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Interferon-mediated intracellular signalling

Modulation of different phospholipase activities in Burkitt lymphoma cells

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The effect of interferon-α on Daudi lymphoma cells either sensitive or resistant to the action of this cytokine has been analysed in terms of phospholipase C (PLC) and D (PLD) activities. Results have shown a combined modulation of PIP_2-specific phospholipase C and phospholipase D. In particular, a decreased activity of PIP_2-specific PLC has been found, concomitant to a PLD-mediated phosphatidylcholine hydrolysis, suggesting that the intracellular signalling activated by interferon in Daudi cells involves a phospholipase D/phospholydrolase pathway.

Interferon; Phospholipase; Daudi cell

1. INTRODUCTION

Molecular events controlling signal transduction generated by the interaction of different agents, like hormones or cytokines, with cell surface receptors are an intriguing aspect of the cell metabolism the knowledge of which has enormously expanded in recent years [1–5]. Among the agents able to regulate cell proliferation, type-I interferons (α and β) are cytokines known to bind to cell-surface receptors inducing resistance to the action of different viruses and producing a decrease in cell growth rate [6]. Such properties identify interferons as potential tumor suppressors. The molecular mechanism by which interferon exerts its antiviral and antiproliferative effect is, anyway, still unclear. Among the molecular changes produced by this cytokine [7–9], an early and transient rise of cellular diacylglycerol (DAG) has been reported, suggesting the possibility that the intracellular cascade of interferon-generated signals could be activated by a degradation of cell-surface phospholipids [10,11]. Recent data on cell metabolism in response to interferon-α treatment, in different cell lines have shown that the interaction of this cytokine with its cell-surface receptors produces different responses of polyphosphoinositide and diacylglycerol metabolism in the whole cells as compared to isolated nuclei [11–13]. In particular, it has been reported that the transient rise of DAG induced by interferon is not accompanied by any modifications in inositol lipid phosphorylation in the cell homogenates, whereas PIP_2-phosphorylation changes have been found to parallel the DAG increase after 90 min of interferon treatment in isolated nuclei [11]. These results allow to speculate on the existence of two distinct pathways, for cytoplasmic and nuclear compartments, involved in the interferon-mediated signal transduction into the cell. To further understand this intriguing aspect of interferon action, in this paper we have focused our attention on a possible PLD and phosphoinositide-specific PLC (phosphoinositidase) involvement in the cascade of molecular events generated at the cytoplasmic level by this cytokine in Daudi lymphoma cells. A characterized line of Daudi cells selected for resistance to the antiproliferative action of interferon-α provided controls for specificity of results.

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Abbreviations: PIP_2, phosphatidylinositol-bisphosphate; IP_3, inositol-trisphosphate; PA, phoshatidic acid; LPL, less polar lipids.

2. MATERIALS AND METHODS

2.1. *Cell culture and interferon treatment*

Daudi lymphoma cells were grown in stationary suspension culture at densities of 4×10^5 to 12×10^5/ml. RPMI 1640 medium was supplemented with 10% foetal calf serum, glutamin, penicillin–streptomycin and neomycin. Viability was determined by the Trypan blue exclusion test. Cells from Daudi interferon-resistant clones were continuously maintained in the presence of 300 IU/ml of recombinant interferon-α 2A (La Roche). The resistant phenotype was stable without any interferon treatment for up to six culture doublings and resistant cells were passaged for at least three doublings without interferon prior to use in experiments. For experiments, cells were prelabelled with [2-^{3}H]glycerol (7 μCi/ml per 10^6 cells) for 40 h. R-IFN-α A treatment of the cells (300 IU/ml) was performed for periods up to 90 min. When required, cells were incubated with 200 μM propranolol as described by Pai [14] before adding interferon.

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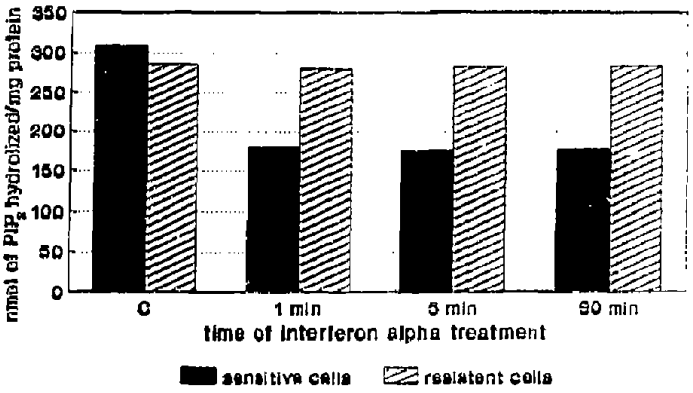

Fig. 1. nmol of [^{3}H]PIP_2 hydrolyzed in interferon-α (300 IU/ml)-treated sensitive and resistant Daudi cells. Results are the mean of 3 separate experiments ± S.D.

2.2. *Assay for PIP_2 specific PLC*

PLC activity was assayed using 3 nmol of [^{3}H]PIP_2 (90,000 dpm) as exogenous substrate, 100 μg protein as enzyme source in the presence of 0.06% taurodeoxycholate and by incubating for 30 min at 37°C. For PIP_2 hydrolysis the buffer used was 100 μM MES pH 6.2 plus 100 μM $CaCl_2$ [15].

