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Signal transduction pathways in the induction of 2′,5′-oligoadenylate synthetase gene expression by interferon α/β

[calcium/protein kinase A and C activators/poly(ADP)-ribose synthetase inhibitor/cell growth factors/quiescent mouse fibroblasts]

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ABSTRACT **Treatment of quiescent BALB/c mouse 3T3 cells with murine interferon α/β (IFN-α/β) (1000 units/ml) leads to the appearance at 4 hr of 1.7-kilobase 2′,5′-oligoadenylate (2′,5′-OAS) mRNA as detected by Northern blot analysis. This mRNA accumulates for at least 18 hr. Two protein kinase C activators, 1,2-dioctanoyl glycerol and phorbol 12-myristate 13-acetate, suppress, whereas the calcium ionophore ionomycin enhances, the IFN-α/β-induced expression of 2′,5′-OAS mRNA. The 8-bromo and dibutyryl analogs of cAMP and the adenylate cyclase activator forskolin did not affect the induction of 2′,5′-OAS mRNA by IFN-α/β. In the absence of IFN-α/β, the above agents used either singly or in combinations, did not induce 2′,5′-OAS mRNA expression nor did platelet-derived growth factor (1–2 units/ml), fibroblast growth factor (6–100 ng/ml), or bovine serum (10–20%). Bovine serum also did not affect 2′,5′-OAS mRNA induction by IFN-α/β. The poly(ADP)-ribose synthetase inhibitor 3-aminobenzamide suppressed IFN-α/β-induced 2′,5′-OAS gene expression. These results suggest that in quiescent BALB/c 3T3 cells (*i*) the 2′,5′-OAS gene is not responsive to the three major signal transduction pathways activated by diacylglycerol, Ca^{2+}, and cAMP; (*ii*) induction of the 2′,5′-OAS gene by IFN-α/β is decreased by activation of the protein kinase C pathway but enhanced by elevation of intracellular $[Ca^{2+}]$.**

Although much has been learned about interferons (IFNs) α and β as inducible cellular regulatory molecules, the pathways whereby these IFNs signal the cell have not been firmly established (reviewed in ref. 1). IFN-α and -β appear to share a common cell-surface receptor to which they bind with high affinity (2, 3). An early response of the cell to IFN-α and -β is the activation of transcription from a group of genes located on at least three different chromosomes (reviewed in refs. 4 and 5; see refs. 6–11). As in other gene systems, the inducibility of IFN-responsive genes is determined largely by 5′-flanking genomic DNA sequences (7, 8).

All types of IFNs (α, β, and γ) have been shown to induce the gene(s) for the double-stranded RNA-dependent enzyme 2′,5′-oligoadenylate synthetase (2′,5′-OAS) and multiple forms of this enzyme have been described (9–11). 2′,5′-OAS, through its 5′-phosphorylated products, plays a key role in the inhibition of picornavirus replication by IFN (12–14). The broader role of 2′,5′-OAS in the regulation of cell proliferation, which may involve 2′,5′-oligoadenylates that are not 5′-phosphorylated, is under continuing investigation (ref. 15; reviewed in refs. 1 and 16).

In human cells, only one 2′,5′-OAS gene has been identified thus far; however, differential splicing produces two mRNAs, which differ only in their 3′ ends. In murine cells, multiple genes encode this enzyme. The 5′-flanking regions of the 2′,5′-OAS genes in both species contain two highly conserved DNA elements (A and B) that act as transcriptional enhancers and appear to perform different functions. In human cells as well as mouse cells, element A appears to function as a constitutive enhancer and element B (also designated E-IRS) seems to be the specific target of IFN-β_1 action (17).

We asked whether activation of one or more of the three major cellular signal transduction pathways would lead to induction of the 2′,5′-OAS gene in quiescent murine 3T3 fibroblasts. In such cells, the c-*fos* and c-*myc* genes can be stimulated via each of the three pathways (18–23), which involve Ca^{2+}, diacylglycerol, and cAMP as second messengers (reviewed in ref. 24; see refs. 25–28). We have used the ionophore ionomycin to activate Ca^{2+}-dependent mechanisms; 1,2-dioctanoylglycerol (DiC_8) and phorbol 12-myristate 13-acetate (PMA) to activate diacylglycerol-dependent protein kinase C; and 8-bromo-cAMP, dibutyryl-cAMP, and forskolin to activate protein kinase A. We have also investigated the inductive effects of combinations of second messenger reagents and the question of whether these reagents affect the induction of the 2′,5′-OAS gene by IFN. We report that none of the second messenger reagents used was capable, alone or in combination with others, to induce detectable levels of 2′,5′-OAS mRNA in BALB/c 3T3 cells, but that ionomycin enhanced and DiC_8 and PMA suppressed induction of this gene by IFN-α/β.

