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# Induction of the transcription factor IRF-1 and interferon-β mRNAs by cytokines and activators of second-messenger pathways

[interferon regulatory factor 1/tumor necrosis factor/interleukin 1/poly(I)·poly(C)/polymerase chain reaction]

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ABSTRACT **Nuclear protein IRF-1 (interferon regulatory factor 1) was earlier shown to bind to cis-acting regulatory elements present on interferon (IFN)-α/β genes and some IFN-inducible genes. Here we show that in both human FS-4 and murine L929 cells, steady-state levels of IRF-1 mRNA were increased by treatment with tumor necrosis factor (TNF), interleukin 1 (IL-1), poly(I)·poly(C), or IFN-β. IRF-1 mRNA induction was also demonstrated in cells treated with calcium ionophore A23187 or with phorbol 12-myristate 13-acetate, but not with epidermal growth factor, dibutyryl-cAMP, or the adenylate cyclase activator forskolin. To determine whether stimulation of IRF-1 mRNA levels correlates with IFN-β induction, we compared IRF-1 and IFN-β mRNA levels in cells exposed to various stimuli. In L929 cells, treatment with poly(I)·poly(C) under conditions that failed to induce significant levels of IFN-β mRNA led to a very low induction of IRF-1 mRNA, but "priming" cells with IFN prior to the addition of poly(I)·poly(C) greatly increased both IRF-1 and IFN-β mRNAs. In FS-4 cells an increase in IFN-β mRNA (examined by the polymerase chain reaction) was seen after treatment with TNF, IL-1, A23187, or poly(I)·poly(C), but not with IFN-β, epidermal growth factor, dibutyryl-cAMP, or forskolin. Thus, all treatments that increased steady-state levels of IFN-β mRNA also enhanced IRF-1 mRNA levels. However, treatment with IFN-β, which caused a marked stimulation in IRF-1 mRNA, failed to produce a detectable increase in IFN-β mRNA. It appears that IRF-1 may be necessary but not sufficient for IFN-β induction. The ability of TNF and IL-1 to increase both IRF-1 and IFN-β mRNAs may be responsible for some similarities in the actions of TNF, IL-1, and the IFNs.**

The induced expression of interferon (IFN)-α/β is controlled by sequences present in the 5′ flanking region of the IFN-α/β genes (1–3). The regulatory region of the human IFN-β gene is composed of distinct functional domains, and inducibility by viruses or double-stranded RNA is thought to be regulated by the interaction of several trans-acting nuclear factors with these sequences (3–9). Virus-induced transcriptional activation of the human IFN-β (10) or IFN-α (11) genes can be mediated by specific repetitive hexanucleotide sequences present within the enhancer region. A nuclear factor termed IRF-1 (interferon regulatory factor 1) was identified on the basis of its ability to bind to these hexameric sequences (5). IRF-1, whose synthesis is strongly inducible by virus infection, acts as a positive transcription factor for the expression of IFN-α/β genes (12, 13).

Recent observations suggest that regulatory mechanisms governing the expression of IFN-inducible genes partly overlap the mechanisms regulating the expression of the IFN-α/β genes themselves. This conclusion is based on the observations of similarities between the sequences controlling inducibility in IFN-α/β genes and several IFN-induced genes (14–16). In addition, it was shown that some IFN-induced genes are also inducible by viruses or double-stranded RNA (14, 15, 17). In agreement with these findings, IRF-1 was shown to bind to regulatory elements in at least one group of IFN-induced genes, the class I major histocompatibility complex genes (12). Furthermore, IRF-1 synthesis is induced not only by virus infection, but also by treatment with IFN (6).

Other observations indicate that some actions of the IFNs can be mimicked by other cytokines or growth factors. For example, the synthesis of some proteins characteristically induced by the IFNs was also shown to be inducible by tumor necrosis factor (TNF) or interleukin 1 (IL-1) (18–22) or by growth factors (17, 23, 24). Furthermore, IL-1 (25) or TNF (26–28) were shown to stimulate IFN-β synthesis in some cells. These observations led us to examine the ability of some cytokines, growth factors, and activators of second-messenger pathways to increase IRF-1 mRNA levels in cultures of murine L929 and human FS-4 cells. We also compared the inducibility of IRF-1 and IFN-β mRNAs by these treatments. We found that IRF-1 and IFN-β mRNAs are inducible by TNF, IL-1, and some activators of second-messenger pathways. We conclude that induction of IRF-1 may be responsible for some IFN-like actions of TNF and IL-1. Induction of IRF-1 by TNF and IL-1 also may contribute to the ability of these cytokines to induce IFN-β.

