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Beta Interferon Subtype 1 Induction by Tumor Necrosis Factor

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Tumor necrosis factor (TNF) induces an antiviral state in various cell lines. This antiviral state is quite similar to that established by interferon (IFN), e.g., TNF treatment of HEp-2 cells induces 2′,5′-oligoadenylate synthetase activity. Both antiviral activity and synthetase induction are greatly reduced when TNF treatment occurs in the presence of a beta interferon subtype 1 (IFN-β_1)-neutralizing antiserum. However, no one has yet directly demonstrated IFN-β_1 induction, either as an antiviral activity in supernatants from TNF-treated cells or as IFN-specific mRNA by Northern (RNA) blot analysis. We have adopted a recently described in vitro DNA amplification protocol for the detection of specific RNAs. By applying this method to RNA from HEp-2 cells, we could demonstrate increased levels of IFN-β_1-specific transcripts after TNF treatment. Dose response and kinetics of IFN-β_1 induction coincided with the TNF-induced antiviral state. Nuclear run-on analysis showed enhanced transcriptional activity of the IFN-β_1 gene in TNF-treated cells. Our data substantiate a role of IFN-β_1 as mediator of the biological activity of TNF in HEp-2 cells.

Current evidence suggests a role for tumor necrosis factor (TNF) in host defense against infectious diseases. TNF has been shown to be induced by different types of infectious agents, i.e., bacteria and viruses (1, 2). Among its numerous biological activities, an antiviral response to TNF in a number of cell lines has been described recently (11, 18, 26). This TNF-induced antiviral activity shares some similarity to the interferon (IFN)-induced antiviral state. In human HEp-2 cells, pretreatment with TNF reduced vesicular stomatitis virus yield and prevented infected-cell lysis (18). Optimal protection required treatment of cells for 6 to 12 h before infection. Also, TNF induced 2′,5′-oligoadenylate synthetase (2-5A synthetase), an enzyme which is otherwise specifically induced only by IFNs, and both induction of 2-5A synthetase and inhibition of virus growth were partially abolished by TNF treatment in the presence of a neutralizing antiserum to beta interferon subtype 1 (IFN-β_1). Treatment with anti-IFN antisera reduced both the TNF-induced antiviral activity and the enhancement of major histocompatibility complex (MHC) class I expression (11, 13, 15). In other studies, Wong and Goeddel (26) showed that TNF induced an antiviral state in human 7860 renal carcinoma cells that was effective against vesicular stomatitis virus, encephalomyocarditis virus, adenovirus type 2, and herpes simplex virus type 2 and induced 2-5A synthetase. In this study, anti-IFN antisera had no effect on the antiviral state. However, in all studies reported to date, induction of IFN-β_1 by TNF has not been directly demonstrated. TNF does enhance the expression of the interleukin 6 (IL-6) gene (also known as IFN-β_2, B-cell stimulatory factor 2 (BSF-2), hybridoma-plasmocytoma growth factor, or 26-kilodalton protein [5, 11, 25, 27]). Several groups have ascribed an antiviral activity to IL-6–IFN-β_2 which could be neutralized by an antiserum to natural IFN-β_1 (11, 12, 28). A role for IL-6 as mediator of the TNF-induced antiviral activity was thus proposed (11). However, this claim has been questioned recently by a number of reports which failed to demonstrate a significant antiviral activity of recombinant IL-6 or inhibition of TNF-induced antiviral activity by antibodies to recombinant IL-6 (21, 24).

By applying a modified in vitro DNA amplification procedure (polymerase chain reaction [PCR]) to total RNA from TNF-treated HEp-2 cells, we provided direct evidence for induction of IFN-β_1 by TNF treatment. In addition, we demonstrated transcriptional activation of the IFN-β_1 gene following TNF treatment. We thus demonstrated that TNF is an endogenous IFN-β-inducer and substantiated a role for IFN-β_1 as a mediator of the TNF-induced antiviral activity in HEp-2 cells.

