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A Cytokine Network in Human Diploid Fibroblasts: Interactions of β-Interferons, Tumor Necrosis Factor, Platelet-Derived Growth Factor, and Interleukin-1

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Earlier studies demonstrated the induction of β_2-interferon (IFN-β_2) in human diploid fibroblasts (FS-4 strain) exposed to tumor necrosis factor (TNF). These studies suggested that IFN-β_2 mediates an antiviral effect in TNF-treated cells and exerts a feedback inhibition of the mitogenic effect of TNF. Here we demonstrate that the expression of the antiviral action of TNF can be enhanced by prior exposure of FS-4 cells to trace amounts of IFN-β_1. IFN-β_1, at a higher concentration, can directly increase the expression of IFN-β_2. Exposure of cells to TNF enhanced IFN-β_2 (but not IFN-β_1) mRNA expression in response to poly(I) · poly(C), an IFN inducer which is also known to stimulate FS-4 cell growth. Platelet-derived growth factor and interleukin-1 also led to the increased expression of IFN-β_2. However, platelet-derived growth factor and interleukin-1 could override the antiviral effect of TNF and also that of exogenously added IFN-β_1. Our data suggest that a complex network of interactions that involves the endogenous production of IFN-β_2 is triggered by several growth-modulatory cytokines. Cellular homeostasis is likely to represent a balance between the induction of IFN-β_2 by these cytokines and their ability to override the inhibitory actions of IFN-β_2.

Many cytokines have antagonistic biological effects on cells in culture. For example, the cell growth-stimulating action of platelet-derived growth factor (PDGF) can be inhibited by the addition of interferon (IFN), and conversely, PDGF is able to inhibit the antiviral action of IFN (8, 11, 16, 26). Similarly, the growth factor-like mitogenic effect of tumor necrosis factor (TNF) in human fibroblasts can be inhibited by the addition of IFN (30). Recent observations that some growth factors themselves induce endogenous IFNs suggest a role for such IFNs in the homeostatic mechanism controlling cell proliferation. Colony-stimulating factor-1 induced IFN in murine bone marrow cells (14); this IFN was subsequently identified as murine IFN-β (17). TNF was shown to induce human IFN-β_2 mRNA in human fibroblast cultures (10). TNF produced an antiviral effect in human fibroblasts, apparently mediated by the induction of IFN-β_2 (10). The development of the antiviral effect of TNF in FS-4 cells could be blocked by anti-IFN-β serum. Moreover, anti-IFN-β serum amplified the mitogenic effect of TNF in human fibroblast cultures (10).

IFNs are classified as α, β, or γ based on neutralization by appropriate antisera (5, 9). At least two functional poly(I) · poly(C)-inducible human IFN-β genes have been characterized (reviewed in references 18 and 24). The IFN-β_1 gene is located on human chromosome 9 and has been extensively investigated (reviewed by C. Weissmann and H. Weber, Prog. Nucleic Acids Res. Mol. Biol., in press). The IFN-β_2 gene (22, 31) is located on chromosome 7 (24) and has been recently expressed into biologically active recombinant human IFN-β in heterologous expression systems (18, 32). Although the IFN-β_1 and -β_2 genes do not cross-hybridize at the nucleic acid level, the two proteins share distinct amino acid sequence similarities (L. T. May, D. C. Helfgott, and P. B. Sehgal, Proc. Natl. Acad. Sci. USA, in press). The two recombinant proteins can be cross-neutralized by several different heterologous polyclonal and monoclonal antibodies (18, 32; J. Vilček, unpublished data). The 1.3-kilobase (kb) IFN-β_2 mRNA is expressed constitutively at a low level in confluent cultures of human fibroblasts maintained for about 1 week without medium replenishment (10, 32). TNF greatly increased the steady-state level of IFN-β_2 mRNA in such fibroblast cultures without the appearance of the 0.9-kb IFN-β_1 mRNA (10).

Experiments described here show that the expression of IFN-β is enhanced when human fibroblasts are first exposed to a low concentration of exogenous IFN-β and then to TNF or when they are first exposed to TNF followed by poly(I) · poly(C). IFN-β_1 by itself, used at high concentration, can directly induce IFN-β_2. We show, in addition, that growth factors such as PDGF and interleukin-1 (IL-1) induce IFN-β_2, but not IFN-β_1, in serum-depleted human diploid fibroblasts. At the same time, PDGF and IL-1 can block the antiviral effect of exogenously added or endogenously produced IFN-β_1 or IFN-β_2. These data support the view that antagonistic or co-agonistic interactions among the exogenously added and the one or more endogenously produced cytokines determine the net biological effects observed. Our experiments document new aspects of the intricate cytokine network that functions in human diploid fibroblast cultures.

MATERIALS AND METHODS

Materials. Recombinant *Escherichia coli*-derived human TNF (specific activity, 4.8×10^7 units/mg) was kindly supplied by the Suntory Institute for Biomedical Research (Osaka, Japan); partially purified human leukocyte IFN-α

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(lot 201P; specific activity, $>2 \times 10^6$ IU/mg) was obtained from the New York Blood Center; partially purified human diploid fibroblast IFN-β (lot 4; specific activity, 2×10^6 IU/mg) was obtained from Dr. Rentschler Arzneimittel GmbH & Co.; recombinant *E. coli*-derived human IFN-β_1^{17Ser} (specific activity, 2×10^8 IU/mg) was obtained from Triton Biosciences; recombinant *E. coli*-derived human IFN-γ (specific activity, $>5 \times 10^6$ IU/mg) was obtained from Suntory Institute for Biomedical Research; highly purified human PDGF was obtained from Elaine W. Raines and Russell Ross (University of Washington School of Medicine, Seattle) and from Cellular Products Inc. (specific activity, $>8 \times 10^5$ units/mg); recombinant *E. coli*-derived human IL-1α (specific activity, 3×10^7 units/mg) was obtained from Hoffmann-La Roche Inc. (Nutley, N.J.); and recombinant *E. coli*-derived human IL-1β (specific activity, 10^7 units/mg) was obtained from Charles A. Dinarello (Tufts University Medical Center, Boston). Poly(I) · poly(C) was obtained from P-L Biochemicals, Inc. (Milwaukee, Wis.), cycloheximide was from Sigma Chemical Co. (St. Louis, Mo.), and actinomycin D was from Calbiochem-Behring (La Jolla, Calif.). Antisera to human IFNs and their use in neutralization assays have been described before (9, 10). The human IFN-α_1 cDNA probe was pLM001 (21); the human IFN-β_1 cDNA probe was pD19 (13); and a 21-nucleotide-long synthetic DNA probe complementary to the IFN-β_2 mRNA (10) was used for Northern blot analyses. [α-^{32}P]dCTP and [γ-^{32}P]ATP were purchased from New England Nuclear Corp. (Boston, Mass.). Reference standards for human IFN-α, -β (expressed as W.H.O. international units per milliliter), and -γ (expressed as N.I.H. reference units per milliliter) were obtained from the National Institutes of Health (Bethesda, Md).

