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INTERFERON INHIBITION OF HEXOSE MONOPHOSPHATE SHUNT ACTIVITY AS THE MECHANISM OF BLOCKING DIFFERENTIATION OF A PREADIPOSE CELL LINE

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SUMMARY: Mouse L cell interferon (IFN α/β) inhibited the differentiation of mouse 3T3-Li fibroblasts into adipocytes. IFN α/β also inhibited hexose monophosphate shunt (HMP) activity in these cells. HMP activity is required for the reducing power necessary for the conversion of 3T3-Li fibroblasts to adipocytes. Both IFN blockage of differentiation and HMP activity were reversed by the reducing agent 2-mercaptoethanol, probably through interaction with membrane receptors and not through direct inactivation of IFN. Several non-antiviral effects of IFN α/β on cellular function, including differentiation and immunoregulation, may be mediated at the biochemical level through blockage of HMP activity.

The biochemical events that are modulated by interferon (IFN), which are expressed in the form of antiviral, anticellular, immunoregulatory, and antitumor activities, remain unclear. Some of these effects of IFN have been thought to be due to the inhibition of protein synthesis via the induction of a ribosome-associated cyclic AMP-independent, double-stranded RNA-dependent protein kinase that phosphorylates peptide initiator factor eIF-2α (1-5) and/or to activation of an RNA endonuclease via induction of a 2'-5'-oligoadenylate synthetase (2,3,6). Recent findings suggest that induction of the above enzyme activities is not sufficient to explain IFN's pleiotropic effects on cellular functions (7-9). We have recently shown that virus-induced mouse L cell IFN (IFN α/β) alters intermediary metabolism in that it inhibits the hexose monophosphate shunt (HMP) pathway in endotoxin-stimulated mouse spleen cells while not affecting the glycolytic or citric acid pathways (10). Concurrently, others showed that IFN inhibited the differentiation of mouse 3T3-Li

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fibroblasts into adipocytes (11,12), which requires HMP activity (13). It was of interest, therefore, to look at the effect of mouse IFN α/β on both HMP activity and fibroblast → adipocyte differentiation in 3T3-Li cells, since information from such a study could provide an opportunity for determining the relationship of a specific biochemical effect of IFN and a specific cellular effect.

MATERIALS AND METHODS

Reagents. IFN α/β, a mixture of alpha and beta interferons, was produced from mouse L cells using Newcastle disease virus as inducer (10). IFN α/β was purified up to 10^8 units/mg protein (14) and was shown to be responsible for the inhibition of 3T3-Li conversion to adipocytes by copurification of the inhibitor activity with IFN α/β and by neutralization of the activity with antibodies specific to IFN α/β. This confirms previous reports that IFN α/β blocks 3T3-Li differentiation (11,12). Reagent grade 2-mercaptoethanol (2-ME) was obtained from Eastman Kodak.

Cell culture. Mouse 3T3-Li fibroblasts (preadipocytes) were the gift of Howard Green, Massachusetts Institute of Technology (15). Cells were grown in complete medium which consisted of Dulbecco's modified Eagle's medium containing 10% donor calf serum, 100 units/ml penicillin, and 100 ug/ml streptomycin. Media were changed every 2 days in maintaining stock cultures. Subcultures were prepared for differentiation experiments from subconfluent stock cultures. The cells were dislodged by incubation (5 min. at 37°C) in Hepes-buffered saline, pH 7.4, containing 1.0 mM EDTA. These cells were diluted in complete medium and seeded at 5×10^5 cells/25 cm^2 in Falcon flasks for differentiation experiments and in Warburg flasks for determination of hexose monophosphate shunt activity.