## MATERIALS AND METHODS

**Materials.** Nick-translation kits, DNase I, and proteinase K were from Boehringer Mannheim. Poly(I)·poly(C) was obtained from P-L Biochemicals. $N^6$,$O^{2'}$-Dibutyryladenosine 3′,5′-cyclic monophosphate (dibutyryl-cAMP), forskolin, phorbol 12-myristate 13-acetate (PMA), and calcium ionophore A23187 were from Sigma. A neutralizing monoclonal antibody to human TNF (29) was provided by M. Tsujimoto (Suntory Institute for Biomedical Research, Osaka, Japan).

**Cells.** Mouse L929 cells were cultured in ES medium (Nissui Seiyaku, Tokyo) supplemented with 5% fetal bovine serum. Human FS-4 foreskin fibroblasts (30) were cultured in Eagle's minimal essential medium (GIBCO) supplemented with 5% fetal bovine serum, 6 mM Hepes, and 3 mM Tricine.

**Induction of IRF-1 and IFN-β mRNAs in Human FS-4 Cells.** FS-4 cells grown in 175-cm² plastic flasks were used 7 days after seeding. The cells were treated with one or more of the following agents: *Escherichia coli*-derived recombinant hu-

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Abbreviations: IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; PMA, phorbol 12-myristate 13-acetate; EGF, epidermal growth factor; PCR, polymerase chain reaction.

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man TNF [20 or 200 ng/ml; supplied by M. Tsujimoto (Suntory Institute for Biomedical Research, Osaka); specific activity, $3 \times 10^7$ units/mg]; *E. coli*-derived recombinant human IL-1$\alpha$ [30 ng/ml; a gift of Alvin Stern and Peter Lomedico (Hoffmann–LaRoche); $3 \times 10^7$ units/mg]; purified mouse epidermal growth factor (EGF; 25 ng/ml; from Collaborative Research); *E. coli*-derived recombinant human IFN-$\beta$ (100 or 1000 units/ml; Betaseron from Triton Biosciences, Alameda, CA; $2 \times 10^8$ units/mg); poly(I)·poly(C) (50 $\mu$g/ml); dibutyryl-cAMP (690 $\mu$M); forskolin (10 $\mu$M); calcium ionophore A23187 (5 $\mu$M); PMA (100 ng/ml). RNA extraction and processing were done as described (28).

**Induction of IRF-1 and IFN-$\beta$ mRNA in Mouse L929 Cells.** Monolayers of L929 cells, cultured in ES medium in 10-cm dishes, were treated with one or more of the following agents: recombinant mouse IFN-$\beta$ (1000 units/ml; Toray Industries, Tokyo; $3 \times 10^7$ units/mg); recombinant human TNF (1000 units/ml; Dainippon Pharmaceutical, Osaka; $3.15 \times 10^6$ units/mg); recombinant human IL-1$\alpha$ (1000 units/ml; Dainippon Pharmaceutical; $2 \times 10^7$ units/mg); dibutyryl-cAMP (690 $\mu$M); forskolin (10 $\mu$M); calcium ionophore A23187 (10 $\mu$M); PMA (100 ng/ml). The IFN inducer poly(I)·poly(C) (100 $\mu$g/ml) was added to L929 cells in the presence of DEAE-dextran (100 $\mu$g/ml) in ES medium for 1 hr. For RNA extraction, cells were harvested with a rubber policeman, washed once with phosphate-buffered saline, and lysed in RSB (10 mM Tris Cl, pH 7.5/10 mM NaCl/1.5 mM $MgCl_2$) containing 0.5% Triton X-100 and 10 mM vanadyl ribonucleoside complex. After the removal of nuclei by centrifugation, the cytoplasmic fraction was extracted twice with phenol, once with phenol/chloroform (1:1, vol/vol), and twice with chloroform and RNA was precipitated with ethanol. To remove vanadyl ribonucleoside complex, RNA was further precipitated with 2 M LiCl at −20°C overnight.

