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The binding of ligands to the 55 kDa component of the interleukin-2 receptor triggers increased turnover of phosphate bound to an 85 kDa protein

Evidence for the role of cyclic AMP

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The cell-surface receptor for interleukin-2 (IL-2) consists of two unlinked polypeptides of 55 and 75 kDa (p55, p75). The monoclonal antibody antiTac binds to p55 alone. We show here that the binding of either IL-2 or antiTac to the surface of T lymphocytes triggered the generation of cAMP. Reagents which activate adenyl cyclase by stimulation of its guanine nucleotide-binding protein (Gs) also stimulated increases in cAMP. All of the above reagents, and cAMP itself, stimulated the turnover of phosphate residues bound to serine and threonine residues of an 85 kDa protein. The data provide evidence that the binding of ligands to the p55 component of the IL-2 receptor generates a biochemical signal by the stimulation of adenyl cyclase via Gs, and that the consequent generation of cAMP and activation of cAMP-dependent protein kinase modulates the turnover of p85-bound phosphate groups.

T lymphocyte; Mitogenesis; Interleukin-2; AMP cyclic

1. INTRODUCTION

The further proliferation of lymphocytes pre-activated by antigen or lectins is stimulated by the binding of the lymphokine interleukin-2 (IL-2) to a specific cell surface receptor [1]. The IL-2 receptor consists of two unlinked glycoprotein chains of 55 kDa (p55) and 75 kDa (p75) [2]. While the biochemical mechanisms by which IL-2 binding transduces its mitogenic signal are poorly understood, IL-2 stimulated protein phosphorylation has been demonstrated in a variety of IL-2-sensitive cells [3–7]. Using a permeabilised lymphocyte system incubated with [^{32}P]ATP, we have shown that both IL-2 and antiTac, a monoclonal antibody directed against p55 [8], stimulated the labelling of an 85 kDa protein (p85) on serine and threonine residues [3]. We have here explored the mechanism by which these ligands stimulate p85 labelling, and show that antiTac, like IL-2 itself [9], triggers a transient elevation of lymphocyte cyclic AMP concentrations. Furthermore, two agents which activate guanine nucleotide-binding regulatory proteins (G-proteins), the fluoroaluminate ion (AlF_4^- [10]) and guanosine 5'-[γ-thio]triphosphate (GTP[S]) elevated lymphocyte cAMP levels and also triggered the phosphorylation of p85. Taken together, the data suggest that both IL-2 and antiTac can trigger the phosphorylation of p85 by a mechanism involving cAMP elevation, and imply a role for the p55 component of the IL-2 receptor in transducing a

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Abbreviations: IL-2, interleukin-2; GTP[S], guanosine 5'-[γ-thio]triphosphate; AlF_4^-, fluoroaluminate ion; p85 (etc.), 85 kDa protein; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; G-protein, guanine nucleotide-binding regulatory protein; PKC, protein kinase C

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biochemical signal across the lymphocyte cell membrane.

2. MATERIALS AND METHODS

2.1. *Cells*

Lymphocytes were purified from the peripheral blood of healthy volunteers and pre-activated by treatment with phytohaemagglutinin for 5 days [11]. The resulting cells were entirely dependent on IL-2 for further proliferation. IL-2 mitogenesis was estimated by measuring the incorporation of [^{3}H]thymidine (41 Ci·mmol^{-1}, Amersham, England) as described [11].

2.2. *cAMP assays*

These were carried out by a radioimmunoassay procedure as described [9]. Duplicate estimations of cAMP did not vary by more than 5%.

2.3. *Detection of ligand-stimulated protein phosphorylation in permeabilized cells*

This procedure has been described in detail previously [3,12]. Briefly, lymphocytes (10^8 cells·ml^{-1}) were treated with L-α-lysophosphatidylcholine (1 mg·ml^{-1}). This procedure renders the cells permeable to small molecules without perturbing their morphology or ability to synthesize DNA, RNA or protein for at least 3 h [13]. The permeable cells were incubated with [γ-^{32}P]ATP (New England Nuclear, England; 3000 Ci·mmol^{-1}) together with IL-2 or other agents for 3 min. Radiolabelled proteins were separated by polyacrylamide gel electrophoresis in the presence of SDS and detected by autoradiography. Autoradiograms were scanned using a Joyce-Loebl Chromoscan II.