Cell culture methods. The human foreskin diploid fibroblast strain designated FS-4 (29) was used in all the experiments. Procedures for cell growth, for antiviral protection assays with encephalomyocarditis (EMC) virus, for detection of IFN activity in samples of cell culture medium, and for the serologic characterization of the IFN activity have been described earlier (10). FS-4 cells were grown in 24-well multiwell plates (1 ml of medium per well), T-175 flasks (30 ml of medium per flask), or 150-mm plastic petri dishes (Flow Laboratories, Inc., McLean, Va.) (30 ml of medium per petri dish) in Eagle minimal essential medium supplemented with 5 or 10% (vol/vol) heat-inactivated (56°C, 30 min) fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.) (10). Unless stated otherwise, experiments were carried out in confluent FS-4 cell cultures 6 to 8 days after the last medium replenishment. Cytokines or other experimental agents were added directly to the conditioned medium in the cultures to achieve the indicated concentrations. IFN titers in some experiments are expressed as laboratory units per milliliter that correspond to approximately 0.1 to 0.2 IU of the appropriate reference standard per ml.

Blot-hybridization analyses of RNA. Procedures for extraction of cellular polyadenylated RNA, blot-hybridization analyses, and autoradiography have been described earlier (10). For RNA blots, polyadenylated RNA (yield approximately 1 μg per T-175 flask or 150-mm petri dish) was denatured in 10 to 20 mM methylmercury hydroxide, electrophoresed through a 1% agarose–10 mM methylmercury hydroxide gel, and blotted onto aminobenzyloxymethyl cellulose paper (ABM paper; Pharmacia, Inc., Piscataway, N.J.) (10, 13, 21, 22). Autoradiography was carried out at −70°C with Kodak XAR-5 film and Du Pont intensifier screens. Although not shown in the Results section, most of the blots were reprobed with a cDNA clone to human β-actin mRNA as part of the last step in each experiment to verify (i) equal mRNA loading in comparable lanes and (ii) adequacy of the procedures used to strip the blots of hybridized probes and those used for rehybridization.

RESULTS

In the following sections particular experimental questions are explored both by biological techniques (for example, by antiviral protection assays) and by nucleic acid hybridization procedures (for example, by Northern blot analyses of polyadenylated RNA). Thus, specific biological observations can be directly correlated with the underlying expression of particular IFN mRNA species.

Enhancement of TNF-induced IFN-β_2 expression by exogenous IFN-β. Earlier studies showed that treatment of confluent cultures of FS-4 cells with TNF at concentrations in the range of 1 to 30 ng/ml for 24 h inhibited virus multiplication and the cytopathic effect of EMC and herpes simplex viruses (10). Incubation of confluent FS-4 cell cultures for a period of 6 to 8 days after the last addition of fresh medium was important for the development of virus resistance. Such continued incubation of confluent cultures appears to be associated with constitutive production of small amounts of IFN-β (10). To determine whether this constitutive production of IFN-β had a role in the ability of TNF to induce an antiviral state, we treated confluent FS-4 cell cultures for 6 h with IFN-β in the concentration range of 0.05 to 0.2 units/ml, starting 2 days after the last addition of fresh medium, and the antiviral activity of TNF at different concentrations was then determined in these cells. [A partially purified preparation of IFN-β obtained from poly(I) · poly(C)-induced FS-4 cell cultures was used in these experiments. This is referred to as IFN-β since the particular mixture of subspecies present is not known. It is likely that IFN-β_1 predominates in this preparation.] TNF alone did not elicit an antiviral state in cultures replenished with fresh medium 2 days before the experiment but did elicit cell protection in cultures that were exposed beforehand to a low concentration of IFN-β (Fig. 1A). The amount of IFN-β used in these experiments was not, by itself, sufficient to protect the cells from EMC virus infection (Fig. 1A). The Northern blot analysis in Fig. 1B confirmed that FS-4 cells exposed to IFN-β at a concentration of 0.5 units/ml for 9 h and then treated with TNF express a higher steady-state level of the 1.3-kb IFN-β_2 mRNA than cells treated with TNF alone or IFN-β (0.5 units/ml) alone. Figure 1B also shows that there is no detectable expression of the 0.9-kb IFN-β_1 mRNA in these cells. Numerous additional experiments failed to reveal any mRNA hybridizable with an IFN-α_1 cDNA probe in TNF- and IFN-β-treated FS-4 cells (data not shown).

Enhancement of poly(I) · poly(C)-induced IFN production by TNF. FS-4 cell cultures treated with TNF for 15 h at concentrations in the range of 3 to 100 ng/ml responded to the IFN inducer poly(I · C) by producing a three- to four-fold-higher yield of IFN-β (Fig. 2A). This increase was blocked when the exposure of cells to TNF occurred in the presence of anti-IFN-β serum, suggesting that endogenous TNF-induced IFN-β_2 was involved in the "priming" of FS-4 cell cultures (see reference 25 for a discussion of the priming effect of IFNs). The role of endogenous TNF-induced IFN-β_2 in regulating its own synthesis in response to poly(I) · poly(C) is illustrated in the Northern blot analysis shown in Fig. 2B. Poly(I) · poly(C) induced both IFN-β_1 and