MATERIALS AND METHODS

Cell culture, virus, and enzyme assays. Human HEp-2 cells were grown in Eagle minimal essential medium supplemented with 10% heat-inactivated fetal bovine serum. Confluent monolayers were used for experiments. Human recombinant TNF-α (specific activity, 2.9×10^7 U/mg as determined in a mouse L-929 cytotoxicity assay) was supplied by Knoll AG, Ludwigshafen, Federal Republic of Germany. Virus yield reduction assays were performed as described previously (18). Activity of 2-5A synthetase was determined by the poly(I):poly(C)-cellulose method and the radiobinding assay with in vitro-labeled 2′,5′-oligoadenylate {ppp 5′(A2′)$_n$5′A 3′p[^{32}P]Cp} as radioligand (23).

Preparation of RNA and Northern (RNA) blotting. Total RNA was isolated by extracting monolayers into hot phenol (60°C), and 0.8 volume of 0.1 M sodium acetate (pH 5.5)–10 mM EDTA–0.2% sodium dodecyl sulfate (SDS) was added. The aqueous phase was extracted twice and subsequently adjusted to 2.5 M LiCl (14). After 5 h at 4°C, RNA was recovered by centrifugation (15,000 × *g*, 4°C), washed in 70% ethanol, and dissolved in H_2O. RNA was fractionated in formaldehyde gels and transferred onto nylon membranes according to the instructions of the manufacturer (Nytran; Schleicher & Schuell) (16). DNA fragments containing parts of the coding regions of IL-6/IFN-β_2 or IFN-β_1 were radiolabeled with [^{32}P]dCTP by the random priming method (7) to a specific activity of 1×10^9 to 3×10^9 cpm/μg. Conditions

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for hybridization and washings were as detailed for nuclear run-on analysis.

Preparation of RNA, reverse transcription, and in vitro amplification of cDNA by PCR. Cells were lysed in 0.5% Nonidet P-40 containing lysis buffer (150 mM NaCl, 10 mM Tris hydrochloride [pH 7.4], 5 mM Vanadyl-ribonucleotide). Nuclei were removed by centrifugation, and cytoplasmic supernatants were extracted into phenol-chloroform containing 0.1 M sodium acetate (pH 5.5), 10 mM EDTA, and 0.2% SDS. After four cycles of phenol-chloroform extraction, the aqueous phase was adjusted to 2.5 M LiCl, kept at 4°C for 5 h, and centrifuged (15,000 × *g*, 4°C, 15 min). The pellet was washed in 70% ethanol and dissolved in RNase-free water, and the RNA concentration was determined by UV spectroscopy. For reverse transcriptase reactions, 2 μg from each sample was mixed with the appropriate antisense oligonucleotide primer (1 μM) in reverse transcriptase buffer (50 mM Tris hydrochloride [pH 8.3], 75 mM KCl, 10 mM dithiothreitol, 3 mM $MgCl_2$, four deoxynucleoside triphosphates [0.5 mM each]), heated to 70°C for 5 min, and then placed on ice. A total of 500 U of Moloney murine leukemia virus reverse transcriptase (Bethesda Research Laboratories, Inc.) was added, and the samples were incubated for 30 min at 37°C. Control reactions were performed at the same time, except that Moloney murine leukemia virus reverse transcriptase was omitted. Any PCR-amplified signal observed in these samples originated from genomic DNA contamination in the RNA preparations. The reverse transcriptase reaction was terminated by incubation of the samples at 90°C for 10 min. Amplification of the single-stranded cDNA was initiated by addition of Taq replacement buffer (final concentrations, 20 mM Tris hydrochloride [pH 8.45], 50 mM KCl, 2.5 mM dithiothreitol, 0.1% gelatin, and 0.75 mM for each of the deoxynucleoside triphosphates), sense primer, and additional antisense primer. The samples were heated again at 90°C for 5 min to completely disrupt the cDNA-mRNA duplex. Three units of Taq polymerase (Perkin-Elmer Cetus) was added, and the samples were overlaid with mineral oil. The PCR was performed for 30 cycles consisting of 90 s at 40°C, 120 s at 72°C, and then 90 s at 90°C. During the last cycle, the extension time was lengthened to 10 min. The mineral oil was extracted from each sample with chloroform, and the samples were prepared for electrophoresis. IFN-β_1 transcripts were made in vitro from a 770-base-pair (bp) *Hin*cII fragment cloned into the polylinker of pSP64 (provided by J. A. Hewitt, Baltimore, Md.).