2.4. *Reagents*

Human recombinant IL-2 was from Amersham; antiTac was kindly provided by Dr T.A. Waldmann, NIH, Bethesda, MD, USA.

3. RESULTS AND DISCUSSION

We have shown previously that the binding of IL-2 to its receptor on intact IL-2 responsive cells triggers the rapid generation of cAMP [9]. These results are confirmed here (fig.1A). In addition, the binding of antiTac also triggered the transient elevation of cAMP (fig.1A). The AlF_4^- anion is known to activate adenyl cyclase via stimulation of Gs, the G-protein which positively regulates its activity [14]. This reagent also triggered the transient elevation of cAMP in lymphocytes (fig.1B), suggesting that adenyl cyclase regulated via a G-protein was present in these cells. IL-2, antiTac, AlF_4^- and GTP[S] (another direct activator of Gs [15]), also stimulated cAMP elevation when added to the permeabilised lymphocyte system used to measure ligand-stimulated protein phosphorylation (fig.1B,C), indicating that the putative coupling between the IL-2 receptor and adenyl cyclase remained intact in the permeable cell system.

Each experiment depicted in fig.1 was repeated on at least three occasions and invariably showed a statistically significant increase in cAMP levels in response to each reagent tested. However, the biphasic response in cAMP levels clearly seen in some experiments (fig.1A,D) showed some variability when cells from different donors were used, with respect to both the time and relative magnitude of each peak.

IL-2 binding to permeabilized lymphocytes in the presence of [^{32}P]ATP stimulated the labelling of an 85 kDa protein (p85, fig.2A). Maximal labelling of p85 occurred within 3 min of IL-2 addition (not shown). The relationship between rapid ligand-stimulated protein phosphorylation and mitogenesis is difficult to establish, due to the complexity of the pathways connecting early events to the initiation of DNA replication [16]. However, the dependence of p85 on IL-2 concentration paralleled the mitogenic dose response to the same ligand (fig.2B), suggesting a role for p85 phosphorylation (or for the phosphotransferase system which phosphorylates p85) in the early steps of IL-2 mitogenesis.

IL-2 stimulated labelling of p85 was clearly detected when IL-2 was added to intact lymphocytes which were then washed free of unbound IL-2, permeabilized and incubated with [^{32}P]ATP (fig.2C). p85 labelling was evident when cells were labelled between 5 and 30 min following IL-2 addition to intact cells, but was reduced to nearly control levels at 60 min. This suggests that p85 labelling was due to an accelerated turnover of protein-bound phosphate rather than to a single stable phosphorylation event. This conclusion has been confirmed by 'chasing' the radiolabel in p85 by incubation with unlabelled ATP (not shown). The experiment shown in fig.2C also suggests that p85 labelling is not an artefact due to addition of IL-2 to permeabilized cells, since labelling was elicited by addition of IL-2 to intact cells which were subsequently washed free of unbound IL-2 prior to permeabilization and incubation with [^{32}P]ATP.

In addition to IL-2 antiTac, AlF_4^- and GTP[S]