3T3-Li differentiation. After 24 hr in 25 cm^2 Falcon flasks in complete medium, differentiation media were added to enhance conversion of fibroblasts to adipocytes. Differentiation media consisted of complete media plus 8 ug/ml d-biotin, 10 ug/ml insulin, and 1 mg/ml glucose (15). Treatment of differentiating 3T3-Li cells with IFN α/β and the reducing agent 2-ME was done as indicated in the RESULTS AND DISCUSSION. Differentiation media containing the appropriate treatment agent was changed every 2 days. The number of lipid-positive cells was determined at the time of media change by microscopic observation without staining. Each point was the average of the number of lipid-positive cells in three 4 mm^2 fields from duplicate flasks. The standard deviation was less than 10% of the mean.

Hexose monophosphate shunt (HMP) activity. Cells at various stages of differentiation were seeded in Warburg flasks as described for Falcon flasks. After 24 hr, differentiation media were removed and cells were washed twice with 2 ml of Hank's balanced salt solution (HBSS). Two ml of HBSS without glucose and containing 10% fetal calf serum, 10 ug/ml insulin, and appropriate additional reagents were added to each flask. Radioactivity was then added at a final concentration of 0.045 uCi (1.5×10^{-5} mmoles) glucose-1-^{14}C. Glucose-6-^{14}C, 0.075 uCi (2.5×10^{-5} mmoles), was added to separate flasks on a selective basis in order to monitor $^{14}CO_2$ release from glucose via the glycolytic and citric acid pathways (10). Only C-1 of glucose is decarboxylated by the HMP pathway, whereas C-1 and C-6 are equally decarboxylated when glucose is oxidized via the glycolytic and citric acid pathways (13). The center well of each Warburg flask was filled with 150 ul of 2.5 N NaOH. Subsequently each flask was tightly sealed with parafilm and incubated for 2 hr at 37°C, after which unlabeled glucose, 5 mmoles final concentration, was added to each flask. The flasks were resealed and incubated as before for 16 to 18 hr. Trichloroacetic acid, 0.1 ml of a 100% solution, was added to each flask, followed by resealing and incubation for 1 hr to ensure maximum $^{14}CO_2$

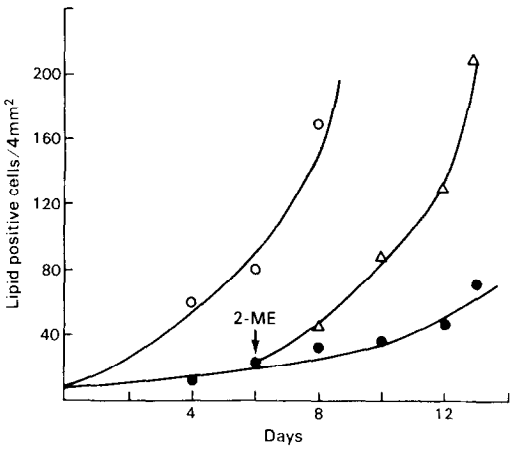

Figure 1. 3T3-Li fibroblasts differentiation to adipocytes. Confluent monolayers were incubated with differentiation media alone (O), differentiation media containing 1000 units IFN α/β/ml (●), or differentiation media containing 1000 units IFN α/β/ml and to which 2-ME ($5x10^{-5}$M) was added on day 6 (Δ). Differentiation media were changed every 2 days. IFN α/β and 2-ME were added to cells in the media.

absorption. Seventy-five ul of the NaOH solution were removed from each flask and spotted onto Gelman glass fiber filter paper discs (Type A-E). The dried paper discs were placed in scintillation counting fluid (Scinti Verse, Fisher Scientific Co.) and counted in a Beckman LS-9000 scintillation counter.