Analysis of PCR products. For detection of the IFN-β_1 mRNA-directed amplification product, 10 μl of each reaction mixture was run on a 2.5% agarose (SeaKem) gel in 1× TBE. For IFN-γ- and IFN-β_1-directed reactions without Moloney murine leukemia virus reverse transcriptase, 25 μl of each reaction was electrophoresed. The amplification products were sized by comparison with the 123-bp markers and the 1-kilobase-pair markers (Bethesda Research Laboratories, Inc.). Following electrophoresis, each gel was blotted to Nytran membrane as described elsewhere (16). After blotting, the membrane was UV cross-linked and baked at 80°C in a vacuum oven. Each blot was hybridized with the appropriate oligonucleotide probe by the method described by Saiki et al. (22). Oligonucleotide probes were end labeled by using [γ-^{32}P]ATP and polynucleotide kinase. After hybridization, each blot was washed two times in 2× SSPE (1× SSPE is 0.18 M NaCl, 10 mM sodium phosphate, and 1 mM EDTA [pH 7.7])–0.1% SDS, for 20 min at room temperature, and two times for 20 min each time at 55°C in 5× SSPE–0.1% SDS. The blots were air dried and exposed to X-ray film for 3 h at −70°C.

Oligonucleotides used in PCR analysis. Oligonucleotides were synthesized with an Applied Biosystems 380A synthesizer by using phosphoramidite technology. The size and extent of purity of each oligonucleotide were determined by electrophoresis and autoradiography of a kinase-labeled portion of each. All oligonucleotides were 90 to 95% of their full length and were used without further purification. For IFN-β_1, the sense primer was 5′-GATTCATCTAGCACTG GCTTG (343 to 364), the antisense primer was 5′-CTTCAG GTAATGCAGAATCC (509 to 529), and the detection oligonucleotide was 5′-GAGAACCTCCTGGCTAATGTC (380 to 400), as numbered in Derynck et al. (6). This set of primers produced an amplification product that was 166 nucleotides long. For IFN-γ, the sense primer was 5′-GCATCCAAAAGAGTGTGGAG (372 to 391), the antisense primer was 5′-GACAGTTCAGCCATCACTTGG (521 to 540), and the detection oligonucleotide was 5′-GACTAAT TATTCGGTAACTGAC (463 to 682), as numbered in Gray and Goeddel (8). For IFN-γ, the amplification product was 168 nucleotides long.