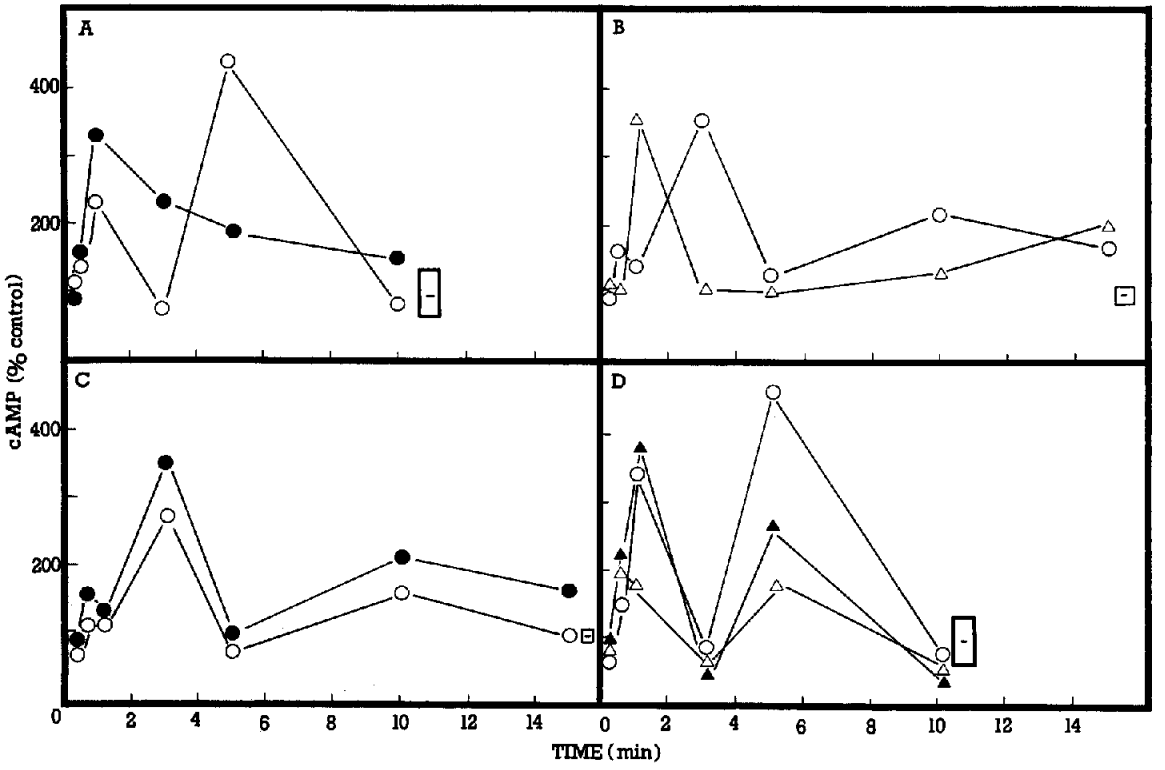

Fig.1. Elevation of cAMP levels in lymphocytes treated with IL-2, antiTac, AlF_4^- or GTP[S]. Intact lymphocytes (A,B) at 10^6 cells·ml^{-1} or permeable lymphocytes (C,D) at 10^8 cells·ml^{-1} were treated with 75 U·ml^{-1} IL-2 (○), 1:100 dilution of antiTac ascites (●), AlF_4^- (10 mM NaF + 1 μM $AlCl_3$) (△) or 1 mM GTP[S] (▲). cAMP was estimated at the times indicated and are expressed as a percentage of cAMP content in untreated control cells which were sampled at each time point. cAMP levels in control cells are indicated as mean ± SD (open bar) on each figure. Actual mean values in control cells varied between 324 and 513 fmol/10^6 cells. The effects of each ligand were found to be significantly different compared to the control by two-way analysis of variance ($p < 0.001$ for A and C, $p < 0.005$ for B and D).

also stimulated p85 phosphorylation as did the direct addition of cAMP (fig.3), implying that the activation of cAMP-dependent protein kinase either directly or indirectly stimulated the turnover of p85-bound phosphate. Phosphoamino acid analysis (not shown) of p85 labelled in response to each of these reagents confirmed our previous conclusions [3] that labelling occurred on serine and threonine residues. By contrast, two reagents which stimulate other phosphotransferase systems did not trigger p85 labelling. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA) which stimulates protein kinase C (PKC) [17] did not elicit p75 labelling (fig.3) suggesting that the kinase C system does not play a role in this aspect of IL-2 mitogenesis. This conclusion is confirmed by our observation that the Ca^{2+} chelator EGTA, which inhibits PKC, did not affect p85 labelling stimulated by either IL-2 or antiTac (not shown). It is unlikely that PKC participates in IL-2 signal transduction in normal lymphocytes, since IL-2 did not provoke the turnover of inositol lipids with the consequent generation of diacylglycerol [9,18,19], the physiological activator of PKC [17]. By contrast, PKC appears to play a crucial role in the early stages of lymphocyte mitogenesis [18,20,21]. cGMP, which activates a specific cGMP-activated kinase, also failed to elicit p85 labelling (fig.3). In conclusion, we have shown here that the binding of either IL-2 or the monoclonal antibody antiTac, which binds only the p55 component of the IL-2 receptor, triggers the generation of cAMP in both intact and permeabilised lymphocytes and stimulates the turnover of phosphate bound to serine and threonine residues of an 85 kDa protein. Since p85 labelling can also be elicited by agents which directly activate G-