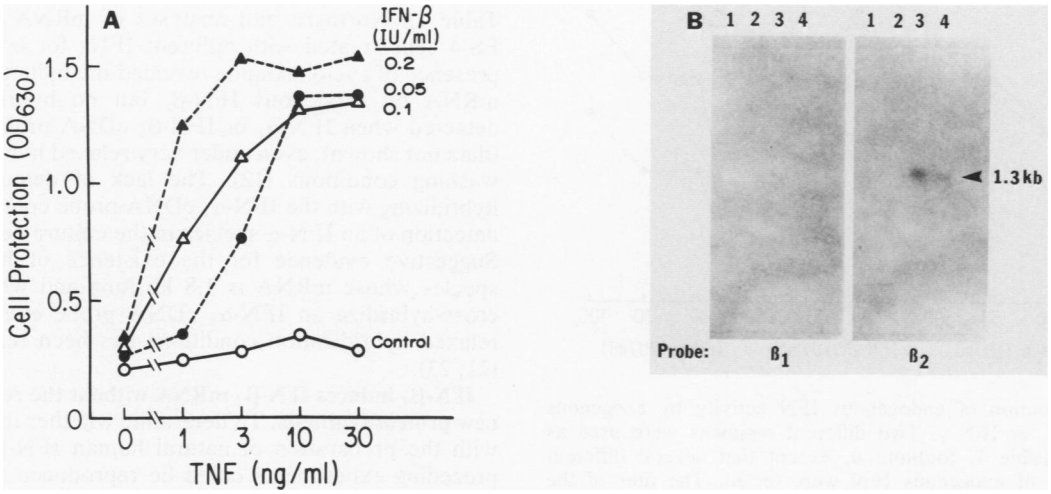

FIG. 1. Enhancement of the antiviral effect (A) and IFN-β_2 mRNA expression (B) by trace amounts of exogenous IFN-β in TNF-treated fibroblasts. (A) Confluent cultures of FS-4 cells in 24-well tissue culture plates were replenished with fresh medium containing 5% fetal calf serum and incubated for only 2 days before use. Trace amounts of IFN-β were added directly to the medium in duplicate sets of wells to give final concentrations in the range from 0.05 to 0.2 IU/ml. Six hours later, TNF was added in increasing concentrations to the medium as indicated. After another 24 h, EMC virus (3,000 PFU per well) was added to the cultures in 100 μl of medium. Protection of cells from the cytopathic effect of EMC virus was determined by the dye uptake method as described previously (10). OD_{630}, Optical density at 630 nm. (B) FS-4 cells (four flasks per group) were treated with IFN-β (0.5 IU/ml) for 9 h and then with TNF (30 ng/ml) for 6 h. Cellular polyadenylated RNA was analyzed by blot hybridization with a ^{32}P-end-labeled IFN-β_2 oligonucleotide probe and then with a nick-translated ^{32}P-labeled IFN-β_1 cDNA probe (10, 13). Lanes: 1, untreated control; 2, IFN-β alone; 3, IFN-β and TNF; 4, TNF alone.

IFN-β_2 in FS-4 cell cultures (Fig. 2B, lane 5). The induction of IFN-β_2 by poly(I) · poly(C), but not IFN-β_1, was enhanced in cells treated with TNF for 15 h (Fig. 2B, lane 6). This increase was less if anti-IFN-β serum was included in the culture medium during the TNF treatment (Fig. 2B, lane 4). Anti-IFN-β serum did not affect the expression of IFN-β_2 mRNA per se in response to TNF alone (compare lanes 2 and 3 in Fig. 2B), but inhibited the ability of the secreted IFN-β to enhance poly(I) · poly(C) induction of IFN-β_2 (compare lanes 4 and 6 in Fig. 2B). The data in Fig. 2 suggest that the increase in IFN titer seen in the medium of TNF- and poly(I) · poly(C)-treated cultures (Fig. 2A) corresponds to the increase in IFN-β_2 expression (Fig. 2B).

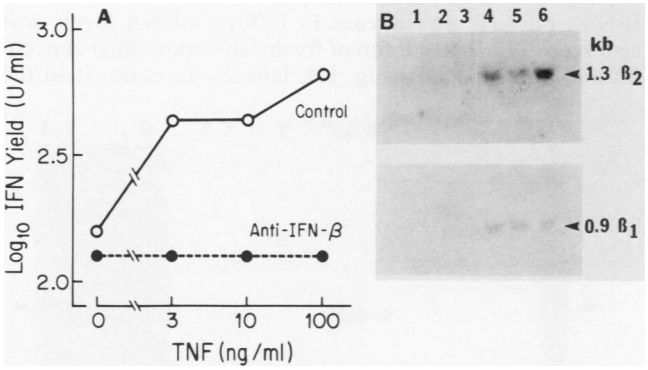

FIG. 2. Enhancement of poly(I) · poly(C)-induced IFN production (A) and IFN-β_2 mRNA expression (B) by TNF. (A) FS-4 cells cultured in 24-well plates were treated with the indicated concentrations of TNF. Rabbit anti-IFN-β serum (5 μl per well) was added to half the cultures together with TNF. After 15 h poly(I) · poly(C) was added (final concentration, 10 μg/ml), and the cultures were incubated for another 1 h. The cultures were then washed extensively with phosphate-buffered saline and incubated for 20 h with fresh medium (0.5 ml per well). The culture fluids were then collected, and the IFN titer was determined as described earlier (10). (B) FS-4 cells (four flasks per group) were treated with TNF (30 ng/ml) for 15 h and then with poly(I) · poly(C) (10 μg/ml) for 2.5 h. Two groups were also treated with TNF in the presence of rabbit anti-IFN-β serum (100 μl per flask). Cellular polyadenylated RNA was analyzed by blot hybridization with an IFN-β_2 probe and then with an IFN-β_1 probe. Lanes: 1, untreated control; 2, TNF alone; 3, TNF in the presence of anti-IFN-β serum; 4, TNF in the presence of anti-IFN-β serum and then poly(I) · poly(C); 5, poly(I) · poly(C) alone; 6, TNF and then poly(I).

Induction of endogenous IFN production by exogenous IFNs. We explored the possibility that other exogenously added IFNs also induce endogenous IFNs in FS-4 cell cultures. Two sets of experiments were carried out to test this possibility. In one set, FS-4 cells were treated with different IFNs at different concentrations for 15 h and then subjected to the cycloheximide-actinomycin D "superinduction regimen" described in the legend to Table 1 (see references 25 and 29 for a discussion of the rationale for the superinduction regimens used; Table 1 describes a representative experiment of several carried out). IFN production in these cultures was monitored over the next 15 h. In the other set, FS-4 cell cultures were treated with different IFNs at different concentrations simultaneously with cycloheximide for 8 h, and IFN production was monitored over the next 15 h (Fig. 3). It is clear from the data summarized in Fig. 3 that all three types of IFNs tested, IFN-α, -β, and -γ, can induce endogenous IFN in FS-4 cells detectable in the culture medium. Comparable IFN yields were obtained with the different protocols tested.