Nuclear run-on analysis. Cells were scraped into ice-cold phosphate-buffered saline, collected by centrifugation at 1,000 × *g* and lysed in buffer containing 0.2% Nonidet P-40, 15 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.5), 60 mM KCl, 15 mM NaCl, 14 mM 2-mercaptoethanol, 5 mM EDTA, 0.5 mM EGTA [ethylene glycol-bis(β aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid], 0.15 mM spermine, and 0.5 mM spermidine. Nuclei were isolated by centrifugation (1,000 × *g*, 4°C, 5 min) and washed in the same buffer without Nonidet P-40. An average of 1.5 × 10^7 to 2 × 10^7 nuclei was obtained from a 15-cm culture dish. The nuclei were suspended (10^7 per reaction) in 100 μl of storage buffer (50 mM Tris hydrochloride [pH 8.3], 0.1 mM EDTA, 5 mM $MgCl_2$, 40% glycerol) and mixed with an equal volume of 2× reaction buffer (140 mM KCl; 10 mM $MgCl_2$; 0.2 mM EDTA; 20 mM phosphocreatine; 20 U of phosphocreatine kinase per ml; 40% glycerol; 1 mM each of ATP, GTP, and CTP; and 100 μCi of [α-^{32}P]UTP (specific activity, 400 Ci/mol). This mixture was incubated for 20 min at 30°C. Run-on transcripts were isolated by sequential treatments of DNase and proteinase K and this process was followed by phenol extraction and trichloroacetic acid precipitation essentially as previously described (9, 10). The eluate from the trichloroacetic acid precipitation was adjusted to 50% formamide–0.9 M NaCl–1% SDS and added to the hybridization mix at 10^6 cpm/ml. Hybridization was performed for 36 h at 42°C in 50% formamide–5× SSPE–1% SDS–5× Denhardt solution–250 μg of yeast RNA per ml. The slot blots were prepared by blotting denatured plasmid DNA onto Nytran membrane. The relevant plasmids were obtained as follows: (i) a 1,000-bp cDNA clone of BSF-2 (IL-6–IFN-β_2) from T. Hirano, Osaka, Japan; (ii) a 770-bp *Hin*cII fragment of the human IFN-β_1 gene from J. A. Hewitt, Baltimore, Md.; (iii) a 1,300-bp cDNA clone of the human 2-5A synthetase from M. Rutherford, Toronto, Ontario, Canada; (iv) a 5,000-bp genomic clone of the HLA/AIII gene from O. Dill, Heidelberg, Federal Republic of Germany; (v) a 3,600-bp genomic clone of a human β-actin-related pseudogene (19a) obtained through L. Gissmann, Heidelberg. The blots were stringently washed twice in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at 68°C for 30 min each.

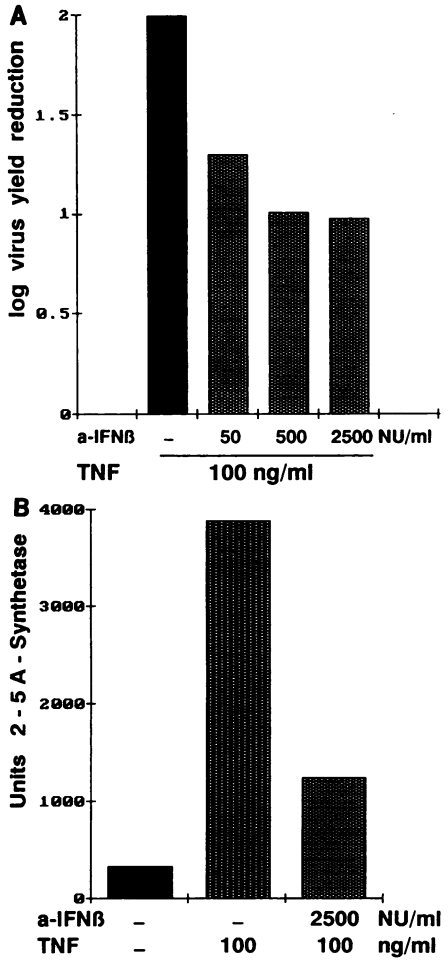

FIG. 1. Effect of anti-IFN-β_1 antibodies on TNF-induced antiviral activity and 2-5A synthetase induction. (A) HEp-2 cells were pretreated for 15 h with TNF alone or in the presence of increasing amounts of a neutralizing antiserum to IFN-β_1. Monolayers were washed and infected with vesicular stomatitis virus at a multiplicity of infection of 0.2. After 24 h of incubation, cells were freeze-thawed twice and virus yield was determined in a plaque assay. Virus yield in untreated controls was 10^8 PFU/ml. (B) Cells were treated for 12 h with TNF in the presence or absence of IFN-β_1 antiserum. 2-5A synthetase activity was determined in cytoplasmic lysates as described in Materials and Methods. NU, Neutralizing unit (1 NU equals the amount of antiserum sufficient to neutralize 10 IU of IFN-β_1).

