-Ig-induced and potentiates the forskolin-induced increase in cAMP. The observations that TPA, diC_8, or anti-Ig can potentiate the forskolin-induced increase in cAMP implies that generators of diacylglycerols, i.e. antigen, can prime the adenylate cyclase system in B-cells. Since changes in the intracellular cyclic nucleotide concentration can modulate B-cell proliferation and differentiation [4,30–32], this priming may act in concert with cytokines and/or prostaglandins secreted by macrophages or T-cells to define the state of proliferation or differentiation in B-cells.

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