Characterization of the induced endogenous IFN, based on neutralization with antisera specific for IFN-α, IFN-β, or IFN-γ, is summarized in Table 1. These data show that when FS-4 cells were treated with IFN-α, -β, or -γ for 15 h before the addition of cycloheximide, the IFN produced was largely IFN-β. When the exogenous IFNs were added at the same time as cycloheximide, the IFN activity in the culture medium was either largely IFN-α or a mixture of IFN-α and

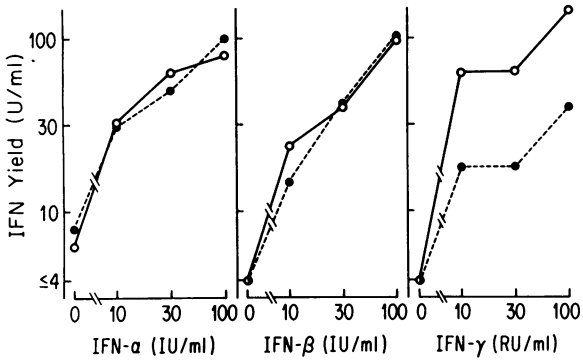

FIG. 3. Induction of endogenous IFN activity by exogenous IFN-α, IFN-β, or IFN-γ. Two different regimens were used as described in Table 1, footnote *a*, except that several different concentrations of exogenous IFN were tested. The titer of the endogenous IFN activity produced when exogenous IFN treatment preceded cycloheximide by 15 h is shown by the open circles, while that produced when exogenous IFN was added simultaneously with cycloheximide is shown by the closed circles.

-β (Table 1). IFN activity (10 to 100 units/ml) of the β serotype was also detected in the culture medium of FS-4 cells appropriately induced with TNF (data not shown).

The Northern blot analysis in Fig. 4 shows that the IFN mRNA detectable in FS-4 cell cultures treated with different IFNs for 18 h is IFN-β_2. No hybridization was detectable with IFN-α_1 or IFN-β_1 cDNA probes (this experiment is comparable to the −15 to 0 h IFN treatment experiment in Table 1). Northern blot analyses of mRNA isolated from FS-4 cells treated with different IFNs for 6 to 8 h in the presence of cycloheximide revealed the induction of IFN-β_2 mRNA by exogenous IFN-β, but no hybridization was detected when IFN-α_1 or IFN-β_1 cDNA probes were used (data not shown), even under very relaxed hybridization and washing conditions (12). The lack of detectable mRNA hybridizing with the IFN-α_1 cDNA probe contrasts with the detection of an IFN-α species in the culture fluids (Table 1). Suggestive evidence for the existence of human IFN-α species whose mRNA is 1.8 kb long and which does not cross-hybridize an IFN-α_1 cDNA probe even under very relaxed hybridization conditions has been reported earlier (21, 23).

TABLE 1. Serologic characterization of endogenous IFN activity produced in FS-4 cells by exogenous IFN-α, IFN-β, and IFN-γ

Exogenous IFN[a]		Residual titer of endogenous IFN (units/ml) after mixing with antiserum[b]:			
Treatment	Type	Anti-IFN-α	Anti-IFN-β	Anti-IFN-γ	No serum
−15 to 0 h	α	130	<4	130	130
	β	140	6	130	160
	γ	190	50	190	180
	None	<4	<4	<4	<4
0 to 8 h	α	10	42	70	60
	β	10	80	72	80
	γ	12	16	30	32
Antiserum[c]					
IFN-α		<4	42	70	60
IFN-β		400	<4	500	400
IFN-γ		64	64	<4	60

[a] FS-4 cells cultured for 2 days after medium replenishment in 24-well plates were treated with exogenous IFN-α, IFN-β, or IFN-γ (each at 300 IU/ml) and incubated for 15 h at 37°C. Cycloheximide (50 μg/ml) was then added for 8 h followed by actinomycin D (2 μg/ml) for 1 h. The cultures were washed with phosphate-buffered saline (six times, 1 ml each time) and incubated with 0.5 ml of minimal essential medium per ml (four wells per group) containing 5% fetal calf serum for another 15 h. The culture fluids were collected, pooled, and stored at −80°C. Another set of cultures was treated similarly except that the exogenous IFNs were added simultaneously with cycloheximide. 0 h refers to the time of addition of cycloheximide. See references 25 and 29 for a discussion of the rationale for the superinduction regimens used.

[b] Each endogenous IFN sample (100 μl) was incubated for 60 min at 37°C with 20 μl of antibodies to IFN-α (rabbit serum, diluted 10 times), to IFN-β (calf serum, diluted five times), to IFN-γ (monoclonal antibody, culture fluid), or with 20 μl of minimal essential medium containing 5% fetal calf serum, and then titrated for residual IFN activity.

[c] To ascertain specificity each antibody was tested in parallel with appropriate reference IFN preparations.

IFN-β_1 induces IFN-β_2 mRNA without the requirement for new protein synthesis. To determine whether the effects seen with the preparation of natural human IFN-β used in the preceding experiments could be reproduced with homogeneous IFN-β_1, we carried out the experiment illustrated in Fig. 5. This Northern blot analysis shows that the steady-state level of IFN-β_2 can be increased by exposure of FS-4 cells to *E. coli*-derived homogeneous IFN-β_1^{17Ser} for 17 h (Fig. 5, lane 2). Cycloheximide by itself increases the steady-state level of IFN-β_2 mRNA (Fig. 5, lane 3) (6, 10, 32). The addition of rIFN-β_1^{17Ser} to FS-4 cells in the presence of cycloheximide caused an even higher level of expression of IFN-β_2 mRNA (Fig. 5, compare lanes 3 and 4). Thus IFN-β_2 is directly inducible by IFN-β_1 in the presence of cycloheximide (Fig. 5) but not by IFN-α or -γ (data not shown).