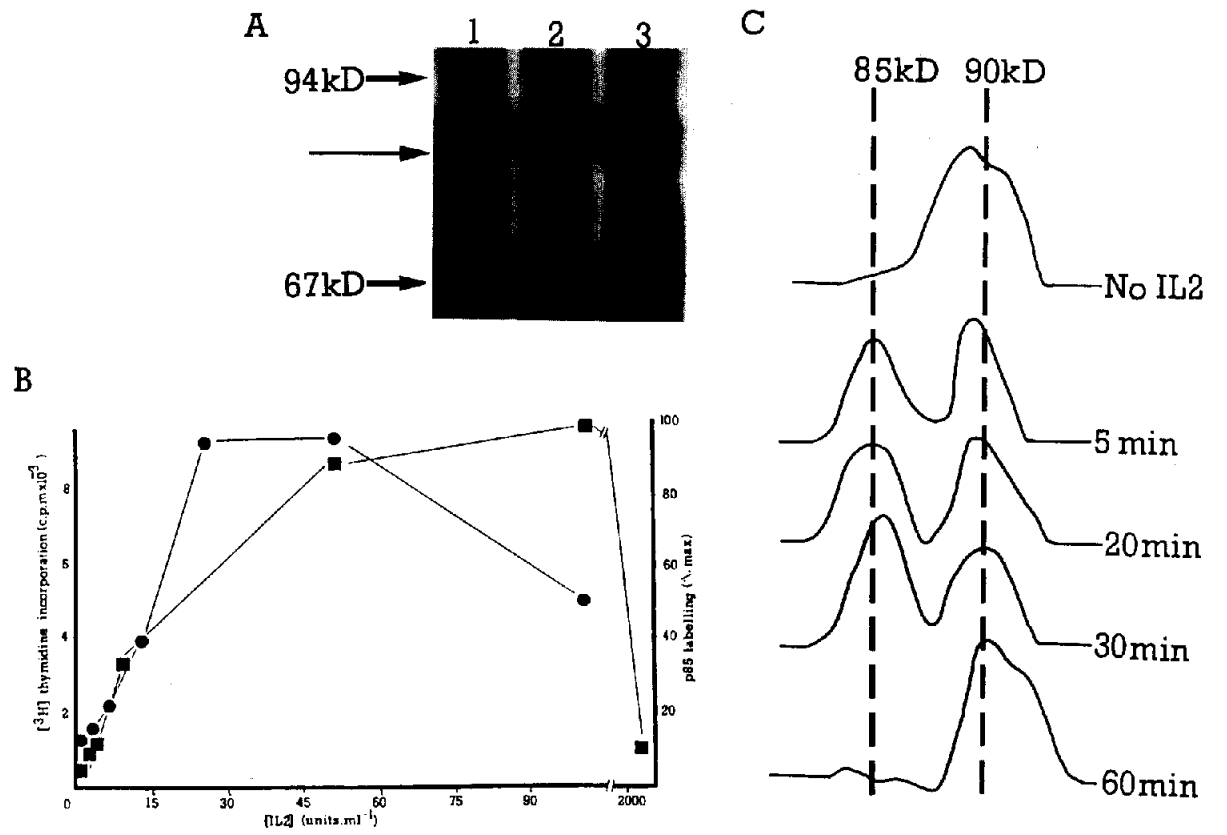

Fig.2. (A) Lymphocytes were permeabilized and incubated with [^{32}P]ATP in the presence of: 1, no additions; 2, 10 $\mu g \cdot ml^{-1}$ BSA albumin; 3, 10 $\mu g \cdot ml^{-1}$ BSA + 75 $U \cdot ml^{-1}$ IL-2. Labelled proteins were separated by gel electrophoresis and visualized by autoradiography. Migration of molecular mass markers is indicated by arrows. The long arrow indicates the position of p85. (B) Dose responses of [^{3}H]thymidine incorporation (●) and p85 labelling (■) in response to increasing concentration of IL-2. p85 labelling was quantitated by densitometric scanning of autoradiogram. (C) Intact lymphocytes were incubated with 75 $U \cdot ml^{-1}$ IL-2 for the indicated times, then washed, permeabilized and incubated with [^{32}P]ATP for 3 min. Scans of the 85 kDa region of the autoradiograms are shown.

proteins and consequently stimulate the generation of cAMP, or by the addition of cAMP itself, we suggest that the binding of either IL-2 or antiTac to the p55 component of the IL-2 receptor is coupled to the stimulation of adenyl cyclase via a G-protein, presumably the well-characterised Gs [14].

