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GTP dependence of the transduction of mitogenic signals through the T3 complex in T lymphocytes indicates the involvement of a G-protein

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The T3 molecule on the surface membrane of T lymphocytes is involved in the transduction of the proliferation signal generated by an interaction between the antigen receptor and an antigen, to the interior of the T cell. Mitogenic monoclonal antibodies against the T3 molecule and mitogenic lectins induce a rapid (within 5 min) protein synthesis-independent activation of ornithine decarboxylase (ODC) in human T lymphocytes. When T cells are selectively depleted of guanine nucleotides by treatment with mycophenolic acid, the early mitogen-induced activation of ODC is completely inhibited. The inhibition rapidly reverted on the addition of guanine a few minutes before the mitogenic stimulation, and even more rapidly by GTP directly introduced into the T cells by a transient membrane permeabilization. GTP can be substituted for by a non-hydrolyzable GTP analogue, GTP-γ-S, which also induces ODC activity by itself in human T cells. These results suggest that a G-protein(s) is involved in the transduction of the proliferation signal in human T cells.

G-protein; Ornithine decarboxylase; Signal transduction; Lymphocyte transformation

1. INTRODUCTION

One of the earliest detectable events in T lymphocytes stimulated to proliferate by mitogenic monoclonal antibodies or lectins, is a severalfold elevation in the activity of ornithine decarboxylase (ODC) within minutes [1–4]. Non-mitogenic ligands to T lymphocytes do not induce ODC activation. The rapid induction of ODC activity by mitogens is independent of de novo protein synthesis but requires energy and an intact cytoskeleton [1]. Earlier we have shown this rapid enzyme activation to be associated with the mitogen-stimulated breakdown of phosphoinositides [3]. In many different cells the ligand-induced enhancement of the turnover of phosphoinositides is mediated by guanine nucleotide binding regulatory proteins, G-proteins [4,5]. Thus it is possible that the early activation of ODC is regulated by a G-protein.

The G-proteins, including the *ras* proto-oncogene encoded proteins, are small proteins on the cytoplasmic surface of the plasma membrane. These are involved in the transduction and regulation of various signals from the external milieu [5–8]. The G-proteins bind guanine nucleotides and display high-affinity GTPase activity [9,10]. The G-proteins are functionally active when GTP is bound. Their function is terminated when the bound GTP is hydrolyzed [6,7,11]. Accordingly, treatment with non-hydrolyzable GTP analogues retains G-proteins in a functional state

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Abbreviations: MyAc, mycophenolic acid; GTP-γ-S, guanosine-5′-(3-*O*-thio)triphosphate; ConA, concanavalin A

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[5–8,12–14], as do point mutations leading to decreased GTPase activity [15,16]. Depletion of the cellular pool of GTP has, on the other hand, been shown to impair the signal transducing capacity of G-proteins [17].

Mycophenolic acid produced by *Penicillium stoloniferum* is a potent inhibitor of guanine nucleotide synthesis in mammalian cells [18–20]. MyAc profoundly inhibits the enzyme inosine monophosphate dehydrogenase which produces the guanine nucleotide precursor xanthosine monophosphate, but does not interfere with the synthesis of other nucleotides [20]. Treatment of cells with MyAc has been shown to lower the cellular GTP content to 10–20% [17,20]. A pool of GTP with rapid turnover is apparently soon exhausted, while a pool of more tightly bound GTP is relatively spared. Thus, the RNA and protein synthesis remains intact for several hours in cells treated with MyAc [20].

2. MATERIALS AND METHODS

2.1. *Materials*

Ficoll-Isopaque was from Pharmacia (Sweden), mycophenolic acid from Sigma (FRG), all nucleotides from Boehringer Mannheim (FRG) and the radioactive compounds from Amersham (England).

2.2. *Cells*

Buffy coats of blood units were kindly provided by the Finish Red Cross Blood Transfusion Service. The mononuclear leukocytes were isolated by centrifugation on Ficoll-Isopaque. The T lymphocytes were further enriched by passage through nylon wool columns [21].

2.3. *Mycophenolic acid treatment*

The T lymphocytes were incubated for 2 h at 37°C in RPMI 1640 medium in the presence of mycophenolic acid (MyAc) to deplete the cells of guanine nucleotides. In experiments where the reversal by guanine, GTP or GTP-γ-S was examined, the cells were always preincubated for 2 h in 100 μM MyAc, prior to permeabilization and/or stimulation. In these experiments the concentration of MyAc was kept at 100 μM at all stages.

2.4. *Transient membrane permeabilization*

The membranes of T lymphocytes were transiently made permeable for small compounds such as GTP and GTP-γ-S, by the ATP^{4-} method as described by Gomperts and co-workers [8,22]. Earlier we have reported the application of this method to T lymphocytes [4].

2.5. *ODC assay*

The activity of ODC was measured by the liberation of $^{14}CO_2$ from [1-^{14}C]ornithine as described [23], in the supernatant of cell samples disrupted by sonication and cleared by centrifugation for 45 min at 42000 × *g*.