**S1 Nuclease Protection Assay.** S1 mapping was performed as described (5), using 5′-end-labeled single-stranded DNA complementary to the mouse IFN-$\beta$ mRNA (5) or IRF-1 mRNA (12). The mRNA copy number was calculated as described (10).

**Detection of IRF-1 by Northern Blot Hybridization.** For detection of IRF-1 mRNA, 10 $\mu$g of RNA extracted from FS-4 cells was denatured and electrophoresed in a denaturing formaldehyde/1% agarose gel (31). RNA was visualized by Northern blot hybridization using a 748-base-pair (bp) fragment (*Kpn* I/*Sac* I) of the human IRF-1 cDNA (32) or an internal reference cDNA, pHe7 (33), both labeled with [$\alpha$-$^{32}$P]CTP by nick-translation.

**Detection of IFN-$\beta$ mRNA by Polymerase Chain Reaction (PCR).** The method for the detection of IFN-$\beta$ mRNA was described before (28). Briefly, 1 $\mu$g of RNA isolated from FS-4 cells after various treatments was reverse-transcribed and the resulting cDNA was used for the PCR, in the presence of 1 unit of *Thermus aquaticus* DNA polymerase, dNTPs (200 $\mu$M each), and two oligonucleotides (1 $\mu$M each) as primers. Primer I (GAA-TGT-CCA-ATG-GAG-GCT-TTG) is complementary to positions 605–625 of the 3′ end of the IFN-$\beta$ mRNA, and primer II (TCT-AGC-ACT-GGC-TGG-AAT-GAG) is complementary to positions 350–370 of the IFN-$\beta$ cDNA. (The numbering of nucleotides in the IFN-$\beta$ DNA sequence is from ref. 34.) Thus, the major product of the PCR is a 276-bp IFN-$\beta$ cDNA fragment. Amplification was carried out for 30 cycles and 10 $\mu$l of the resulting reaction mixture was visualized by Southern blot hybridization, using a fragment of the 3′ end of the IFN-$\beta$ cDNA as a probe.

## RESULTS

**Stimulation of IRF-1 mRNA Levels by TNF, IL-1, and Activators of Second-Messenger Pathways.** A cDNA encoding human IRF-1 was recently isolated (32), enabling us to analyze the levels of IRF-1 mRNA in human cells. Control human FS-4 fibroblasts had a very low level of IRF-1 mRNA (Fig. 1). Treatment with IFN-$\beta$ for 2.5 hr increased IRF-1 mRNA >10-fold. Interestingly, TNF treatment led to a similar enhancement of IRF-1 mRNA. When treatment with IFN-$\beta$ or TNF was continued for 7.5 hr, the stimulation of IRF-1 mRNA levels was greatly diminished.

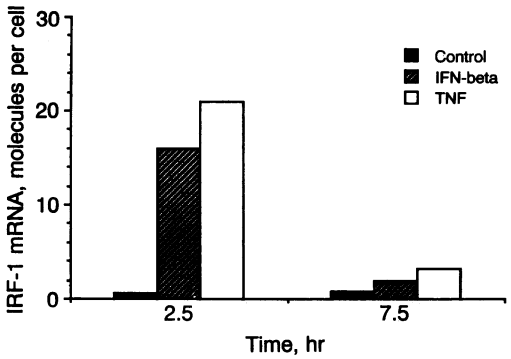


FIG. 1. Induction of IRF-1 mRNA by treatment with TNF or IFN-$\beta$ in human FS-4 fibroblasts. Cultures were exposed to control medium (solid bars), human IFN-$\beta$ (100 units/ml; hatched bars), or human TNF (20 ng/ml; open bars). At the indicated times, RNA was isolated and IRF-1 mRNA levels were determined by S1 nuclease mapping.