MATERIALS AND METHODS

Cells and Reagents. BALB/c mouse 3T3 (clone A31) cells were obtained from W. J. Pledger (Vanderbilt University, Nashville, TN). Mouse IFN-α/β (specific activity, 1.8×10^8 units per mg of protein) was purchased from Lee Biomolecular Research Laboratories (San Diego, CA) and platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) were from Collaborative Research. DiC_8 was purchased from Avanti Polar Lipids; ionomycin and forskolin were purchased from Calbiochem; and arachidonic acid, 8-bromo-cAMP, and dibutyryl-cAMP were from Sigma. 3-Aminobenzamide was purchased from Aldrich. Human glyceraldehyde-3-phosphate dehydrogenase cDNA was obtained from American Type Culture Collection. 2′,5′-OAS cDNA (L3 clone) was a kind gift from Judith Chebath and Michel Revel (Weizmann Institute, Rehovot, Israel).

Cell Culture. Frozen cells (in 10% dimethyl sulfoxide in liquid nitrogen) were thawed and seeded in dishes or flasks at a density of 2.3×10^3 cells per cm^2 in Dulbecco's medium supplemented with 10% calf serum (GIBCO). Passages were carried out when cells reached ≈60% confluence, and the cells were discarded after 6–8 weeks of serial passages. For

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Abbreviations: IFN, interferon; 2′,5′-OAS, 2′,5′-oligoadenylate synthetase; DiC_8, 1,2-dioctanoylglycerol; PMA, phorbol 12-myristate 13-acetate; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor.

experiments, BALB/c 3T3 cells were seeded at a density of 5×10^3 cells per cm^2 in T-175 flasks or 150-mm Petri dishes in 30 ml of Dulbecco's medium with 10% calf serum. The cultures were incubated at 37°C for 3 or more days until they had reached confluence (5×10^4 cells per cm^2). The medium was then replaced with fresh serum-free Dulbecco's medium containing test reagents, and incubation continued at 37°C for desired periods.

mRNA Extraction and Isolation and Northern Blot Analysis. For hot-phenol extraction (29, 30), supernatants from trypsinized Dounce homogenized cells were used. For guanidine thiocyanate extraction (31), cell monolayers were used directly. Procedures for isolation of poly(A)$^+$ mRNA, electrophoresis in 1% agarose gels containing 10 mM methylmercury hydroxide, transfer to aminobenzoyloxymethyl cellulose paper (ABM paper; Schleicher & Schuell), and hybridization with cDNA probes have been described (29, 30). Autoradiography was carried out for 1–4 days at −70°C using XAR-5 film and Quanta III Dupont intensifying screens. The autoradiograms were scanned with the LKB Ultroscan XL laser densitometer.

Probes. All probes were prepared by random-primed labeling of DNA fragments using the Klenow fragment and [α-^{32}P]dCTP. The 2′,5′-OAS cDNA probe was linearized by *Bgl* I digestion of clone L3 (32). The glyceraldehyde-3-phosphate dehydrogenase probe was prepared by *Hin*dIII/*Xba* I digestion of human glyceraldehyde-3-phosphate dehydrogenase plasmid (33) and the 546-base-pair fragment was separated by agarose gel electrophoresis and then isolated from the gel using an NA-45 DEAE membrane followed by elution in a buffer consisting of 10 mM Tris·HCl (pH 8.0), 1 mM EDTA, and 1 M NaCl according to the supplier's recommendations (Schleicher & Schuell).

RESULTS

Induction of 2′,5′-OAS Gene Expression by IFN-α/β. In initial experiments, quiescent BALB/c 3T3 cells were treated with IFN-α/β (1000 units/ml) for various periods to establish the time course of accumulation of IFN-induced 2′,5′-OAS mRNA. Fig. 1 shows that two mRNA signals were detected. The 1.7-kilobase (kb) mRNA was first detected 4 hr after beginning of treatment with IFN-α/β, and it accumulated for at least 18 hr. A 5-kb RNA was also first detected at 4 hr. In subsequent experiments, we mainly focus on the 1.7-kb mRNA.

