Volume 220, number 1, 52–56 FEB 04916 August 1987

Interleukin-2 binding to activated human T lymphocytes triggers generation of cyclic AMP but not of inositol phosphates

R. Gitendra Wickremasinghe, Anthony R. Mire-Sluis and A. Victor Hoffbrand

Department of Haematology, Royal Free Hospital, Pond Street, Hampstead, London NW3 2QG, England

Received 5 May 1987

Human T lymphocytes stimulated with phytohaemagglutinin undergo a single round of cell division. Further proliferation is dependent on the lymphokine interleukin-2 (IL2) [(1987) Immunology 60, 7–12]. We show here that binding of IL2 to its receptors on the lymphocyte surface triggers the generation of cyclic AMP. In contrast, generation of inositol phosphates from the breakdown of inositol lipids was not detected. We suggest that cyclic AMP may play a role in the transduction of the IL2 proliferative signal in T lymphocytes.

Lymphokine; Second messenger; Inositol lipid; Protein phosphorylation; Growth factor

1. INTRODUCTION

The binding of the lymphokine interleukin-2 (IL2) to specific cell-surface receptors present on activated T lymphocytes triggers DNA synthesis and mitogenesis in these cells [1]. The IL2 receptor has recently been shown to consist of two non-sulfhydryl linked transmembrane polypeptides with molecular masses of 75 and 55 kDa, respectively [2]. Nevertheless, the mechanisms involved in transduction of the IL2 mitogenic signal are unclear. The binding of some mitogens to their cognate cell-surface receptors stimulates the generation of biochemical second messengers within target cells. These second messengers activate cellular phosphotransferase systems, which are presumed to initiate cascades of biochemical reactions which culminate in DNA synthesis and cell division [3–5]. For example, binding of the mitogen prostaglandin E_1 to its receptor on Swiss 3T3 fibroblasts results in the elevation of cyclic AMP (cAMP) [6], the allosteric activator of cAMP-dependent protein kinase [7]. In contrast, PDGF binding to cells triggers the breakdown of inositol-containing phospholipids with the generation of a pair of second messengers, diacylglycerol (DG) and inositol trisphosphate (IP_3) [8]. DG is an allosteric activator of protein kinase C, while IP_3 stimulates release of Ca^{2+} from intracellular stores and consequent activation of calmodulin-dependent protein kinases [9,10]. Since IL2 stimulation of T lymphocytes rapidly stimulates protein phosphorylation [11,12], we measured the generation of second messengers in IL2-dependent lymphocytes. While increase in cAMP levels were clearly detectable, we found no evidence for the breakdown of inositol lipids following IL2 binding.

Correspondence address: R.G. Wickremasinghe, Department of Haematology, Royal Free Hospital, Pond Street, Hampstead, London NW3 2QG, England

2. MATERIALS AND METHODS

2.1. *Lymphocyte preparations*

Lymphocytes were prepared by sedimentation of heparinized venous blood from normal donors on

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Lymphoprep gradients (Nyegaard, Oslo). Monocytes and platelets were removed and the lymphocytes activated by treatment for 5 days with 10 $\mu l \cdot ml^{-1}$ PHA (Wellcome, Beckenham). The activated lymphocytes were completely dependent on exogenous IL2 for further proliferation [13].

2.2. *Cyclic AMP measurement*

For cAMP measurements, cells were resuspended at 5×10^6 ml^{-1} in fresh RPMI 1640 plus 10% fetal calf serum and allowed to equilibrate at 37°C, 5% CO_2 for 1 h. Recombinant human IL2 (10 units $\cdot ml^{-1}$, Amersham) or its vehicle were then added. At various times cells were harvested by centrifugation at $800 \times g$ for 3 min and cAMP extracted by addition of 0.5 ml ice-cold ethanol followed by sonication for 30 s. Radioimmunoassay was carried out using a rabbit antiserum against succinyl cAMP (Miles Laboratories, Stoke Poges) by a protocol supplied by the manufacturer.