IRF-1 mRNA levels were also analyzed in murine L929 cells after various treatments (Fig. 2). Untreated cells had very little IRF-1 mRNA. IFN-$\beta$ caused a rapid increase in IRF-1 mRNA, which peaked at 1 hr and then gradually decreased. TNF and IL-1 also increased IRF-1 mRNA, with quite similar kinetics. We also examined the effect of various activators of second-messenger pathways. The adenylate cyclase activator forskolin and dibutyryl-cAMP failed to increase IRF-1 mRNA, but the phorbol ester PMA did produce an increase with kinetics somewhat similar to those seen with IFN-$\beta$, TNF, or IL-1. In contrast, calcium iono-

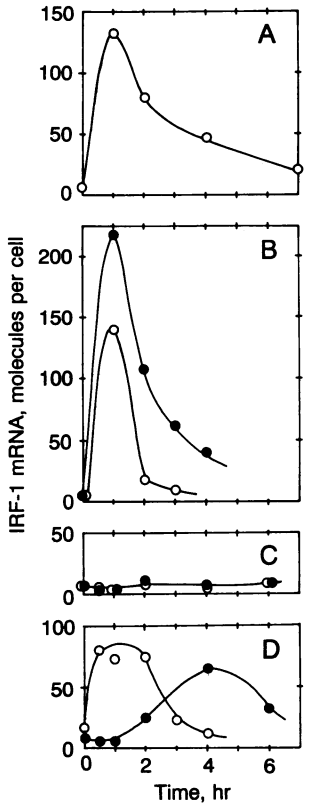


FIG. 2. Induction of IRF-1 mRNA by cytokines and activators of second-messenger pathways in L929 cells. (*A*) Recombinant mouse IFN-$\beta$ (1000 units/ml). (*B*) Recombinant human TNF (1000 units/ml; ●) or IL-1$\alpha$ (1000 units/ml; ○). (*C*) Dibutyryl-cAMP (690 $\mu$M; ●) or forskolin (10 $\mu$M; ○). (*D*) Calcium ionophore A23187 (10 $\mu$M; ●) or PMA (100 ng/ml; ○). At the indicated times, the cells were harvested and IRF-1 mRNA levels were determined by S1 mapping.

phore A23187 increased IRF-1 mRNA with delayed kinetics, with a peak reached only at 4 hr.

**Comparison of IRF-1 and IFN-β mRNA Levels After Different Treatments.** If IRF-1 is necessary for IFN-β induction, then treatments that induce IFN-β mRNA should also increase IRF-1 mRNA. Infection of L929 cells with Newcastle disease virus led to an increase in both IRF-1 and IFN-β mRNA levels (12). In addition, the expression of IRF-1 in COS cells led to an induction of IFN-α and IFN-β (13).

To gain more insight into the relationship between the induction of IRF-1 mRNA and that of IFN-β mRNA, we examined the effect of a double-stranded RNA, poly(I)·poly(C), in L929 cells. Exposure of L929 cells to poly(I)·poly(C) led to no detectable increase in IFN-β mRNA and to a barely demonstrable increase in IRF-1 mRNA 1 hr after the addition of poly(I)·poly(C) to the cells (Fig. 3*A*). This finding reflects the fact that poly(I)·poly(C) is a relatively weak inducer of IFN-β in these cells (35). Induction of IFN-β can be enhanced by "priming" cells with IFN prior to the addition of the inducer (35, 36). Fig. 3*B* shows that exposure of L929 to IFN-β for 3 hr before the addition of poly(I)·poly(C) led to a peak of IRF-1 mRNA at 1 hr (due to the addition of IFN-β at 0 hr) and a second, larger peak of IRF-1 mRNA at 4 hr [which was most likely a response to poly(I)·poly(C)]. In addition, these cells showed an increase in IFN-β mRNA at 7 and 10 hr [i.e., 3 and 6 hr after stimulation with poly(I)·poly(C)]. Thus, priming with IFN led to an increase in both IRF-1 and IFN-β mRNAs produced in response to poly(I)·poly(C).