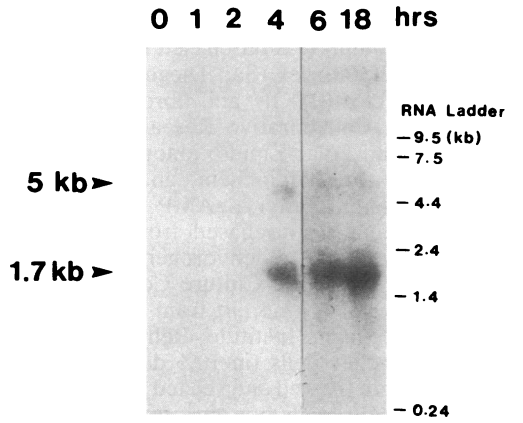

FIG. 1. Time course of accumulation of 2′,5′-OAS mRNA in IFN-α/β-treated BALB/c 3T3 cells. Cells (8×10^7) in confluent cultures were treated with IFN-α/β at 1000 units/ml for 0, 1, 2, 4, 6, and 18 hr. The procedures for cell harvest, mRNA isolation, agarose gel electrophoresis, mRNA transfer, and hybridization are described in *Materials and Methods*. A ladder of single-stranded RNA molecular size markers was electrophoresed in parallel. Arrowheads point to two 2′,5′-OAS mRNA signals.

Effects of Protein Kinase C Activation and Intracellular [Ca^{2+}] Elevation. In quiescent BALB/c 3T3 cells, 150 μM DiC_8 did not induce 2′,5′-OAS mRNA in detectable amounts. Moreover, 150 μM DiC_8 suppressed the induction of 1.7-kb 2′,5′-OAS mRNA by IFN-α/β (1000 units/ml) by at least two-thirds (Fig. 2). These results suggest not only that the diacylglycerol–protein kinase C pathway may not serve in the transduction of the IFN signal, but that its activation can have a negative effect on the induction of 2′,5′-OAS gene expression by IFN in the mouse BALB/c 3T3 cell line.

Similar results were obtained with PMA, which did not induce 2′,5′-OAS mRNA when used at either 16 or 150 nM (data not shown). Also, as shown in Fig. 3, 16 nM PMA suppressed the IFN-induced 2′,5′-OAS mRNA expression by ≈50%; however, increasing PMA concentration to 50 or 150 nM did not cause a further reduction in 1.7-kb 2′,5′-OAS mRNA expression. Thus, the maximal suppressive effect of PMA was less than that of 150 μM DiC_8.

In contrast, 100 nM ionomycin increased the IFN-α/β-induced expression of the 1.7-kb 2′,5′-OAS mRNA in quiescent BALB/c 3T3 cells, although by itself ionomycin, like DiC_8 and PMA, did not activate 2′,5′-OAS mRNA expression (Fig. 2). Among the concentrations of ionomycin tested, 100 nM gave the highest enhancing effect on IFN-α/β-induced 2′,5′-OAS mRNA expression, with 1600 nM ionomycin causing a suppression (Fig. 4). Our [^{35}S]methionine labeling studies have shown that ionomycin markedly stimulates incorporation into two specific proteins of approximately 31 and 75 kDa (I.T. and T. Kikuchi, unpublished data).

Arachidonic acid (25 μM) did not cause a detectable induction of 2′,5′-OAS mRNA (Fig. 2 *Left*). In a separate experiment, prostaglandin E_1 (30 μM) also did not cause detectable induction of this mRNA, and the 1.7-kb mRNA signal was weaker and the 5-kb signal was absent in cells treated with IFN-α/β (1000 units/ml) and prostaglandin E_1 (30 μM).

Lack of Effect of Protein Kinase A Activation. To investigate the response of the 2′,5′-OAS gene to activation of the cAMP second messenger pathway, we used two cAMP analogs (8-bromo-cAMP, 1.45 mM; and dibutyryl-cAMP, 1.22 mM) and an adenylate cyclase activator (forskolin, 1 μM). Table 1 shows that these agents used alone did not induce 2′,5′-OAS gene expression in quiescent BALB/c 3T3 cells nor did they either increase or decrease the IFN-α/β-induced expression of this gene. As shown in Table 2, combinations of ionomycin and DiC_8; ionomycin and forskolin; DiC_8 and forskolin; and ionomycin, forskolin, and DiC_8 also did not induce 2′,5′-OAS gene expression in quiescent BALB/c 3T3 cells.