RESULTS

Cellular analysis of the TNF-induced antiviral state. Pretreatment of HEp-2 cells with human TNF-α induces an antiviral activity which inhibits replication of virus and protects cells against virus-mediated lysis. Treatment of HEp-2 cells with 100 ng of TNF per ml led to a 100-fold reduction in yield of vesicular stomatitis virus (Fig. 1A). When TNF was mixed with neutralizing antiserum to human IFN-β_1, an antiserum-concentration-dependent reversion of virus inhibition was observed. Antiserum against IFN-α or IFN-γ did not affect TNF-mediated antiviral activity (data not shown). In parallel with the antiviral state, TNF induced the 2-5A synthetase activity in HEp-2 cells (Fig. 1B). Inclusion of the antiserum to IFN-β_1 also reduced the induction of 2-5A synthetase by 80%. Together these data suggested the involvement of an IFN-like activity in the TNF response, possibly IFN-β_1 itself.

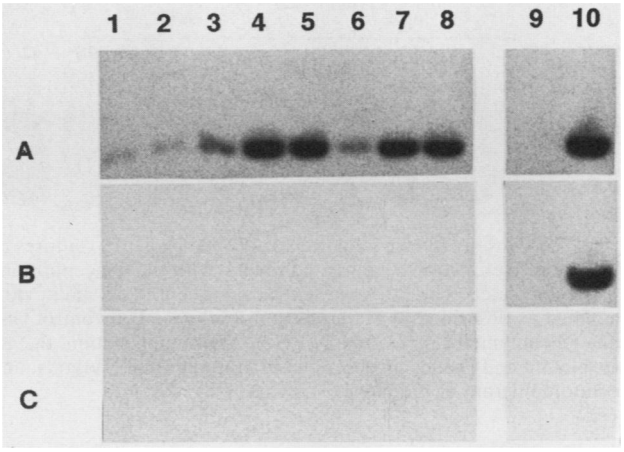

FIG. 2. PCR analysis of IFN-β_1 mRNA levels in HEp-2 cells. Cultures of HEp-2 cells were treated with 500 ng of TNF per ml for 0, 1, 2, 3, and 4 h (lanes 1 to 5). Lanes 6 to 8 were from 4-h cultures treated with IFN-γ (250 U/ml), TNF plus IFN-γ, and rI:rC (50 μg/ml), respectively. RNA was extracted at the times indicated, purified, and quantitated as described in Materials and Methods. (A) PCR analysis for IFN-β_1 mRNA. Lanes 9 and 10 contain 0.1 pg of SP-6-produced IFN-β_1 mRNA without and with Moloney murine leukemia virus reverse transcriptase added in the cDNA synthesis reaction. (B) Control reaction for PCR. The PCR method was performed as for panel A, except that the reverse transcriptase was omitted from the cDNA synthesis step. Lane 9 contained 0.1 pg of SP-6-produced IFN-β_1 mRNA, and lane 10 contained 0.1 pg of the corresponding cDNA clone. (C) PCR detection of IFN-γ mRNA was performed as described in Materials and Methods. Lanes 9 and 10 were as listed for panel A.