PDGF induces IFN-β_2. The growth factor PDGF was tested for its ability to increase IFN-β_1 and -β_2 mRNA expression in human fibroblasts. The blot-hybridization data in Fig. 6 indicate that the addition of PDGF for 8 h in the concentration range of 1 to 10 units/ml to confluent, serum-depleted FS-4 cell cultures led to an increase in the level of IFN-β_2 mRNA. An increase in IFN-β_2 mRNA levels was also seen after the addition of fresh calf serum (final concentration, 10%, vol/vol) (Fig. 6B, lane 2). In contrast to the

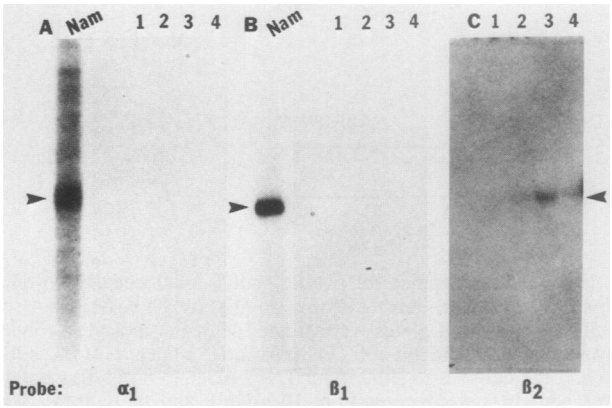

FIG. 4. Increased expression of IFN-β_2 mRNA in FS-4 cells treated with IFN-α, IFN-β, or IFN-γ (300 IU/ml). FS-4 cells (four flasks per group) were treated with IFN-α, IFN-β, or IFN-γ (without cycloheximide or actinomycin D) at a final concentration of 300 IU/ml for 18 h. Cellular polyadenylated RNA was extracted and analyzed by blot hybridization with IFN-α_1, IFN-β_1, and IFN-β_2 probes. A blot of mRNA from Sendai virus-induced human Namalwa cells was included as an internal control in the IFN-α_1 and IFN-β_1 hybridizations (13). These probes detect the 0.8- to 1.4-kb IFN-α_1-hybridizable mRNA and the 0.9-kb IFN-β_1 mRNA in induced Namalwa (Nam) cells as indicated by the arrowheads. Lanes: 1, untreated control; 2, IFN-α-treated cells; 3, IFN-β-treated cells; 4, IFN-γ-treated cells.

observations of Zullo et al. (33) with murine BALB/c-3T3 cells, no hybridization was detected when these blots were probed with an IFN-β_1 cDNA. Similarly, no hybridization was detected by using an IFN-α_1 cDNA probe.

IL-1 induces IFN-β_2. The growth factor IL-1 was also tested for its ability to affect IFN-β_1 and IFN-β_2 expression in human fibroblasts. The Northern blot analysis shown in Fig. 7 shows that treatment of FS-4 cells with IL-1α for 12 h greatly increased the steady-state level of IFN-β_2 mRNA but not that of IFN-β_1 mRNA.

PDGF and IL-1 override the antiviral effect of TNF. It is known that mitogens such as PDGF can interfere with the antiviral and antiproliferative effects of IFNs (11, 16, 26). We have confirmed that PDGF can decrease the antiviral effect of rIFN-β_1^{17Ser} in FS-4 cells (data not shown). Thus, it appeared that PDGF could both induce IFN-β and block its biological effects. This possibility was tested in an experiment in which we attempted to determine whether PDGF could elicit an antiviral state in FS-4 cells (Fig. 8A). We also tested the effect of PDGF on the antiviral state elicited by TNF in these cultures. It is clear from the data in Fig. 8A that PDGF by itself did not elicit an antiviral state in FS-4 cell cultures even though it led to the increased expression of IFN-β_2 (Fig. 6). Furthermore, PDGF could abolish the antiviral state elicited by TNF.

IL-1 also did not induce an antiviral state by itself in FS-4 cell cultures (Fig. 8B). It blocked the antiviral effect of TNF in these cells (Fig. 8B) and inhibited the antiviral activity of exogenously added IFN-β (data not shown).

DISCUSSION

Many of the interactions described in this paper involve the increased expression of the cytokine IFN-β_2 in diploid fibroblast culures (Fig. 9). This endogenous cytokine is a glycosylated protein of apparent molecular weight of 21,000; it is translated from an mRNA of 1.3 kb that contains an open reading frame of 212 amino acids of which the first 30 to 33 amino acids form a hydrophobic stretch that could act as the secretory signal (19, 22, 24, 31, 32; May et al., in press). Biologically active recombinant human IFN-β_2 has been produced in heterologous rodent cell expression systems (18, 32). IFN-β_2 shares distinct amino acid sequence similarities with IFN-β_1 (May et al., in press), and the antiviral activity of IFN-β_2 can be neutralized by polyclonal and monoclonal antisera raised against the IFN-β_1 protein (18–20, 22, 31, 32). These findings are the basis for the designation "IFN-β_2" in keeping with the guidelines of the Committee on Interferon Nomenclature (5), although the 1.3-kb IFN-β_2 mRNA and the 0.9-kb IFN-β_1 mRNA do not cross-hybridize the respective heterologous cDNA probes and can thus be readily distinguished from each other in Northern blot analyses (22, 31).

The expression of IFN-β_2 mRNA in human diploid fibroblasts is markedly affected by the physiological state of the

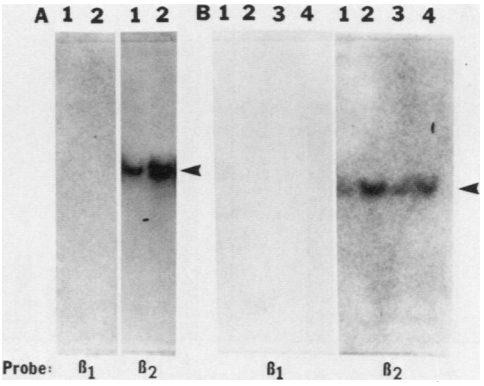

FIG. 6. PDGF and bovine serum increase expression of IFN-β_2 mRNA in FS-4 cells. (A) Quiescent FS-4 cells (10 150-mm plastic petri dish cultures per group), which were given their last medium replenishment 8 to 10 days earlier, were treated with PDGF (10 units/ml) in 5 ml of conditioned medium per culture for 8 h, and the cellular polyadenylated RNA was analyzed by blot hybridization with first the IFN-β_2 probe and then the IFN-β_1 probe. Lane 1, Untreated control; lane 2, PDGF-treated cells. (B) Quiescent FS-4 cells (seven 150-mm plastic petri dish cultures per group) were treated with 10% (vol/vol) calf serum (GIBCO) or PDGF for 8 h. Lane 1, Untreated control; lane 2, 10% calf serum; lane 3, PDGF (1 unit/ml); lane 4, PDGF (10 units/ml).