Induction of IRF-1 and IFN-β mRNAs was also compared in human FS-4 cells. Treatment with TNF at either 20 or 200 ng/ml increased IRF-1 mRNA levels; this stimulation was greatly reduced by an antibody to TNF, indicating that TNF was indeed responsible for this action (Fig. 4*A*). Recombinant human IL-1α showed a similar stimulation of IRF-1 mRNA levels, but a 2-hr treatment with EGF did not. To measure IFN-β mRNA we employed a highly sensitive method in which cDNA derived from IFN-β mRNA by reverse transcription is amplified in the PCR. This method allowed Reis *et al.* (28) to establish that IFN-β is present even in unstimulated FS-4 cells and that exposure of FS-4 cells to TNF for 2 hr led to an ≈16-fold increase over the basal steady-state level of IFN-β mRNA. Fig. 4*B Left* shows that a band corresponding to a 276-bp amplified fragment of IFN-β cDNA was demonstrable in all samples tested. The higher intensity of the bands in the samples originating from TNF- or IL-1-treated cells indicates a higher concentration of IFN-β mRNA in these cell extracts. Since amplification by PCR over a large number of cycles is not strictly linear (37), the degree of increase in mRNA concentration after TNF or IL-1 treatment may actually be greater than the increase in the radioactivity of the corresponding bands. To prove that the amplified IFN-β cDNA was not in part or in whole due to contamination with genomic DNA, we carried out the PCR with the same RNA preparations while omitting the reverse-transcription step (Fig. 4*B Right*). No bands were discernible, proving a lack of contamination with genomic IFN-β DNA.

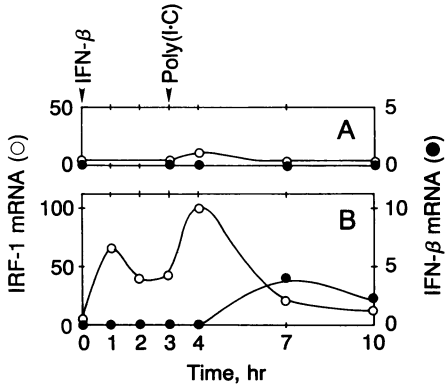


FIG. 3. Relationship between the inductions of IRF-1 and IFN-β mRNAs by poly(I)·poly(C) in L929 cells. Cultures were treated with control medium (*A*) or murine IFN-β (1000 units/ml) (*B*) from 0 to 3 hr. Thereafter both groups of cultures were exposed to poly(I)·poly(C) (100 μg/ml) in the presence of DEAE-dextran (100 μg/ml) for 1 hr (from 3 to 4 hr on the time scale shown). At the indicated times the cells were harvested and IRF-1 and IFN-β mRNA levels (molecules per cell) were determined by S1 mapping.

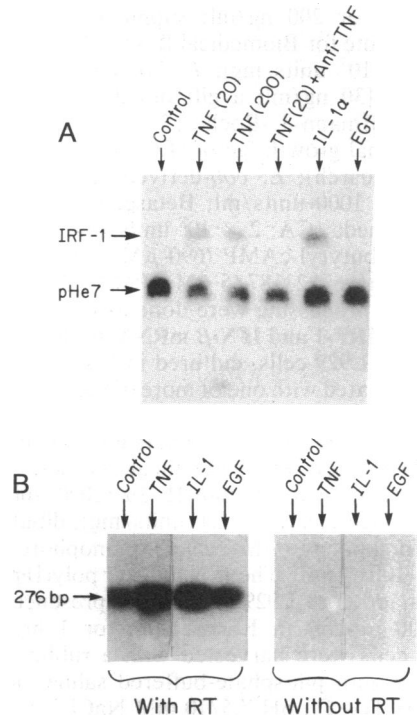


FIG. 4. Comparison of IRF-1 and IFN-β mRNA induction by cytokines in FS-4 cells. (*A*) Cultures were treated for 2 hr with control medium, human TNF (20 or 200 ng/ml), a mixture of TNF (20 ng/ml) and monoclonal antibody to TNF (50 μg/ml), IL-1α (30 ng/ml), or EGF (25 ng/ml). RNA was extracted and IRF-1 mRNA levels were determined by Northern blot analysis. To ascertain that equal amounts of RNA were applied the blots were also probed with internal reference cDNA pHe7. (*B*) RNA samples from the same control, TNF (20 ng/ml)-treated, IL-1α-treated, and EGF-treated cultures were also examined for the presence of IFN-β mRNA by reverse transcription and PCR, followed by Southern blot analysis of IFN-β cDNA (With RT). The same RNA samples were processed identically except for omission of the reverse transcription step (Without RT).

