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Transient shift of diacylglycerol and inositol lipids induced by interferon in Daudi cells

Evidence for a different pattern between nuclei and intact cells

A. Cataldi[1], S. Miscia[1], R. Lisio[2], R. Rana[1] and L. Cocco[1]

[1]*Istituto di Morfologia Umana Normale, Università di Chieti* and [2]*Istituto di Citomorfologia Normale e Patologica del CNR, Chieti, Italy*

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The effect of human recombinant DNA interferon-α type A on inositol lipid and diacylglycerol metabolism was investigated in Daudi lymphoma whole cells and isolated nuclei. In isolated nuclei after 90 min of interferon treatment an enhanced rate of PIP_2 phosphorylation and an increase of DAG mass were observed. In whole cells, after 1 min of interferon treatment, there was a rapid and transient shift of DAG mass apparently not related to inositol lipid modifications, thus indicating the presence in nuclear and cytoplasmic compartments of inositol lipid fractions with different metabolic features in response to interferon-α.

Interferon; Diacylglycerol; Inositol lipid; Daudi cell

1. INTRODUCTION

Investigations of the response of cell metabolism to interactions between external agents and cell surface receptors have shown the hydrolysis of a membrane bound PIP_2, which produces at least two second messengers: DAG and IP_3. This pathway seems to regulate the transduction mechanism which controls many cellular processes such as metabolism, secretion, contraction, neural activity and cell proliferation [1].

Recently, the presence of these molecules in the nucleus showing a different metabolic behaviour in response to external stimuli compared to cytoplasmic polyphosphoinositides, has suggested a possible role of nuclear inositol lipids in the transduction to the nucleus of stimuli-generated signals [2-6].

Among the agents reported to be able to modulate cell response, interferon has been largely investigated in terms of antiviral and antiproliferative activity [7]. In particular, a number of results have demonstrated that the r-IFN-α A antiproliferative effect is accompanied by modifications of nucleic acid and nuclear phospholipid metabolism [8,9] in Daudi lymphoma cells. Indeed these cells are extremely sensitive to human α-interferons which progressively inhibit cell proliferation over 1-4 days when added in culture [10]. In our experiments we have tested the effect of r-IFN-α A action, both at cytoplasmic and nuclear levels, in Daudi lymphoma cells on the metabolism of inositol lipids and DAG.

Correspondence address: L. Cocco, Istituto di Anatomia Umana Normale, Via Irnerio 48, 40126 Bologna, Italy

Abbreviations: DAG, diacylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5 trisphosphate; rIFNA, recombinant DNA human interferon A; PMSF, phenylmethanesulfonylfluoride; TLC, thin layer chromatography; LPL, less polar lipids

2. MATERIALS AND METHODS

2.1. *Cell culture and interferon treatment*

Daudi cells were grown in stationary suspension culture at densities of 5×10^5 to 15×10^5/ml and viability was determined by trypan blue exclusion test. Cells were prelabelled with [2-^{3}H]glycerol (5 μCi/ml per 10^6 cells) for 40 h. Cells were treated with r-IFN-α A (100 IU/ml) for times up to 24 h.

2.2. *Isolation of nuclei*

Cells were resuspended in a buffer containing 2 mM $MgCl_2$, 10 mM Tris HCl, pH 7.4, 0.1 mM PMSF and 0.6% Triton X-100. The suspension was filtered 4 times through a syringe and then $MgCl_2$ concentration was brought to 5 mM.

2.3. *Phosphorylation of whole cell homogenates and isolated nuclei*

The standard phosphorylation mixture (100 μl) contained 360 μg of nuclear protein (or 180 μg of total homogenate protein), 0.32 M sucrose, 5 mM β-mercaptoethanol, 5 mM $MgCl_2$, 10 mM Tris-Cl, pH 8.0, 10 μM ATP, and 25 μCi of [γ-^{32}P]ATP (5000 Ci/mmol, Amersham, UK). Incubation was 4 min at 30°C and the reaction was stopped by addition of trichloroacetic acid to a final concentration of 10% (v/v). The pellets were washed twice with distilled water and extracted with acidic chloroform-methanol and phase separation and washes were exactly as in Dawson and Eichberg [11]. DAG and inositol lipids were separated by TLC as previously described [11,12] and respectively fluorographed at -80°C and autoradiographed at room temperature. Spots corresponding to internal standards were scraped off and then counted in a liquid scintillation counter.