RESULTS AND DISCUSSION

Conversion of a confluent monolayer of 3T3-Li cells to adipocytes in the presence of differentiation media is demonstrated in Figure 1 in a representative experiment. IFN α/β blocked the differentiation. The reducing agent 2-ME, at $5x10^{-5}$ M, completely reversed the IFN α/β induced blockage. A 2-ME control did not show enhanced differentiation, so this is not the mechanism by which the IFN α/β effect was reversed. We have previously shown that 2-ME blocked IFN α/β induced suppression of the *in vitro* antibody response by mouse spleen cells (16). This blockage was due to an interaction of 2-ME with the spleen cells and not to inactivation of soluble IFN α/β, since IFN α/β does not lose antiviral activity against virus infected L cells in the presence of $5x10^{-5}$ M 2-ME (16). Similarly, IFN α/β was not affected by 2-ME in inhibiting mengo virus replication in 3T3-Li adipocytes (data not shown). Thus, 2-ME exerted its effects by acting on the 3T3-Li cells and not on IFN α/β. The blockage of 3T3-Li conversion to adipocytes and reversal by 2-ME can be visualized in Figure 2.

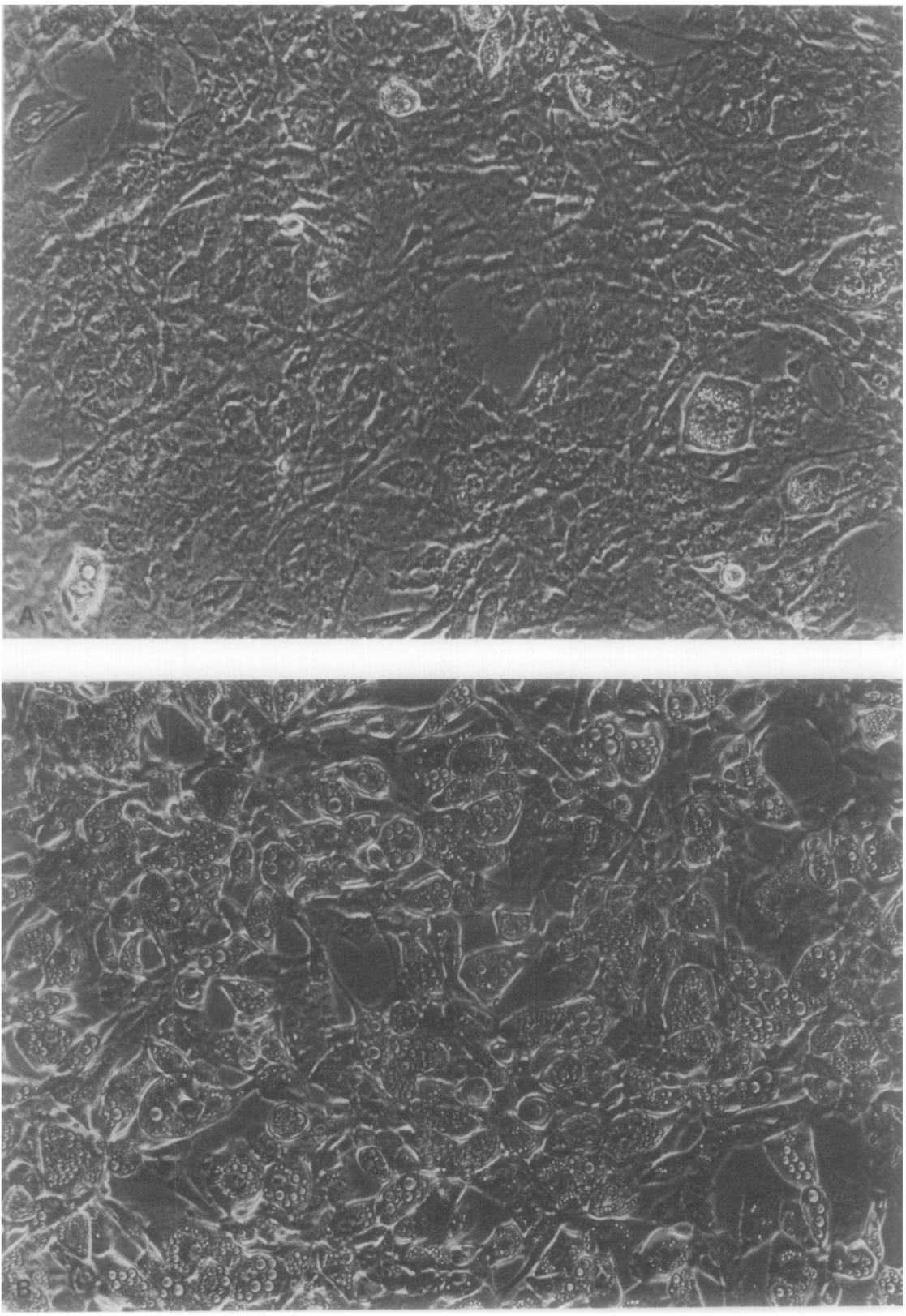
A
B

Table 1

The effect of IFN α/β on HMP activity in 3T3-Li cells

Treatment[a]	Days exposed to differentiation media	CPM (Mean±SD)[b]	Inhibition (% control)	P-value (n=6)
IFN α/β	0	5499±1489	12	NS
None	0	6296±1505		
IFN α/β	4	9122±3000	29	≤0.05
IFN α/β + 2-ME	4	12573±1143	1	NS
None	4	12738± 900		

[a] IFN α/β added to cultures at 1000 units/ml. 2-ME added to cultures at 5×10^{-5} M. Both reagents were incorporated into the differentiation media.

[b] $^{14}CO_2$ from glucose-1-^{14}C oxidation by 3T3-Li cells.

We have recently shown that IFN α/β alters intermediary metabolism in that it inhibits the HMP pathway in endotoxin-stimulated mouse spleen cells while not affecting the glycolytic or citric acid pathways (10). HMP activity is required for the production of the NADPH necessary for the conversion of 3T3-Li fibroblasts to adipocytes (13,15). We determined if IFN α/β could block HMP activity in differentiating 3T3-Li cells, and if 2-ME