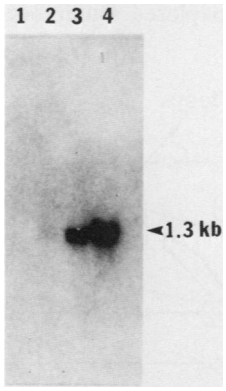

FIG. 5. Recombinant *E. coli*-derived IFN-β_1^{17Ser} induces IFN-β_2 mRNA. FS-4 cells were treated with rIFN-β_1^{17Ser} (500 IU/ml) for 17 h (lane 2; five flasks per group) or with αIFN-β_1^{17Ser} (500 IU/ml) in the presence of cycloheximide (50 μg/ml) for 9 h (lane 4; three flasks per group). Lane 1 represents an untreated control group (five flasks), and lane 3 represents a control group treated only with cycloheximide (three flasks). The blot was hybridized with the IFN-β_2 probe.

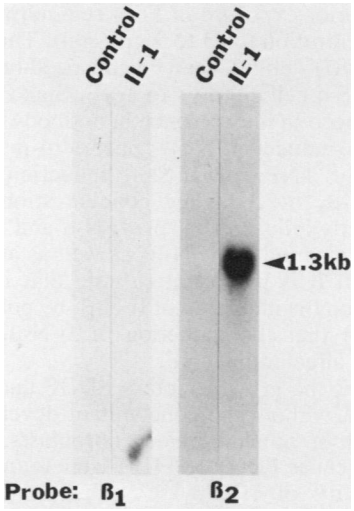

FIG. 7. IL-1α increases expression of IFN-β_2 mRNA in fibroblasts. FS-4 cells (five flasks per group) were treated with IL-1α (20 units/ml) for 12 h, and the cellular polyadenylated RNA was analyzed by blot hybridization with first the IFN-β_2 probe and then the IFN-β_1 probe. Lane 1, Untreated control; lane 2, IL-1α-treated cells.

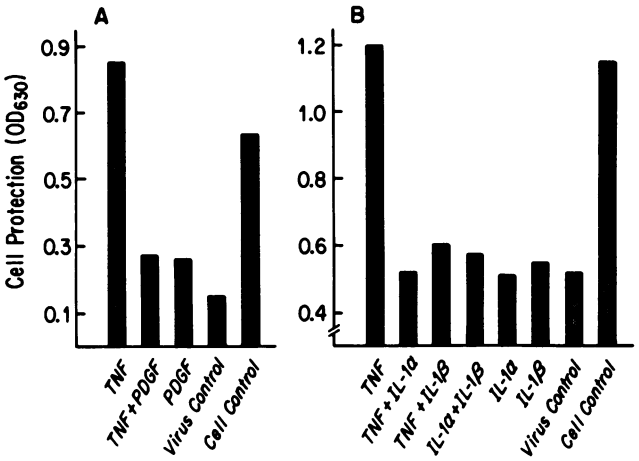

FIG. 8. PDGF and IL-1 override the antiviral effect of TNF. FS-4 cells were seeded in 24-well plates and replenished with fresh medium as described in the legend to Fig. 1. Six (A) and seven (B) days after medium change, TNF (30 ng/ml), PDGF (2.5 units/ml), IL-1α (1 ng/ml; 30 units/ml), IL-1β (30 ng/ml), and combinations as indicated were added directly to duplicate cultures. Protection of cells from the cytopathic effect of EMC virus was determined as described in the legend to Fig. 1 and in reference 10. OD_{630}, Optical density at 630 nm.

cells in culture. Confluent, quiescent FS-4 cell cultures constitutively express IFN-β_2 at a low level (10). The present experiments show that such cultures respond to a variety of growth stimuli [TNF, PDGF, IL-1, bovine serum, and poly(I) · poly(C)] by increasing the amount of the endogenous IFN-β_2 mRNA. The subsequent secretion of endogenous IFN-β_2 into the culture medium appears to be important in modulating or mediating several of the biological effects of some of these growth factors.

Complex cytokine interactions in FS-4 cell cultures. IFN-β_2 appears to suppress the cell growth-stimulating action of TNF while mediating its antiviral effect in FS-4 cells (10). Furthermore, the induction of IFN-β_2 by TNF can be enhanced by prior exposure of FS-4 cell cultures of IFN-β_1 at a low concentration (0.03 to 3 units/ml). The induction of IFN-β_2 by poly(I) · poly(C) can be enhanced further by prior exposure of FS-4 cell cultures to TNF. Since this enhancement was reduced in the presence of antibody to IFN-β, the secreted, TNF-induced IFN-β_2 appears to mediate the enhancement, by TNF, of IFN-β_2 induction by poly(I) · poly(C). IFN-β_1 at a higher concentration (10 to 300 units/ml) directly induces IFN-β_2. IFN-α and IFN-γ at high concentration also induce IFN-β_2 as well as an endogenous IFN-α species. It is important to note that the enhancing effect of TNF on the induction of IFN-β_2 by poly(I) · poly(C) is selective in that the induction of IFN-β_1 by poly(I) · poly(C) is not affected by TNF.

Unlike TNF, the growth factors PDGF and IL-1 induce IFN-β_2 mRNA without the concomitant development of an antiviral state in human diploid fibroblasts. This is not unexpected because PDGF and IL-1 were found to block the antiviral activity of IFN-β_1^{17Ser}. Thus, although strong mitogens such as PDGF induce endogenous IFN-β_2, they can apparently override at least some of the effects of the endogenous IFN in FS-4 cells. Action of IL-1 is dependent on cell type because IL-1 produced an antiviral state in human osteosarcoma MG63 cells (27, 28; M. Kohase and J. Vilček, unpublished data).