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3. RESULTS AND DISCUSSION

The fluorographic analysis of nuclear lipids has evidenced, respect to controls, a progressive increase of ^{3}H-glycerol incorporation in DAG fraction up to 90 min of r-IFN A treatment followed by a progressive restoration to control levels within 24 h (Fig. 1a). A different DAG profile has been found in the analysis carried out on intact cells. In the latter, in fact, the incorporation of ^{3}H precursor reaches the maximal level within 1 min after interferon treatment decreasing to control values at 24 h (Fig. 1b). The percent values of the counts obtained from the scraping of TLC spots, are reported in Table I. The values point out once more the difference in the profile of DAG between isolated nuclei and intact cells. The labelling time we have used and the early response evidenced in intact cells, as well as in purified nuclei, suggest that labelling equilibrium has been reached so that the modifications reported could be ascribed to actual changes of DAG mass. Interestingly, the changes occurring in the nuclei isolated from cells treated with interferon for 90 min are accompanied by modifications of in vitro γ-^{32}P incorporation from ATP into nuclear phosphoinositides and specifically into PIP_2. As shown in Table II, the in vitro incorporation of [γ-^{32}P]ATP into this fraction is increased by 50% with respect to controls. This suggests that PIP_2 could be the target of the interferon generated signal at the nuclear level and consequently that the increased hydrolysis of this fraction at this time of treatment may represent a crucial event for the generation of DAG. This possibility is supported by the evidence that no modification is detectable at 24 h after interferon treatment, neither in nuclear DAG nor in PIP_2 profile. The [γ^{32}P]ATP incorporation in polyphosphoinositides from whole cells (Fig. 2b; Table II) failed to reveal any changes of PIP_2 profile within 1 min after interferon treatment suggesting different metabolic features in the response to interferon stimuli with respect to the nuclear compartment. The shift of DAG fraction in the intact cells is in accordance with data reported by other authors [13] concerning an early and transient increase of DAG in Daudi cells by 30 s after human interferon treatment. Moreover, the modifications of DAG metabolism within 90 min, observed in isolated nuclei, parallel a number of findings previously reported concerning transient changes of nucleic acid biosynthesis and phospholipid profile in nuclei isolated from Daudi cells treated for up to 24 h with α-interferon. In particular a progressive decrease of nuclear ^{3}H-UMP incorporation up to 90 min of r-IFN-α A treatment has been found followed by a restoration which reaches