Induction of IRF-1 and IFN-β mRNAs was also analyzed in FS-4 cultures exposed to various activators of second-messenger pathways, to poly(I)·poly(C), or to IFN-β. In general agreement with the results obtained in L929 cells, an increase in IRF-1 mRNA was seen after treatment with calcium ionophore A23187 or phorbol ester, but not after treatment with dibutyryl-cAMP or forskolin (Fig. 5*A*). Unlike L929 cells, FS-4 cells showed a strong increase in IRF-1 mRNA in response to poly(I)·poly(C) or IFN-β. [In addition to the characteristic band of IRF-1 mRNA, a second, slower migrating band was also seen in the extract of cells treated with poly(I)·poly(C) or IFN-β. This larger mRNA was always observed in samples with high concentrations of IRF-1 mRNA and it may represent an unprocessed form of IRF-1 transcript.] The same RNA preparations were analyzed for the presence of IFN-β by reverse transcription and PCR (Fig. 5*B*). No increase in the intensity of the IFN-β cDNA band

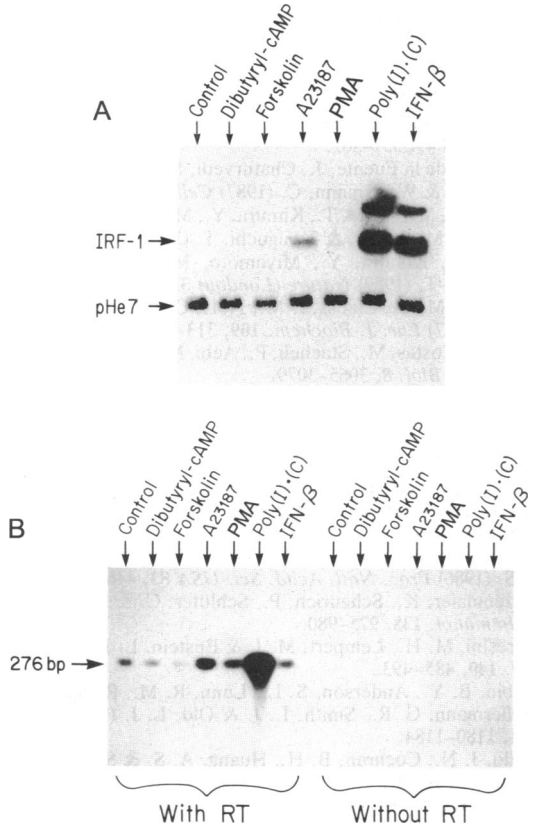


FIG. 5. Comparison of IRF-1 and IFN-$\beta$ mRNA induction by activators of second-messenger pathways, poly(I)·poly(C), and IFN-$\beta$ in FS-4 cells. (*A*) Cultures were treated for 2 hr with control medium, dibutyryl-cAMP (690 $\mu$M), forskolin (10 $\mu$M), A23187 (5 $\mu$M), PMA (100 ng/ml), poly(I)·poly(C) (50 $\mu$g/ml), or human IFN-$\beta$ (1000 units/ml). RNA was extracted and IRF-1 mRNA levels were determined by Northern blot analysis. The blots were also probed with internal reference cDNA pHe7. (*B*) The same RNA samples were also examined for the presence of IFN-$\beta$ mRNA by using reverse transcription and PCR. Details of procedure were the same as in the experiment of Fig. 4.