The IFN-β_1 and IFN-β_2 inducer poly(I) · poly(C) also stimulates cell growth in human diploid fibroblast cultures (2; J. Vilček, M. Kohase, and D. Henriksen-DeStefano, J. Cell. Physiol., in press). Anti-IFN-β serum further enhanced the growth stimulatory effect of poly(I) · poly(C), suggesting that the induction of IFN-β_1 or IFN-β_2 or both by poly(I) · poly(C) exerts a feedback inhibition on cell proliferation (Vilček et al., in press).

TNF induces IL-1 expression in macrophages (1, 7), endothelial cells (15), and FS-4 fibroblasts (J. Le, D. Weinstein, U. Gubler, and J. Vilček, submitted for publication). Available results (10; present studies) suggest that in FS-4 cells the induction of IFN-β_2 by TNF, leading to the development of an antiviral state, is dominant over both the induction of IL-1 by TNF and the blocking effect of this IL-1 on the IFN-β_2-mediated antiviral state.

In the present experiments, TNF and rIFN-β_1^{17Ser} increased the steady-state levels of IFN-β_2 mRNA in the presence of cycloheximide, indicating that new protein synthesis is not required for this induction. The observation that rIFN-β_1^{17Ser} directly induced IFN-β_2 expression, whereas IFN-α did not, suggests that although IFN-α and -β_1 compete for the same cell surface receptor (3), there can be differences in the intracellular events that they elicit.

The increase in gene expression mediated by the endogenous production of a second cytokine is sensitive to inhibition by cycloheximide. Thus, the increase in the expression of class I HLA mRNA in TNF-treated human endothelial cells and fibroblasts can be blocked by cycloheximide (4) and by anti-IFN-β serum (May et al., in press). Therefore, the increase in class I HLA gene expression by TNF is likely to be, at least in part, an indirect effect of the endogenous secretion of IFN-β_2 in these cultures.

There are differences in the behavior of the IFN-β_2 gene in different human cells. Human lymphoblastoid cells (Namalwa), which appear to contain an intact IFN-β_2 gene as judged by restriction fragment analysis, did not express detectable IFN-β_2 mRNA in response to inducers that led to the expression of IFN-β_1 mRNA in Namalwa cells and IFN-β_1 and IFN-β_2 in FS-4 cells (24; P. B. Sehgal and L. T. May, *in* K. Cantell and H. Schellekens, ed., *The Biology of The Interferon System 1986*, Martinus Nijhoff, in press). We anticipate that similar cell-type- and tissue-specific differences will be uncovered as the cytokine networks operative in other systems are explored. Differences in the endogenous

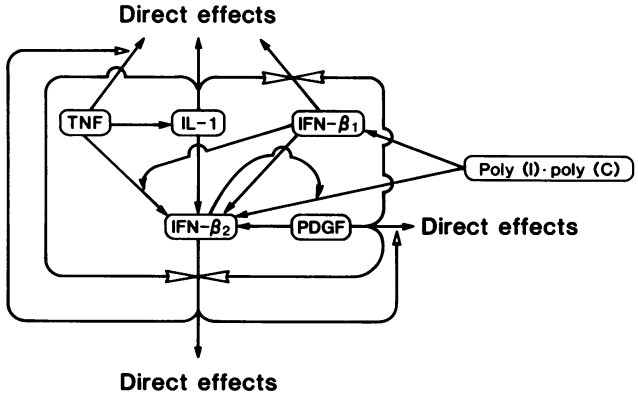

FIG. 9. Cytokine network in human diploid fibroblast cultures. Solid arrowheads represent stimulatory effects, open arrowheads represent inhibitory effects, and the relative size of the open arrowheads represents the relative strength of the inhibitory effects.

production of cytokines in local tissue environments in response to particular stimuli may contribute to the diversity of the biological responses observed.

Conclusions. IFNs have emerged as important autocrine regulators of cell growth and function. In human diploid fibroblasts, the endogenous production of IFN-β_2 is highly responsive to the presence of other growth-modulatory cytokines such as TNF, PDGF, IL-1, and IFN-β_1. A complex network of interactions is triggered when one or more of these agents is present in the cellular environment (Fig. 9). IFN-β_2 appears to mediate the antiviral effect of TNF and to modulate the mitogenic effect of TNF. The production of IFN-β_2 in response to TNF can be increased further by trace amounts of IFN-β_1. At higher concentrations, IFN-β_1 can directly induce IFN-β_2 expression. Some of the growth factors such as PDGF and IL-1 can, at appropriate concentrations, override the biological effects of endogenous or exogenous IFN-β. A growth factor such as TNF that does not interfere with IFN-β action can induce another endogenous growth factor, such as IL-1, that does. Thus, the apparent biological effects of a particular cytokine in human diploid fibroblast cultures represent the cumulative result of the qualitative, quantitative, and temporal interrelationships summarized briefly in Fig. 9. Cellular homeostasis is likely to represent a balance among these several antagonistic and coagonistic interactions.