2.6. *Measurement of RNA and protein synthesis*

The incorporation of [^{3}H]uridine and [^{3}H]leucine during a 3 h incubation into trichloroacetic acid-precipitable material was measured from samples of 1×10^6 cells, cultured in RPMI 1640 medium at 37°C and harvested by a multichannel cell harvester (Scatron, Norway) after the addition of 1/10 vol. trichloroacetic acid. The radioactivity was counted in ACS scintillation fluid (Amersham, England).

3. RESULTS

When human T lymphocytes were incubated in the presence of 100 μM MyAc for 2 h, the activation of ODC normally seen within 10 min after treatment with a mitogenic ligand (the monoclonal antibody OKT3 or ConA) was abolished. This effect of MyAc was dose-dependent (fig.1). The inhibition was rapidly reversed in a dose-dependent fashion by guanine (fig.2), added a few minutes before the mitogenic stimulation (fig.3). The results from experiments using the monoclonal antibody OKT3 are shown in table 1. Guanosine also reverted the MyAc-induced inhibition of ODC activation, while all other compounds tested (adenosine, cytosine, uridine, thymidine) were without effects (not shown). Treatment of resting or mitogen-stimulated T lymphocytes with guanine alone did not alter their ODC activities (not shown).

To further demonstrate the specific requirement for GTP in the activation of ODC by mitogens, exogenous GTP was introduced directly into MyAc-treated, transiently membrane permeabilized T

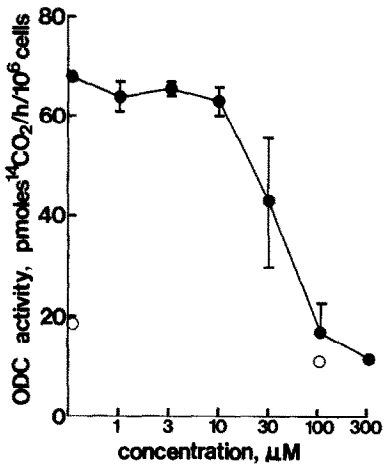

Fig.1. The effects of MyAc on the inducibility of ODC activity by ConA (●) and in unstimulated cells (○). The cells were preincubated for 2 h in the presence of the indicated concentrations of MyAc before ConA (10 μg/ml) was added. The activity of ODC was measured 10 min later and is expressed as pmol $^{14}CO_2$/h per 10^6 cells.

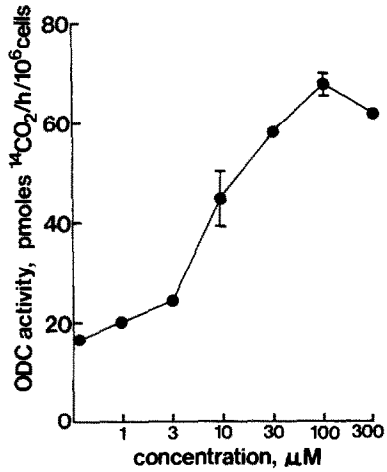

Fig.2. Dose-dependent reversal of the MyAc-induced inhibition of ODC activation by guanine. The cells were preincubated for 2 h in 100 μM MyAc and then 10 min in the presence of the indicated concentrations of guanine (MyAc still present). The activity of ODC was measured as in fig.1.

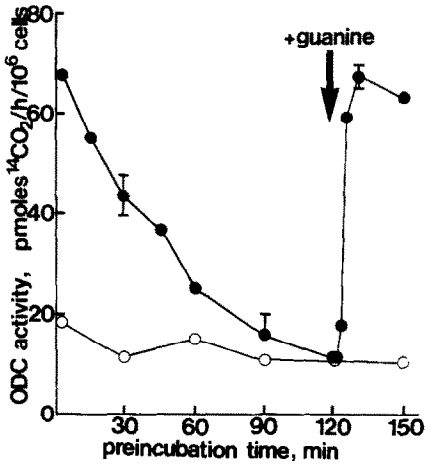

Fig.3. Time course of the MyAc-induced inhibition of the activation of ODC and reversal of the inhibition by addition of 100 μM guanine. The activity of ODC was measured after 10 min in the presence (●) or absence (○) of ConA, as in fig.1.