was seen after treatment with dibutyryl-cAMP or forskolin. Treatment with poly(I)·poly(C) caused a very marked increase in the amount of IFN-$\beta$ cDNA; this result was not unexpected, since poly(I)·poly(C) is a potent IFN-$\beta$ inducer in FS-4 cells (30). An increase in the IFN-$\beta$ cDNA band was also seen with calcium ionophore A23187; although this effect was modest in comparison with poly(I)·poly(C), it was reproducible. PMA also appeared to produce a slight increase in IFN-$\beta$ cDNA, but this finding will require confirmation. Finally, treatment with IFN-$\beta$ had no significant effect on the intensity of the IFN-$\beta$ cDNA band. Thus, all treatments producing a demonstrable increase in IFN-$\beta$ mRNA [TNF, IL-1, A23187, poly(I)·poly(C), and possibly PMA] also led to an increase in IRF-1 mRNA. However, the converse was not true, because IFN-$\beta$ is a potent inducer of IRF-1 mRNA and yet we failed to detect an increase in IFN-$\beta$ mRNA in IFN-$\beta$-treated cells.

## DISCUSSION

Some actions characteristically associated with the IFNs are also expressed by other cytokines or growth factors. For example, TNF and IL-1 were shown to induce the synthesis of several IFN-inducible proteins (18–22). Furthermore, TNF and IL-1 can modulate IFN-$\beta$ synthesis; under proper conditions both cytokines can prime cells to produce increased levels of IFN-$\beta$ (38), and in some cells TNF (26–28) or IL-1 (25) was shown to induce IFN-$\beta$. These observations suggest that TNF, IL-1, and IFNs share the ability to activate regulatory factors that drive the expression of the same cellular genes, including the IFN-$\alpha/\beta$ genes themselves. IRF-1 was recently identified as a positive transcription factor regulating the expression of IFN-$\alpha/\beta$ genes (12, 13). Furthermore, IRF-1 was shown to bind to a sequence in the regulatory region of the murine *H-2D$^d$* gene, suggesting a role for IRF-1 in the regulation of some IFN-inducible genes (12). In the present study we tried to answer two major questions. (*i*) Could induction of IRF-1 be one of the common denominators underlying the similarities in the actions of the IFNs, TNF, and IL-1? (*ii*) Is there a correlation between the induction of IRF-1 and IFN-$\beta$ mRNAs by different agents?

Addressing the former question, we found that IFN-$\beta$, TNF, and IL-1 all produced a similar marked and rapid increase in IRF-1 mRNA, in both human FS-4 and murine L929 cells. These results suggest that IRF-1 indeed might mediate the stimulation of the expression of some target genes that are inducible by TNF or IL-1 as well as the IFNs—e.g., the genes for 2′-5′ oligoadenylate sythetase (18), class I and II major histocompatibility complex antigens (19, 20), IL-6 (39), and some other, incompletely identified gene products (21, 22). On the other hand, no evidence for the stimulation of IRF-1 mRNA was seen with EGF (Fig. 4*A*). Platelet-derived growth factor also failed to produce a significant stimulation of IRF-1 mRNA levels in both L929 and FS-4 cells (data not shown), suggesting that some IFN-like actions seen with these growth factors (17, 23, 24) are probably not mediated by an increased synthesis of IRF-1.

The second major question asked concerned the role of IRF-1 in IFN-$\beta$ induction by various agents. It was shown earlier that in L929 cells the IRF-1 gene is inducible by Newcastle disease virus under conditions also leading to IFN-$\alpha/\beta$ induction (6, 12). Here we demonstrated the inducibility of IRF-1 mRNA by an IFN-inducing double-stranded RNA, poly(I)·poly(C), in both L929 and FS-4 cells. In L929 cells stimulation of IRF-1 mRNA by poly(I)·poly(C) was greatly enhanced by the priming of cells with IFN, a treatment also leading to a marked enhancement of IFN-$\beta$ induction (Fig. 3). These results suggest that IRF-1 might be an important element in the phenomenon of IFN priming, demonstrated with many different cells and IFN inducers (reviewed in ref. 36).