Whereas IL-2 is mitogenic for lymphocytes, antiTac is not, and under some experimental conditions blocks IL-2 mitogenesis [22]. Therefore, additional signals consequent to IL-2 binding to the p75 receptor component must also be of importance in securing the commitment of lymphocytes to mitosis. The stimulation of phosphorylation of a 75 kDa protein on unspecified amino acids following IL-2 addition to antiTac immunoprecipitates of lymphocyte membranes has been reported and may represent ligand-stimulated autophosphorylation of the p75 receptor component [6]. In addition, IL-2 binding has been shown to increase the phosphorylation on tyrosine residues of several proteins, suggesting the direct or indirect activation of tyrosine-specific protein kinases [7]. Taken together with the IL-2-stimulated phosphorylation of 63 and 68 kDa proteins on serine and threonine residues [4,5] it is likely that signalling via the IL-2 receptor involves the activation of multiple phosphotransferase systems. This complexity of the signalling mechanisms is also evident in other growth factor systems [16].

The role of the p55 IL-2 receptor component in signalling is controversial. Dose response studies

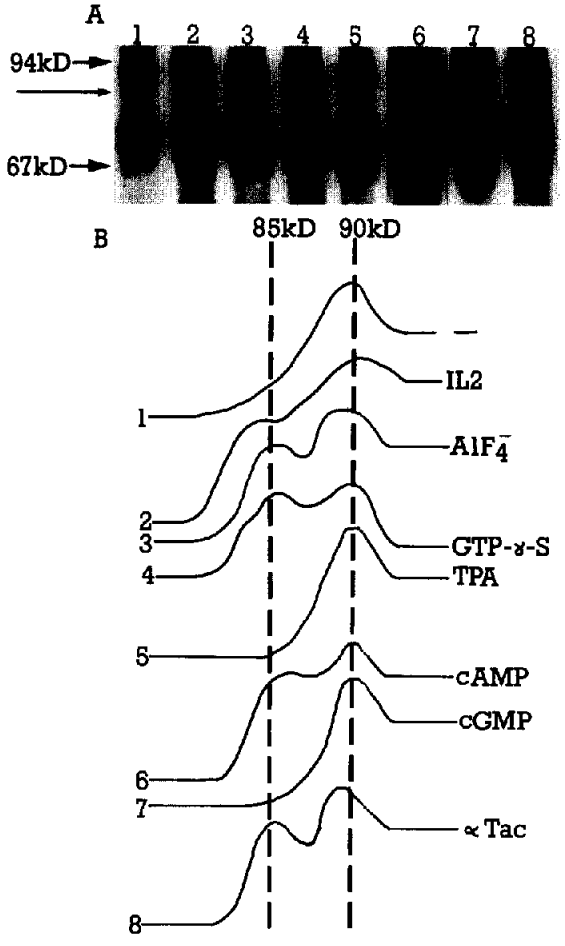

Fig.3. (A,B) Labelling of p85 by [^{32}P]ATP in response to various reagents. The final concentrations were: IL-2, 75 U·ml^{-1}; AlF_4^-, 10 mM NaF + 1 μM $AlCl_3$; GTP[S], 1 mM; TPA, 100 nM; cAMP, 1 μM; cGMP, 1 μM. Densitometric scans of each lane of the autoradiograms are shown.

using IL-2 in the presence or absence of antiTac have led to the suggestion that the only role of p55 is to co-operate with the p75 component in order to generate a high affinity IL-2 binding site and that signal transduction is mediated by p75 alone [20]. However, fibroblasts expressing p55 in the absence of p75 can respond mitogenically to IL-2 suggesting that p55 alone is capable of transducing a signal across the plasma membrane [23]. The data reported here also suggest strongly that the binding of ligands to the p55 component of the IL-2 receptor is capable of generating biochemical signals within the cell. The precise role of these signals in the complex cascades of events leading to the initiation of mitosis remain to be elucidated.

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