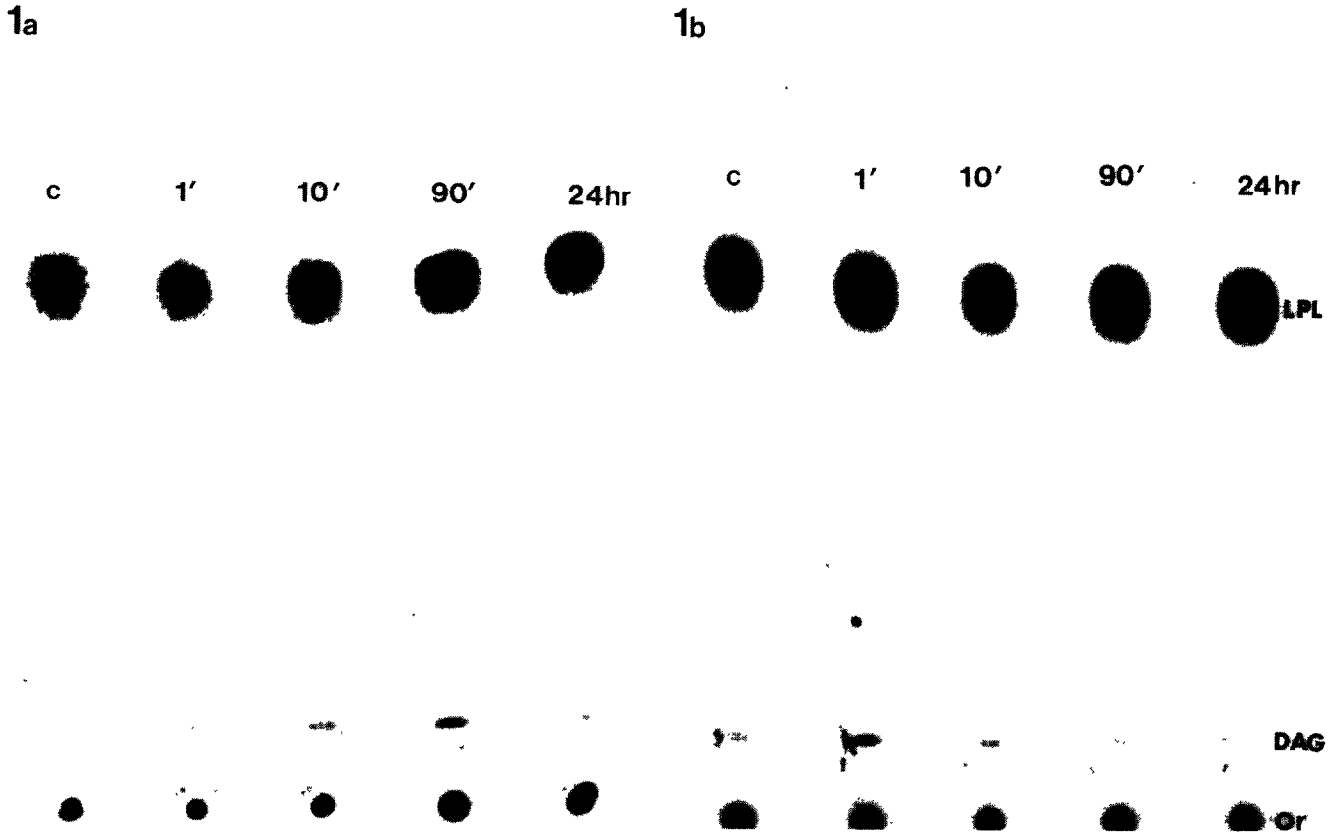

Fig. 1. (a) [^{3}H]glycerol incorporation into DAG in nuclei isolated from Daudi cells treated with r-IFN-α A for 1 min, 10 min, 90 min and 24 h. C, control. (b) [^{3}H]glycerol incorporation into DAG in intact Daudi cells treated with r-IFN-α A for 1 min, 10 min, 90 min and 24 h. C = control.