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LITERATURE CITED

1. **Bachwich, P. R., S. W. Chensue, J. W. Larrick, and S. L. Kunkel.** 1986. Tumor necrosis factor stimulates interleukin-1 and prostaglandin E_2 production in resting macrophages. Biochem. Biophys. Res. Commun. **136**:94–101.
2. **Barrack, E. R., and M. D. Hollenberg.** 1981. Mitogenesis in normal human fibroblasts by polyinosinic·polycytidylic acid and other synthetic acidic polymers: enhancement of action by glucocorticoids. J. Cell. Physiol. **108**:445–454.
3. **Branca, A., and C. Baglioni.** 1981. Evidence that types I and II interferons have different receptors. Nature (London) **294**: 768–770.
4. **Collins, T., L. A. Lapierre, W. Fiers, J. L. Strominger, and J. S. Pober.** 1986. Recombinant tumor necrosis factor increases mRNA levels and surface expression of HLA-A, B antigens in vascular endothelial cells and dermal fibroblasts *in vitro*. Proc. Natl. Acad. Sci. USA **83**:446–450.
5. **Committee on Interferon Nomenclature.** 1980. Interferon nomenclature. Nature (London) **286**:110.
6. **Content, J., L. De Wit, P. Poupart, G. Opdenakker, J. Van Damme, and A. Billiau.** 1985. Induction of a 26-kDa-protein mRNA in human cells treated with interleukin-1-related, leukocyte-derived factor. Eur. J. Biochem. **152**:253–257.
7. **Dinarello, C. A., J. G. Cannon, S. M. Wolff, H. A. Bernheim, B. Beutler, A. Cerami, I. Figari, M. A. Palladino, Jr., and J. V. O'Connor.** 1986. Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. J. Exp. Med. **163**:1433–1450.
8. **Gresser, I.** 1977. On the varied biologic effects of interferon. Cell. Immunol. **34**:406–415.
9. **Havell, E. A., B. Berman, C. A. Ogburn, K. Berg, K. Paucker, and J. Vilček.** 1975. Two antigenically distinct species of human interferon. Proc. Natl. Acad. Sci. USA **72**:2185–2190.
10. **Kohase, M., D. Henriksen-DeStefano, L. T. May, J. Vilček, and P. B. Sehgal.** 1986. Induction of β_2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. Cell **45**:659–666.
11. **Lin, S. L., T. Kikuchi, W. J. Pledger, and I. Tamm.** 1986. Interferon inhibits the establishment of competence in G_0/S phase transition. Science **233**:356–359.
12. **May, L. T., and P. B. Sehgal.** 1985. On the relationship between human interferon α_1 and β_1 genes. J. Interferon Res. **5**:597–604.
13. **May, L. T., P. B. Sehgal, K. S. LaForge, and M. Inouye.** 1983. Expression of the native α and β interferon genes in human cells. Virology **129**:116–126.
14. **Moore, R. N., H. S. Larsen, D. W. Horohov, and B. T. Rouse.** 1984. Endogenous regulation of macrophage proliferative expansion by colony-stimulating factor-induced interferon. Science **223**:178–181.
15. **Nawroth, P. P., I. Bank, D. Handley, J. Cassimeris, L. Chess, and D. Stern.** 1986. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. J. Exp. Med. **163**:1363–1375.
16. **Oleszak, E., and A. D. Inglot.** 1980. Platelet-derived growth factor (PDGF) inhibits antiviral and anticellular action of interferon in synchronized mouse or human cells. J. Interferon Res. **1**:37–48.
17. **Resnitzky, D., A. Yarden, D. Zipori, and A. Kimchi.** 1986. Autocrine β-related interferon controls c-*myc* suppression and growth arrest during hematopoietic cell differentiation. Cell **46**:31–40.
18. **Revel, M., R. Ruggieri, and A. Zilberstein.** 1986. Expression of the human IFN-β_2 genes in rodent cells, p. 207–216. *In* H. Schellekens and W. E. Stewart II (ed.), The biology of the interferon system 1985. Elsevier Biomedical Press, Amsterdam.
19. **Sagar, A. D., P. B. Sehgal, D. L. Slate, and F. H. Ruddle.** 1982. Multiple human β interferon genes. J. Exp. Med. **156**:744–755.
20. **Sehgal, P. B., M. Inoue, and Y. H. Tan.** 1984. Detection of human β_2 interferon using a radioimmunoassay. J. Interferon Res. **4**:63–66.
21. **Sehgal, P. B., L. T. May, K. S. LaForge, and M. Inouye.** 1982. Unusually long mRNA species coding for human α and β interferons. Proc. Natl. Acad. Sci. USA **79**:6932–6939.
22. **Sehgal, P. B., and A. D. Sagar.** 1980. Heterogeneity of poly(I) · poly(C)-induced human fibroblast interferon mRNA species. Nature (London) **288**:95–97.
23. **Sehgal, P. B., A. D. Sagar, and I. A. Braude.** 1981. Further heterogeneity of human α interferon mRNA species. Science **214**:803–805.
24. **Sehgal, P. B., A. Zilberstein, M.-R. Ruggieri, L. T. May, A. Ferguson-Smith, D. L. Slate, M. Revel, and F. H. Ruddle.** 1986. Human chromosome 7 carries the β_2 interferon gene. Proc. Natl. Acad. Sci. USA **83**:5219–5222.
25. **Stewart, W. E., II.** 1979. The interferon system. Springer-Verlag, New York.
26. **Taylor-Papadimitriou, J., M. Shearer, and E. Rozengurt.** 1981. Inhibitory effect of interferon on cellular DNA synthesis: modulation by pure mitogenic factors. J. Interferon Res. **1**:401–409.
27. **Van Damme, J., M. De Ley, G. Opdenakker, A. Billiau, P. De Somer, and J. Van Beeumen.** 1985. Homogeneous interferon-inducing 22K factor is related to endogenous pyrogen and interleukin-1. Nature (London) **314**:266–268.
28. **Van Damme, J., G. Opdenakker, A. Billiau, P. De Somer, L. De Wit, P. Poupart, and J. Content.** 1985. Stimulation of fibroblast interferon production by a 22 K protein from human leukocytes. J. Gen. Virol. **66**:693–700.

29. **Vilček, J., and E. A. Havell.** 1973. Stabilization of interferon messenger RNA activity by treatment of cells with metabolic inhibitors and lowering of the incubation temperature. Proc. Natl. Acad. Sci. USA **70**:3909–3913.
30. **Vilček, J., V. J. Palombella, D. Henriksen-DeStefano, C. Swenson, R. Feinman, M. Hirai, and M. Tsujimoto.** 1986. Fibroblast growth-enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. J. Exp. Med. **163**:632–643.
31. **Weissenbach, J., Y. Chernajovsky, M. Zeevi, L. Shulman, H. Soreq, U. Nir, D. Wallach, M. Perricaudet, P. Tiollais, and M. Revel.** 1980. Two interferon mRNAs in human fibroblasts: *in vitro* translation and *Escherichia coli* cloning studies. Proc. Natl. Acad. Sci. USA **77**:7152–7156.
32. **Zilberstein, A., R. Ruggieri, and M. Revel.** 1985. Human interferon-β_2: is it an interferon inducer?, p. 73–83. *In* G. B. Rossi and E. Dianzani (ed.), The interferon system, Serono Symposia, vol 24. Raven Press, New York.
33. **Zullo, J. N., B. H. Cochran, A. S. Huang, and C. D. Stiles.** 1985. Platelet-derived growth factor and double-stranded ribonucleic acids stimulate expression of the same genes in 3T3 cells. Cell **43**:793–800.