Table 1

The effects of MyAc and guanine on the activation of ODC by a monoclonal antibody against the T3 molecule (OKT3)

	Ornithine decarboxylase activity (pmol $^{14}CO_2$/h per 10^6 cells)
Unstimulated cells	18.1 ± 3.4
Cells treated with OKT3 (10 min)	59.0 ± 5.7
MyAc-treated cells + OKT3 (10 min)	13.3 ± 3.3
MyAc-treated cells + guanine (100 μM, 10 min) + OKT3 (10 min)	66.5 ± 7.5

The cells were preincubated for 2 h in 100 μM MyAc and 10 min after the addition of 100 μM guanine prior to stimulation with 25 ng/ml OKT3. The activity of ODC was measured 10 min later and is expressed as pmol $^{14}CO_2$/h per 10^6 cells; the data represent means ± SD, from three experiments

lymphocytes. In these cells the ODC activation was fully inducible immediately after the permeabilization step (fig.4). Approx. 5 μM GTP was needed for 50% reversal. The introduced GTP did not by itself activate ODC. When GTP-γ-S was introduced into MyAc-treated cells, an activation of ODC was observed after the addition of ConA (fig.4). ATP or the non-hydrolyzable GDP

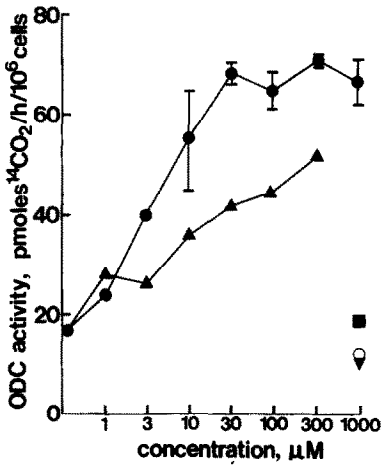

Fig.4. Reversal of the MyAc-induced inhibition of ODC activation by GTP (●,○), GTP-γ-S (▲), GDP-β-S (■) and ATP (▼). The cells were preincubated for 2 h in 100 μM MyAc, permeabilized in the presence of indicated nucleotides, resealed and treated with (filled symbols) or without (open symbols) ConA (10 μg/ml) for 10 min, as in fig.1.

Table 2

RNA and protein synthesis in human T cells pretreated with 100 μM MyAc and 100 μM guanine for 2 h

	Incorporation of	
	[^{3}H]Leucine (cpm/10^6 cells)	[^{3}H]Uridine (cpm/10^6 cells)
Untreated cells	6577 ± 785	1085 ± 404
MyAc-treated cells	6124 ± 452	1156 ± 240
MyAc-treated cells + guanine 100 μM	6510 ± 632	1492 ± 129
Cycloheximide-treated cells	4391 ± 525	not done

Cycloheximide (20 μg/ml) treated cells were used as controls

analogue GDP-β-S could not replace GTP (fig.4) and all other nucleotides tested (ATP-γ-S and dTTP) were also ineffective (not shown).

To exclude the possibility that the MyAc-induced inhibition of ODC activation was caused by significantly decreased protein or RNA synthesis during the preincubation in MyAc, incorporation of radiolabeled leucine and uridine into trichloroacetic acid-precipitable material was measured. In cells treated with MyAc for 5 h, the incorporation of these compounds during the last 3 h was only slightly decreased. In the presence of guanine this small decrease was absent (table 2).

4. DISCUSSION

Mature T cells express an idiotypic antigen receptor on their surface. When this receptor binds its specific antigen the T cell undergoes blast-transformation and starts to proliferate. Since the receptor polypeptides only have a few amino acids inside the plasma membrane, it is generally thought that the tightly associated T3 molecule is responsible for transducing the activation signal from the antigen receptor to the inside of the T cell. The mechanism and immediate target of this mitogenic signal transduction are, however, unknown.

The presented results indicate that GTP is needed for the rapid activation of ODC in human T lymphocytes by an antibody against the T3 molecule (OKT3) or the lectin ConA. This is the first demonstration of the involvement of a G-protein in the transduction of the proliferation signal from the antigen receptor-T3 complex. We have demonstrated earlier that non-hydrolyzable GTP analogues (without addition of mitogens) induce a rapid ODC activation, suggesting the participation of a G-protein [4], but it has not until now been clear which receptor is linked to this G-protein.

Pertussis and cholera toxins have been widely used in the studies of G-proteins. Unfortunately these toxins do not affect the *ras* encoded p21s. On the other hand, a selective depletion of the cellular pool of guanine nucleotides by treatment with MyAc apparently affects all G-proteins, including the p21s. The rapid reversal by guanine, guanosine or GTP further emphasizes the usefulness of MyAc in the studies of G-protein mediated events in whole cells.

Of the hitherto identified G-proteins, only the *ras*-proto-oncogene encoded proteins (p21) have clearly been implicated in the regulation of proliferation [24–28]. Several recent reports indicate that *ras* encoded proteins may be involved in the regulation of phosphatidylinositol turnover [29–31]. Sistonen et al. [32] have shown that transfection of fibroblasts with the Ha-*ras*

(mutated in codon 12) leads to high and dose-dependent ODC activities in the cells. Furthermore, the early ODC activation obtained with mitogenic ligands in human T cells is insensitive to pretreatment of the cells with either pertussis or cholera toxins (unpublished). Therefore it seems possible that the G-protein involved in the activation of ODC (by stimulating phosphatidylinositol breakdown) during the onset of proliferation of human T lymphocytes is in fact a c-*ras*-encoded p21.

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