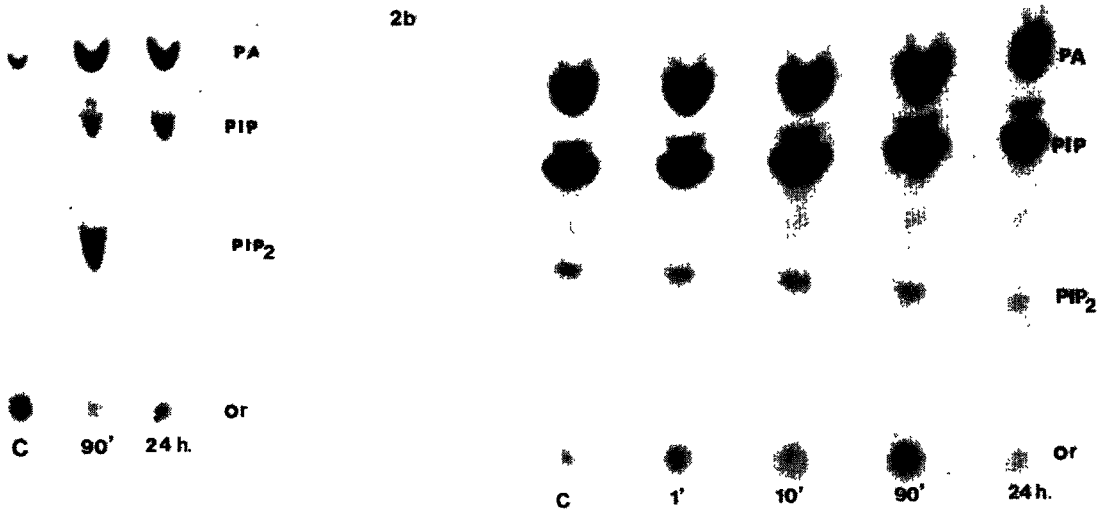

Fig. 2. (a) [γ-^{32}P]ATP phosphorylation in nuclei isolated from Daudi cells treated with r-IFN-α A for 90 min and 24 h. C = control. (b) [γ-^{32}P]ATP phosphorylation in Daudi cells treated with r-IFN-α A for 1 min 10 min, 90 min and 24 h. C = control.

Table I

Percentage of diacylglycerol fraction referred to total lipid extracts*

Time of r-IFN A treatment	Intact cells (% of total cpm)	Nuclei (% of total cpm)
C	6.1 ± 0.8	5.1 ± 0.7
1 min	12.1 ± 0.9	5.8 ± 0.6
10 min	8.2 ± 0.8	5.9 ± 0.6
90 min	5.5 ± 0.6	9.8 ± 0.8
24 h	5.3 ± 0.7	5.4 ± 0.6

* Total counts per 1×10^8 cells = 136 000 cpm. Total counts per 2×10^8 nuclei = 56 000 cpm. The data are the average of 5 separate experiments ± SD

control values within 24 h [14]. These modifications might be related to receptor down regulation mechanisms. It might be possible that the cells undergo the effects of a transient initiating signal induced by the interaction between surface receptors and interferon. The simultaneous changes of polyphosphoinositide and DAG profiles in the nucleus, with different features compared to cytoplasm, allows one to speculate on a possible involvement of nuclear lipid fraction in the regulation of cell metabolism [14]. Moreover, the modifications induced by interferon on the metabolism

Table II

Percentage of in vitro [γ-^{32}P]ATP incorporation into phosphoinositides in Daudi intact cells and isolated nuclei

	time of rIFN A treatment				
	C	1′	10′	90′	24h
Nuclei					
PIP_2	20.5 ± 1.2	18.4 ± 0.8	22.4 ± 0.7	31.1 ± 1.1	16.8 ± 1.2
PIP	49.4 ± 0.8	48.8 ± 1.0	45.0 ± 0.6	35.7 ± 0.8	46.5 ± 0.9
PA	30.1 ± 1.1	32.7 ± 0.9	32.5 ± 0.8	33.1 ± 0.6	35.6 ± 1.2
Intact cells					
PIP_2	5.8 ± 0.4	5.2 ± 0.3	4.6 ± 0.3	4.8 ± 0.2	6.2 ± 0.6
PIP	54.7 ± 0.9	51.1 ± 0.8	52.1 ± 0.6	59.1 ± 1.1	48.2 ± 0.8
PA	39.4 ± 0.8	40.2 ± 0.7	43.4 ± 1.0	35.5 ± 0.9	45.3 ± 1.0

Total counts per 1×10^8 cells = 185 000 cpm. Total counts per 2×10^8 nuclei = 74 000 cpm. The data are the average of 5 separate experiments ± SD. Valves are % of total cpm

of nuclear inositol lipids are supported by results obtained in other experimental systems in which an agonist induced hydrolysis of PIP and PIP_2 at nuclear level has been shown [14]. In conclusion, these results are in agreement with previously reported data on the presence of inositol lipids in the nuclear compartment with a possible role in the modulation of intranuclear signalling [2–6].

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