Lack of Effect of PDGF and FGF. It has been reported that PDGF at very high concentration (200 units/ml) induces 2′,5′-OAS gene expression upon 8-hr exposure of quiescent cells to the growth factor (34). The physiological significance of this finding is not clear, as PDGF was used at a much higher concentration than required to render the quiescent cells competent to respond to additional cell growth factors that mediate traverse of the cells through the G_1 phase (35). In our experiments, activation of the cell cycle in 50% or more of cells in quiescent cultures with PDGF at 1–2 units/ml or with 10–20% calf serum was not associated with detectable expression of the 2′,5′-OAS gene expression 4 and 16 hr later (data not shown). Induction of the 1.7-kb 2′,5′-OAS mRNA by IFN-α/β (1000 units/ml) in quiescent cells activated with 10% or 20% calf serum was indistinguishable from that observed in unactivated cells (data not shown).

As FGF is also a well-characterized competence factor in BALB/c 3T3 cells (36), we exposed quiescent cells to FGF at 6.25, 25, or 100 ng/ml and measured the steady-state level of the 1.7-kb 2′,5′-OAS mRNA analysis 6 hr later. Fig. 5

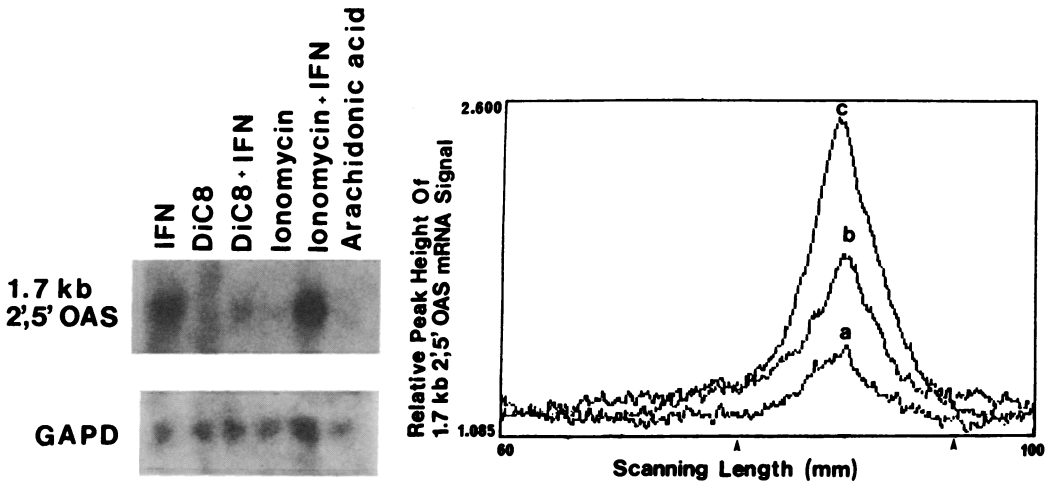

FIG. 2. Suppression by DiC_8 and stimulation by ionomycin of the IFN-α/β-inducible expression of 1.7-kb 2',5'-OAS mRNA. Confluent BALB/c 3T3 cells (8×10^7) were treated with IFN-α/β (1000 units/ml), DiC_8 (150 μM), DiC_8 (150 μM) + IFN-α/β (1000 units/ml), ionomycin (100 nM), ionomycin (100 nM) + IFN-α/β (1000 units/ml), or arachidonic acid (25 μM) for 6 hr. The procedures used are described in *Materials and Methods*. (*Left*) Northern blot signal of 1.7-kb 2',5'-OAS mRNA. GAPD, glyceraldehyde-3-phosphate dehydrogenase. (*Right*) Densitometric analysis of *Left* expressed as relative areas of peaks measured from 78 to 94 mm (cf. arrowheads) as follows: a, IFN + DiC_8, 1.444; b, IFN, 4.862; c, IFN + ionomycin, 8.798.

shows that FGF also failed to induce detectable levels of this mRNA even at the highest concentration (100 ng/ml), at which 86% of nuclei became labeled after continuous 24-hr labeling with [^{3}H]thymidine in the presence of 5% platelet-poor plasma-derived serum.