2.3. *Generation of inositol phosphates*

Resting lymphocytes (5×10^6 ml^{-1} were labelled for 18 h at 37°C with 2.5 $\mu Ci \cdot ml^{-1}$ *myo*-[^{3}H]inositol (Amersham) in RPMI 1640 medium without inositol, supplemented with 10% dialyzed calf serum and antibiotics. Activated lymphocytes were labelled in the same medium at 10^7 cells $\cdot ml^{-1}$ for 4 h. (Labelling in inositol-free medium did not impair the ability of lymphocytes to respond to appropriate mitogens.) Labelled cells were washed twice in Hanks' salts, resuspended in unlabelled medium (2×10^6 cells $\cdot ml^{-1}$) and incubated at 37°C for 1 h in order to deplete free *myo*-[^{3}H]inositol. They were then resuspended at 10^7 cells $\cdot ml^{-1}$ in RPMI 1640 (without serum). Following a 20 min incubation at 37°C the appropriate mitogen or its vehicle was added. Inositol phosphates were extracted as described [14] and the radiolabel in individual inositol phosphates was determined by chromatography on Dowex A1-X8 columns [15]. Recovery of the radiolabel following analysis was typically 85–90%.

3. RESULTS AND DISCUSSION

We have recently shown that monocyte-depleted resting human lymphocytes undergo a single IL2-independent cell division when stimulated with phytohaemagglutinin (PHA) [13]. The resulting daughter cells (activated cells) were completely dependent on exogenous IL2 for further proliferation. We have used these activated lymphocytes to measure the generation of second messengers following IL2 treatment. Fig.1 shows that the basal levels of cAMP in activated lymphocytes from different donors showed considerable variability. However, addition of IL2 to these cells resulted in an increase in cAMP levels of 1.6–2.3-fold 30 min following growth factor addition. Fig.2 shows the time course of this cAMP elevation. A detectable increase was seen 3.5 min following IL2 addition with maximal levels between 20 and 30 min. A similar time course of cAMP elevation was observed in two further experiments.

We then assayed the generation of the soluble inositol phosphates following 5 min incubation of activated lymphocytes with IL2. In this experiment the lymphocytes were pre-labelled with *myo*-[^{3}H]inositol to label the inositol-containing

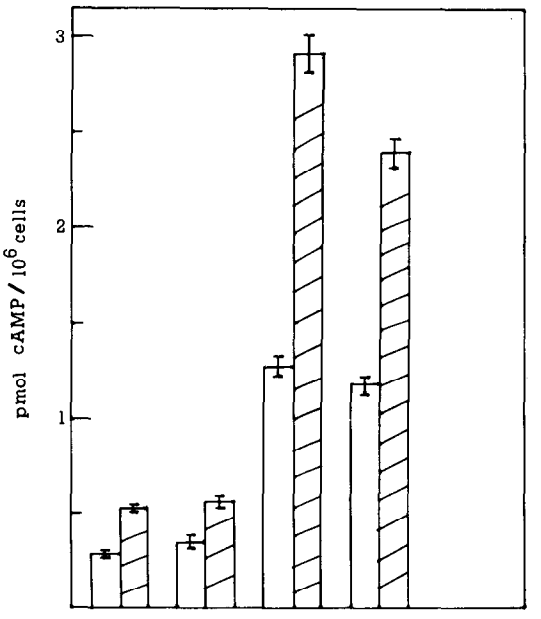

Fig.1. Increase of cAMP levels in activated lymphocytes treated with IL2. cAMP levels in untreated (open bars) and IL2 treated (hatched bars) activated lymphocytes from different donors. Assays were carried out in triplicate. Bars indicate standard errors. The IL2 stimulated increases in cAMP levels were significant ($P < 0.001$, Student's t-test).