2.3. *Lipid extraction and thin layer chromatography*

Cells (7×10^6) were processed for lipid extraction and TLC separation using the procedure of Bligh and Dyer [16]. When appropriate, a different solvent system (chloroform/methanol/acetic acid: 65:15:2, by volume) was used to separate phosphatidic acid (PA). Lipids were fluorographed at −80°C and spots corresponding to internal standards were scraped off and counted in a liquid scintillation counter.

3. RESULTS AND DISCUSSION

The effect of interferon on diacylglycerol levels in Daudi cells is reported in Table I. Since interferon produces a rapid and transient increase of this lipid, it appeared of interest to evaluate the possible involvement of PIP_2-specific phospholipase C in DAG production. This enzyme, in fact, is responsible for PIP_2 hydrolysis generating the two well-known second messengers DAG and IP_3 [1]. Fig. 1 shows the PLC activity on PIP_2 in the samples treated for up to 90 min with interferon-α with respect to the control, suggesting an inhibitory effect of this cytokine on PIP_2-specific phospholipase C activity. This result prompts the question from which phospholipid source DAG is produced. Among the cellular phospholipids believed to exert a role on cell metabolism regulation, phosphatidylcholine

Table I

Effect of recombinant interferon-α (300 IU/ml) on DAG levels in Daudi sensitive cells (dpm per 7×10^6 cells)

	Control	1 min	5 min	90 min
DAG	431 ± 16	467 ± 14	791 ± 18	501 ± 11
LPL	2316 ± 37	2431 ± 31	2471 ± 42	2936 ± 29

Data are the mean of 3 separate experiments ± S.D.

Fig. 2. Effect of propranolol on interferon induced DAG production in Daudi sensitive cells. Cells were labelled with [^{3}H]glycerol as stated in section 2. Results represent at least 3 separate experiments. Lane 1, control; lane 2, 5 min of interferon-α; lane 3, 5 min of interferon-α plus propranolol (200 μM).

(PC), the principal phospholipid class in mammalian tissues, is supposed to be involved in mechanisms which regulate signal transduction [17]. There is considerable evidence that cellular PC is a major source of DAG via phosphodiesteratic cleavage by a PLD-generating choline and PA. The latter is converted into DAG by a PA-specific phosphohydrolase [14,17,18]. Since this latter enzyme is specifically inhibited by propranolol, a compound also reported to be uneffective on phosphoinositide-specific phospholipase C [18], we have checked the effect of interferon, in the presence of this PA phosphohydrolase inhibitor, on DAG production. Results have disclosed that the rapid DAG increase, usually induced by interferon, is dramatically restored to

Table II

Effect of propranolol on PA levels in interferon-treated Daudi cells (dpm per 1×10^6 cells)

	Control	5 min IFN	5 min IFN+propranolol
PA	577 ± 13	618 ± 15	803 ± 24

Data are the mean of 3 separate experiments ± S.D.

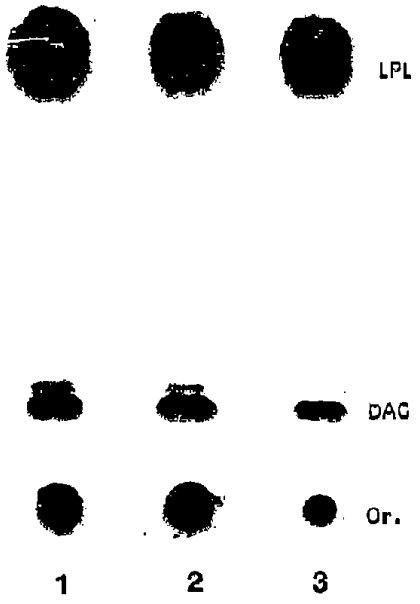

Fig. 3. Effect of propranolol on interferon-induced DAG production in Daudi resistant cells. Cells were labelled with [^{3}H]glycerol as stated in section 2. Results represent at least 3 separate experiments. Lane 1, control; lane 2, 5 min of interferon-α; lane 3, 5 min of interferon-α plus propranolol (200 μM).

control levels when cells are incubated in the presence of this compound (Fig. 2), indicating that interferon may exert its action at the cytoplasmic level through a rapid and transient hydrolysis of PC via the phospholipase D/phosphohydrolase pathway. The reduced hydrolysis of the PIP_2 fraction in the interferon-treated samples concomitant with the rise of DAG levels, allows for speculation on the possibility that inhibition of PIP_2 specific phospholipase C might be a crucial metabolic modulation required by the signalling system activated by interferon in the transduction of its molecular signals. PA accumulation, observed when propranolol is added to the system (Table II), further supports the involvement of the PLD-mediated PC hydrolysis and reasonably excludes the possibility that PC hydrolysis would occur via a non-specific PLC involvement, in which case DAG would have been produced also in the presence of propranolol. The results here reported are in accordance with a number of findings in other cell systems, in which PC hydrolysis through the phospholipase D/phosphohydrolase pathway is supposed to occur when stimulated by different external agents [17]. Interestingly, interferon does not affect both PIP_2-PLC and DAG levels in the clone of Daudi cells selected for resistance to this cytokine (Figs. 1 and 3). It must be noted, anyway, that the addition of propranolol in control cells both sensitive and resistant to interferon, results in a slight decrease in the recovery of the label in the DAG fraction (not shown), strongly indicating that the phospholipase D/phosphohydrolase pathway is likely to contribute to DAG formation also at basal levels, possibly becoming the major route involved in the intracellular transduction system activated by the interaction between interferon and its receptors in Burkitt lymphoma cells.

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