Effect of Inhibition of Poly(ADP)-Ribose Synthetase. Poly(ADP)-ribosylation is an important posttranslational modification event in nuclei, which affects many cellular functions (for review, see refs. 37 and 38). To obtain evidence as to whether poly(ADP)-ribosylation is involved in IFN action, we treated quiescent BALB/c 3T3 cells with IFN-α/β in the presence of 6 mM 3-aminobenzamide, an inhibitor of poly(ADP)-ribose synthetase. As Fig. 6 shows, 6 mM 3-aminobenzamide inhibited 2',5'-OAS gene expression, and densitometric scanning analysis indicated at least 50% inhibition. This suggests that poly(ADP)-ribosylation may play a role in IFN-α/β-induced 2',5'-OAS gene expression.

DISCUSSION

We have investigated the ability of second messenger reagents to stimulate genes for 2',5'-OAS in quiescent BALB/c 3T3 cells by measuring the steady-state level of 1.7-kb 2',5'-OAS mRNA by Northern blot analysis and densitometric scanning.

After exposure of cells to IFN-α/β (1000 units/ml), two RNA species could be detected by hybridization with a 2',5'-OAS cDNA probe (L3 clone) (39). The major species of 1.7 kb represents 2',5'-OAS mRNA and the minor species of 5 kb may represent a polyadenylylated unprocessed precursor RNA (M. Revel, personal communication). Both were first detected 4 hr after the beginning of IFN treatment. Nuclear run-on experiments in the HeLa cell line have revealed a maximal transcription rate of 2',5'-OAS RNA 3 hr after the start of IFN treatment and a decrease to the base-line level by 9 hr (40). If the course of transcription of 2',5'-OAS RNA is similar in quiescent BALB/c 3T3 cells, then the 1.7-kb mRNA is highly stable since our steady-state level determinations indicate the presence of considerable amounts of the 1.7-kb 2',5'-OAS mRNA 18 hr after the beginning of IFN treatment.

In our experiments, none of the following second messenger reagents induced the 2',5'-OAS gene: ionomycin (in-

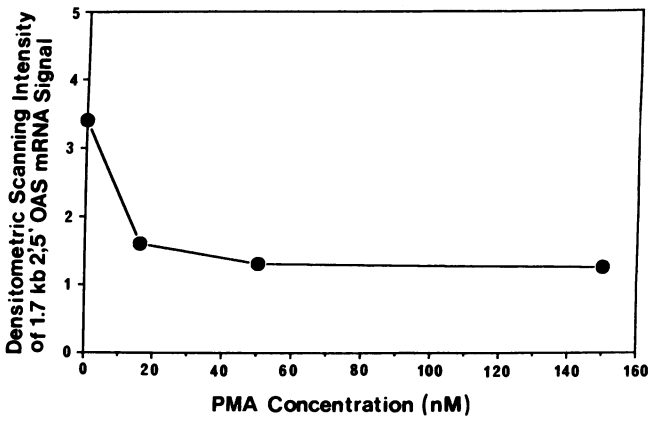

FIG. 3. Suppression by PMA of the IFN-α/β-inducible expression of the 1.7-kb 2',5'-OAS mRNA. Confluent BALB/c 3T3 cells (8×10^7) were treated with IFN-α/β (1000 units/ml) at different concentrations of PMA (0, 15, 50, and 150 nM). Cells were harvested and processed for Northern blot analysis as described in *Materials and Methods*. X-ray films were scanned in a densitometer to obtain relative peak areas for the 1.7-kb 2',5'-OAS mRNA signal.

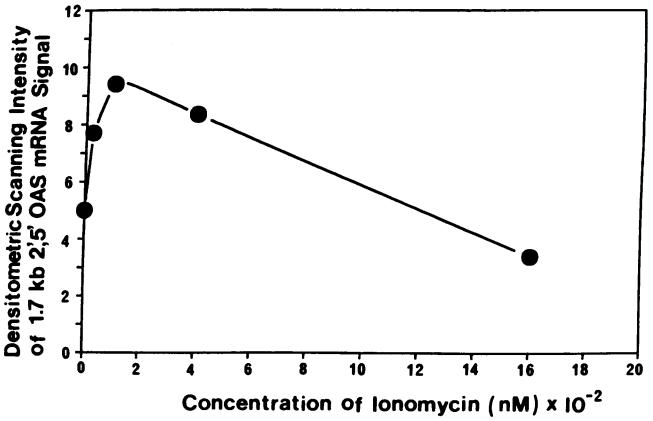

FIG. 4. Concentration dependence of the enhancing effect of ionomycin on the IFN-α/β-inducible expression of the 1.7-kb 2',5'-OAS mRNA. Confluent BALB/c 3T3 cells (8×10^7) were treated with IFN-α/β (1000 units/ml) at different concentrations of ionomycin (0, 25, 100, 400, and 1600 nM). Cells were harvested and processed for Northern blot analysis as described in *Materials and Methods*. X-ray films were scanned in a densitometer to obtain relative peak areas for the 1.7-kb 2',5'-OAS mRNA signal.