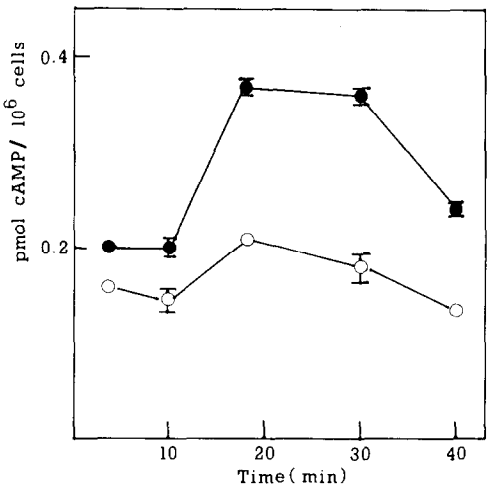

Fig.2. Time course of increase of cAMP in activated lymphocytes treated with 10 units·ml^{-1} IL2 at time zero. (○) Control; (●) IL2. Assays were carried out in duplicate.

phospholipids [14]. The small (1.3-fold) increase in inositol phosphate (IP) evident here was not reproduced in other experiments (fig.3A). No increase in inositol bisphosphate (IP_2) or inositol trisphosphate (IP_3) was evident. The latter observation is significant, since IP_3 is the initial breakdown product of phosphatidylinositol bisphosphate (PIP_2), which is thought to be the key substrate in ligand-stimulated inositol lipid breakdown [9,10]. As a positive control we also assayed the generation of labelled inositol phosphates from *myo*-[^{3}H]inositol pre-labelled resting lymphocytes following PHA stimulation. In agreement with earlier reports [16,17], increases in levels of IP, IP_2 and IP_3 were detectable. The largest increase was in radiolabelled IP_3 (3-fold) (fig.3B). The overall increase in soluble inositol phosphates stimulated by PHA was 1.7-fold, whereas no overall increase was observed following IL2 stimulation of activated cells (fig.3A).

The addition of 10 mM LiCl to cell cultures causes an inhibition of breakdown of IP to inositol. This inhibition has been exploited to facilitate detection of inositol phosphate production, since the sequential breakdown of IP_3 via IP_2 and IP is blocked, with consequent accumulation of high levels of IP [14]. The experiment depicted in fig.4A shows that no significant accumulation of any inositol phosphate occurred when *myo*-

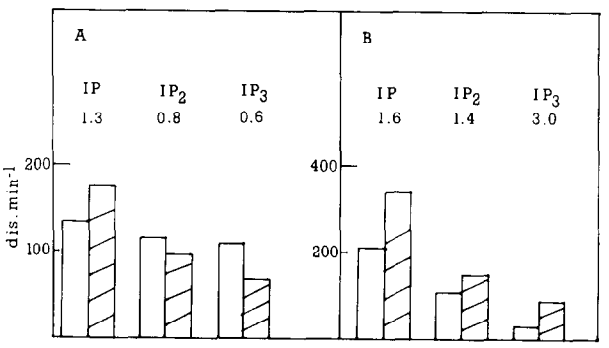

Fig.3. Changes in levels of radiolabelled inositol phosphates following IL2 treatment of *myo*-[^{3}H]inositol pre-labelled, activated lymphocytes (A) or PHA treatment of resting lymphocytes (B). Open bars, control; hatched bars, mitogen added 5 min before extraction. Figures above each pair of bars give the ratio of radiolabel in each inositol phosphate from mitogen-treated cells compared to the control.

[^{3}H]inositol pre-labelled, LiCl-treated, activated lymphocytes were treated for 30 min with IL2. In contrast, a very large increase in IP, accompanied by smaller increases in IP_2 and IP_3 was clearly evident when resting lymphocytes were treated with PHA (fig.4B). The difference could not be attributed to different levels of labelling of inositol lipids in resting and activated cells; in the experiment shown here the resting cells contained 5664 dpm/10^6 cells and the activated cells 4320 dpm/10^6 cells respectively in inositol lipids, as measured by scintillation counting of the organic phase residues following inositol