could reverse the effect of IFN α/β. IFN α/β did not significantly inhibit $^{14}CO_2$ production from cells in the fibroblast state at day 0 in culture prior to addition of differentiation media, while $^{14}CO_2$ production in converting cells at day 4 in differentiation media was significantly inhibited by IFN α/β (Table 1). The latter cells were active in HMP activity based on comparison of $^{14}CO_2$ release by glucose-1-^{14}C and glucose-6-^{14}C, while the former were not. 2-ME completely reversed the effect of IFN α/β. Thus, IFN α/β was capable of blocking HMP activity in 3T3-Li adipocytes and the reducing agent 2-ME was capable of reversing this block. The data are consistent with those on the effect of IFN α/β on HMP activity in endotoxin-stimulated spleen cells (10). 2-ME did not initiate or enhance HMP activity in either 3T3-Li preadipocytes, adipocytes, or mouse spleen cells, so its reversal of IFN α/β inhibition was not due to its possible HMP enhancing activity.

Figure 2. Blockage of 3T3-Li fibroblasts differentiation to adipocytes by IFN α/β and reversal by 2-ME. (A) IFN α/β; (B) IFN α/β and 2-ME. Magnification was 90X.

We have shown here that IFN α/β inhibition of differentiation of 3T3-Li fibroblasts into adipocytes may be due to IFN blockage of HMP activity in these cells. Both the blockage of differentiation and HMP activity are reversed by the reducing agent 2-ME. 2-ME does not exert its effect by direct interaction with IFN α/β, but could act through interaction with receptor-associated disulfide bonds. Breakage of such bonds could reverse or "neutralize" IFN-induced allosteric changes that may be responsible for inhibition of HMP activity. IFN α/β induction of the antiviral state is not due to inhibition of HMP activity, since reversal of inhibition by 2-ME does not negate the establishment of the antiviral state. The data presented here and elsewhere on IFN-induced immunosuppression (16) suggest that IFN α/β may exert some of its non-antiviral effects on cellular function via regulation of HMP activity.

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