Table 1. Densitometric analysis of the 1.7-kb 2',5'-OAS mRNA signal in a Northern blot representing quiescent BALB/c 3T3 cells treated with cAMP analogs or adenylate cyclase activator

Reagent	Densitometric intensity*	
	No IFN	IFN, 1000 units/ml
Control	0.864	3.771
8-Bromo-cAMP (1.45 mM)	0.839	3.882
Dibutyryl-cAMP (1.22 mM)	0.850	4.057
Forskolin (2 μM)	0.810	3.620

*Arbitrary units.

creases intracellular calcium and arachidonic acid release); DiC_8 and PMA (activate protein kinase C and increase arachidonic acid release); 8-bromo-cAMP and dibutyryl-cAMP (activate protein kinase A); and forskolin (activates adenylate cyclase). The following combinations of reagents also did not induce this gene: ionomycin + DiC_8, ionomycin + forskolin, DiC_8 + forskolin, and ionomycin + forskolin + DiC_8. This suggests either that the 2',5'-OAS gene lacks response elements to the putative effector molecules that carry the signal transduced via these distinctive pathways to the nuclear DNA or that response elements are present, but that in the absence of an as yet unidentified IFN-activated signaling event, the effector molecules of the three major pathways are in and of themselves not sufficient to elicit a response. Our findings that ionomycin enhances and DiC_8 and PMA suppress the induction of the 2',5'-OAS gene expression by IFN-α/β favor the second possibility. If the regulatory elements (A or B, or both) in the 2',5'-OAS gene are responsive to effector molecules of the calcium and diacylglycerol–protein kinase C signal transduction pathways, it will be of considerable interest to determine the basis for the opposite effects of the activation of these two pathways and the specific reason for the inability of ionomycin to induce 2',5'-OAS gene expression by itself. Is ionomycin unable to act on the IFN-responsive enhancer element B but able to enhance 2',5'-OAS gene expression via the constitutive element A provided that element B is acted upon by IFN? Does activation of protein kinase C have a negative effect through alteration of a positively acting protein that in IFN-treated cells binds to the regulatory element B? It is of course also possible that either ionomycin or DiC_8 and PMA affect IFN-induced 2',5'-OAS gene expression through mechanisms that do not directly involve regulatory elements A and B and the proteins that bind to these elements. Such alternative mechanisms could, e.g., involve the binding of IFN to its receptors on the cell surface.

The relationship of our findings to the reported potentiation of the antiviral activity of IFN by the calcium ionophore A23187 (32) is not clear. The expression of IFN-inducible genes was not measured and, furthermore, A23187 by itself inhibited the production of infectious virus (32).

Table 2. Densitometric analysis of the 1.7-kb 2',5'-OAS mRNA signal in a Northern blot representing quiescent BALB/c 3T3 cells treated with combinations of second messenger reagents

Reagent	Densitometric intensity*
Control	0.363
IFN	0.970
Ionomycin	0.351
DiC_8	0.366
Forskolin	0.370
Ionomycin + DiC_8	0.355
Ionomycin + forskolin	0.340
DiC_8 + forskolin	0.360
Ionomycin + forskolin + DiC_8	0.397

The concentrations used were as follows: IFN-α/β, 1000 units/ml; ionomycin, 100 nM; DiC_8, 150 μM; forskolin, 2 μM.
*Arbitrary units.

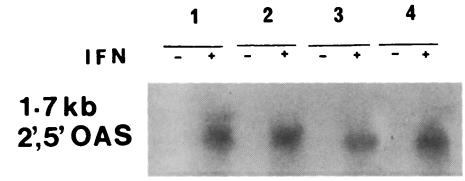

FIG. 5. Induction of 1.7-kb 2',5'-OAS mRNA expression by IFN-α/β in the presence of FGF. Confluent BALB/c 3T3 cells (8×10^7) were incubated for 6 hr with or without IFN-α/β (1000 units/ml) in the presence of FGF at the following concentrations: lanes 1, 0 ng/ml; lanes 2, 100 ng/ml; lanes 3, 25 ng/ml; lanes 4, 6.25 ng/ml. Cells were harvested and processed for Northern blot analysis as described in *Materials and Methods*.