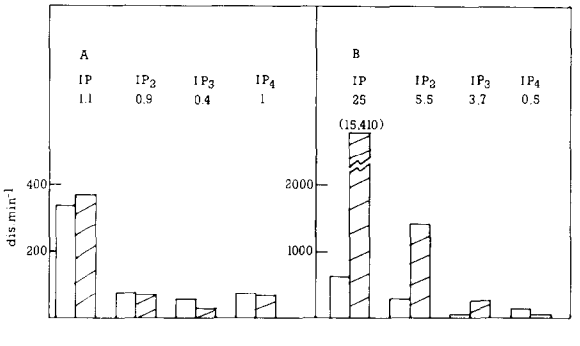

Fig.4. Changes in levels of radiolabelled inositol phosphates following IL2 treatment of *myo*-[^{3}H]inositol labelled activated lymphocytes (A) or resting lymphocytes (B) in the presence of 10 mM LiCl. See legend to fig.3 for details.

phosphate extraction. Results very similar to those depicted in fig.4A and B were obtained on four further occasions, confirming that inositol lipid breakdown was a consequence of PHA treatment of resting lymphocytes but not of IL2 binding to activated lymphocytes.

The results described here show that binding of mitogenic levels of IL2 to cell surface receptors of activated T lymphocytes stimulates elevation of cellular cAMP. The IL2-stimulated cAMP elevation observed here is slower than the increases elicited by acute hormones. This time course is, however, compatible with the observation that IL2 binding reaches equilibrium at about 15 min [18] and is comparable with the time course of cAMP elevation in Swiss 3T3 cells treated with platelet-derived growth factor [19]. Accordingly, we suggest that cAMP elevation contributes to the mechanism by which the IL2 proliferation signal is transduced in activated T lymphocytes. We have been unable to stimulate cell proliferation by addition of agents which increase cAMP levels, e.g. dibutyryl cAMP, isobutylmethylxanthine (not shown) suggesting that other transducing mechanisms may also contribute to securing commitment to mitosis. However, no breakdown of inositol lipids was observed, in agreement with observations on cloned murine cytotoxic lymphocytes [20] and on human short-term lymphoid cell lines [21]. This is in contrast with the conclusions of Farrar and co-workers [22,23] who showed that IL2 stimulation of the murine CT6 cytotoxic T-cell line triggered the breakdown of inositol lipids [22] and the translocation of protein kinase C from the cytosol to the particulate fraction [23], presumably resulting from its activation by DG generated from inositol lipids. Beckner and Farrar [24] have shown that IL2 actually reduces cAMP levels in CT6 cells, thereby reversing the antiproliferative effect of cAMP levels produced by prostaglandin treatment. Furthermore, activation of protein kinase C was required for the IL2-induced reduction in cAMP levels, suggesting a reciprocal relationship between the cAMP-dependent kinase system and protein kinase C. In contrast, protein kinase C activation causes cAMP elevation in lymphomas [25]. In order to reconcile these observations we suggest that the IL2 receptor may be coupled to different intracellular second messenger generating systems in different cell types. Both the generation of cAMP by adenylate cyclase [26] and the breakdown of inositol phospholipids [27] appears to be coupled to receptor stimulation via GTP binding proteins (G proteins). The IL2 receptor of CT6 cells has recently been shown to be coupled to a G protein [28]. It is therefore plausible that the association of the IL2 receptor with different G proteins in different contexts may stimulate the generation of different second messengers and the activation of alternative phosphotransferase systems. This is in accord with the observations that epidermal growth factor stimulates polyphosphoinositide breakdown in A431 cells [29] but not in hamster fibroblasts [30]. Therefore, the same growth factor may stimulate mitogenesis via alternative second messenger systems.

ACKNOWLEDGEMENTS

This work was supported by the Cancer Research Campaign. We are grateful to Jamie Jeremy, Gerry Klaus and Richard Bruckdorfer for advice on second messenger assays and to Mrs Megan Evans for word processing.

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