Since both TNF (26–28) and IL-1 (25) were shown to act as inducers of IFN-$\beta$ in some cells, it is tempting to speculate that IRF-1 induction by these cytokines is related to their IFN-$\beta$-inducing activity. Some evidence supporting the role of IRF-1 in driving IFN-$\beta$ expression has been obtained. Thus, in addition to TNF, IL-1, and poly(I)·poly(C), the calcium ionophore A23187 was found to stimulate both IRF-1 and IFN-$\beta$ mRNA levels, whereas three other agents (EGF, dibutyryl-cAMP, and forskolin) failed to produce a detectable increase in either IRF-1 or IFN-$\beta$ mRNA. PMA did produce an increase in IRF-1 mRNA, and at the one time point examined it produced a questionable increase in IFN-$\beta$ mRNA levels (Fig. 5). In addition, when the kinetics of IRF-1 and IFN-$\beta$ mRNA stimulation were compared in FS-4 cells exposed to either TNF or IL-1, the peak of IRF-1 mRNA stimulation ($\approx$1 hr) preceded the peak of IFN-$\beta$ mRNA stimulation ($\approx$2 hr), consistent with a positive regulatory role for IRF-1 (data not shown).

Although our data are consistent with the idea that IRF-1 induction is a necessary step, it is also apparent that it is not sufficient for IFN-$\beta$ induction. Treatment with IFN-$\beta$ was similar to treatment with poly(I)·poly(C) in the stimulation of IRF-1 mRNA levels produced in FS-4 cells; however, while poly(I)·poly(C) was a potent inducer of IFN-$\beta$ mRNA, treatment with IFN-$\beta$ failed to increase IFN-$\beta$ mRNA (Fig. 5 and data not shown). Our data are consistent with a multitude of

earlier studies showing that treatment of cells with IFNs can enhance ("prime") subsequent induction of IFN-$\beta$ by viruses or poly(I)·poly(C), but does not by itself lead to IFN-$\beta$ induction (reviewed in ref. 36). It is possible that in addition to quantitative changes, IRF-1 must also undergo qualitative changes (e.g., phosphorylation) in order to function efficiently. Poly(I)·poly(C) or virus infection might be both increasing IRF-1 synthesis and leading to its necessary post-translational modification, whereas IFN-$\beta$ treatment might produce only the former. Alternatively, IRF-1 alone might be insufficient to efficiently drive IFN-$\beta$ transcription, and some other transcription factor also may have to be synthesized or activated. Indeed, several recent studies have implicated a NF-$\kappa$B-like nuclear protein and a $\kappa$B-like enhancer sequence in the regulatory region of the IFN-$\beta$ gene in the control of IFN-$\beta$ induction by viruses or poly(I)·poly(C) (7–9, 40).

It is conceivable that both IRF-1 and a NF-$\kappa$B-like factor are required for the efficient induction of IFN-$\beta$ gene expression. It has been postulated that a cooperative interaction between the positive regulatory domains I and II (PRDI and PRDII) of the IFN-$\beta$ gene promoter is required for virus induction (41). PRDI contains the IRF-1 binding site (5), whereas PRDII corresponds to the $\kappa$B-like element (7–9). In this context it is interesting that TNF was recently shown to induce activation of nuclear proteins binding to $\kappa$B-like sites on the gene for the $\alpha$ chain of the IL-2 receptor (42), on the human immunodeficiency virus 1 genome (43), or on the IL-6 gene (Y. Zhang and J.V., unpublished data). In addition, preliminary evidence suggests that TNF can drive the expression of a reporter gene linked to multimerized $\kappa$B elements, whereas IFN-$\beta$ treatment does not (N.W., unpublished data). Failure of IFN-$\beta$ treatment to activate NF-$\kappa$B-like protein(s) might account for the lack of IFN-$\beta$ mRNA induction by IFN-$\beta$.

Another possible reason why IRF-1 induction does not always correlate with IFN-$\beta$ induction is that negative regulatory factors also are likely to be involved in the control of IFN-$\beta$ gene expression. A putative negative regulatory factor, IRF-2, has been identified and cloned (6). The IRF-2 protein binds to the same nucleotide sequence element and also shares extensive sequence homology with IRF-1 in its DNA-binding portion. Although the exact role of IRF-2 in the control of IFN-$\beta$ expression is still under study, its slower kinetics of induction by some agents and its ability to down-regulate the function of IRF-1 under some conditions suggest that it acts as a repressor.

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