Our results showing that activation of protein kinase C in quiescent 3T3 cells does not lead to the induction of the IFN-response gene 2',5'-OAS and that in IFN-treated cells it suppresses the expression of this gene raises a question as to the significance of the reported rapid and transient increase in diacylglycerol in IFN-α- or -β-treated human fibroblasts or lymphoblastoid (Daudi) cells (41, 42). The increase was reported to be proportional to the number of IFN receptors, and the IFN dose–response relationships for the increase in diacylglycerol and for the inhibition of vesicular stomatitis virus replication and Daudi cell proliferation were similar. It was also reported that PMA, at concentrations from 0.3 to 30 nM, inhibited Daudi cell proliferation in a dose-dependent manner (42). Considered together the findings obtained in the present study and those of Yap *et al.* (41) raise the possibility that activation of 2',5'-OAS gene expression is by a signal transduction mechanism other than that which operates in the establishment of the antiviral and anticellular effects of IFN. Alternatively, the reported transient elevation in the diacylglycerol level (41, 42) may not play a role in the induction of 2',5'-OAS gene or the biological effects of IFN-α or -β. A way to approach this question would be to use variant cells defective with respect to the diacylglycerol–protein kinase C pathway and determine the ability of IFN to exert its effects in such cells. Finally, the findings themselves need to be confirmed by other laboratories.

There is considerable evidence that IFNs can antagonize cell growth factor action and, conversely, that cell growth

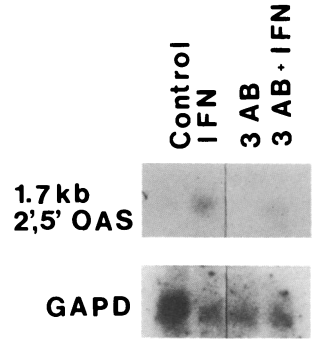

FIG. 6. Suppression of the IFN-α/β-inducible expression of the 1.7-kb 2',5'-OAS mRNA by 3-aminobenzamide (3-AB). Confluent BALB/c 3T3 cells (8×10^7) were not treated, and 4×10^7 cells were treated with IFN-α/β (1000 units/ml), 3-AB (6 mM), or 3-AB (6 mM) + IFN-α/β (1000 units/ml) for 6 hr. Cells were harvested and processed for Northern blot analysis as described in *Materials and Methods*. (*Upper*) Northern blot showing signal of 1.7-kb 2',5'-OAS mRNA. (*Lower*) A 1.4-kb mRNA in the same blot probed with glyceraldehyde-3-phosphate dehydrogenase (GAPD) cDNA. As twice as many cells were used for the control as for the experimental samples, the GAPD signal is stronger in the control.

factors can antagonize IFN effects (reviewed in ref. 1). These mutual effects are dependent on the concentration of growth factors and IFNs and the number of growth factors used. We have shown previously that IFN-α/β, at concentrations inhibitory for cell growth, suppresses the PDGF-induced early synthesis of proteins pI and pII (31 and 35 kDa, respectively) (43). In the present study, PDGF (1–2 units/ml) and FGF (6–100 ng/ml) did not suppress IFN-induced 2′,5′-OAS gene expression, nor did they themselves induce expression of this gene, suggesting that any effects they might have at higher concentrations would involve a cellular response over and above that which is sufficient for a quiescent cell to become competent for an epidermal growth factor- and somatomedin C-mediated traverse of G_1.

Our finding that inhibition of poly(ADP)-ribose synthetase with 3-aminobenzimide decreases the expression of the 2′,5′-OAS gene in response to IFN-α/β is consistent with the previous findings of Exley *et al.* (44) obtained in Daudi lymphoblastoid cells. 3-Aminobenzamide was reported to suppress the IFN-α-induced appearance on the Daudi cell surface of antigens characteristic of mature plasma cells (44). It is of interest that this inhibitor of poly(ADP)-ribose synthetase also protected Daudi cells against the cell-growth inhibitory effect of IFN-α, suggesting that this effect may be associated with cell differentiation induced by IFN. The results in Daudi and BALB/c 3T3 cells indicate a function for poly(ADP)-ribose synthetase in the induction of specific genes by IFNs.

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