Molecular and PCR amplification analysis of IFN-β_1 induction. Attempts to directly demonstrate induction of IFN-β_1 either as biological activity in supernatants from TNF-treated cells or by Northern blot analysis were unsuccessful (data not shown). Since it has been shown previously that rather small amounts of exogenously added IFN-β_1 (less than 1 U/ml) were sufficient to greatly boost the antiviral activity of TNF (12), it was conceivable that undetectable levels of endogenously induced IFN-β_1 were critically involved in the antiviral action of TNF. We thus decided to apply a more sensitive method for the detection of IFN-β_1-specific transcripts. An in vitro DNA amplification method which allows the amplification of specific DNA sequences by several orders of magnitude has recently been described (22). We have adopted this PCR for the detection of specific mRNAs. By using appropriate oligonucleotide primers specific for the sequences they are designed to amplify, IFN-β_1-specific transcripts in total cytoplasmic RNA from HEp-2 cells treated with TNF was reverse transcribed to single-stranded cDNA. This cDNA was further amplified by using 30 cycles of PCR. Figure 2 shows the results of a PCR experiment designed to detect IFN-β_1 RNA in HEp-2 cells treated for various times with TNF. A weak signal was observed in untreated cells (lane 1). With increasing length of exposure to TNF, an enhanced signal which reached maximum strength at about 3 h was obtained (lane 4). Increased expression was also observed after treatment with a synthetic double-stranded RNA (polycytidylic acid-polyinosinic acid [rI:rC]; lane 8). Double-stranded RNA is an established IFN-β_1 inducer; however, HEp-2 cells are low producers of IFN-β_1 after rI:rC induction (less than 30

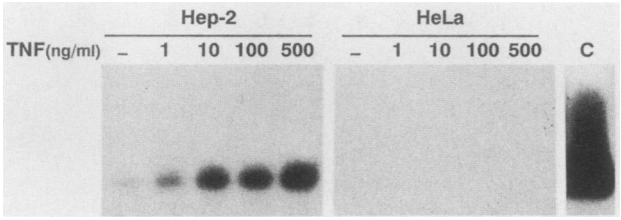

FIG. 3. Dose-response induction of IFN-β_1 by TNF. Cultures of HEp-2 and HeLa cells were treated for 4 h with the concentrations of TNF indicated. The RNA was extracted, quantitated and in vitro amplified as described in Materials and Methods. The control lane (lane C) contained 2 pg of IFN-β_1 RNA. After amplification, the gel was blotted and probed as described in Materials and Methods, and an autoradiogram of the blot is shown.

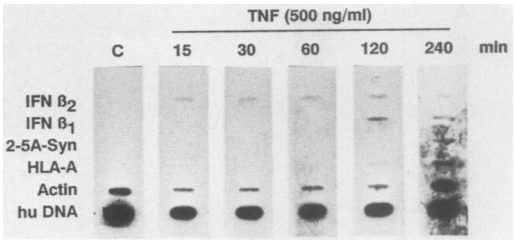

FIG. 4. Transcriptional activation in HEp-2 and HeLa cells following TNF treatment. Nuclei were prepared from HEp-2 cells at the indicated times after the beginning of TNF treatment and immediately used for a nuclear run-on analysis (see Materials and Methods for details). Lane C, Untreated control. HLA-A, human lymphocyte antigen type A; hu DNA, human DNA.

IU/ml in the culture supernatants [data not shown]). In addition, the level of IFN-β_1 mRNA usually peaks 2 to 2.5 h after double-stranded RNA treatment (3). Treatment with IFN-γ led to a minor increase, if any, in IFN-β_1 expression (lane 6). Combined treatment with TNF plus IFN-γ, which leads to a synergistic antiviral effect, did not significantly increase the IFN-β_1 signal above that level obtained with TNF alone.

The results indicate induction of IFN-β_1 by TNF as predicted from the neutralization data in Fig. 1. To assess the significance of these results, two critical questions were addressed. (i) Is the signal of the PCR indeed due to cDNA from reverse transcription rather than contaminating genomic DNA? (ii) Is there a biological relevance to the signal obtained or does it result from "transcriptional noise," i.e., random transcription due to leaky control of gene expression in cell lines? The PCR was repeated on the same RNA preparation, but the reverse transcriptase reaction was omitted (Fig. 2B, lanes 1 through 8). Under these conditions, no signal was obtained, thus excluding the possibility of contamination by genomic DNA. IFN-γ is a lymphokine and is thus produced only by lymphocytes, probably exclusively by T cells. Detection of IFN-γ RNA in HEp-2 cells could thus be an indication for amplification of transcriptional noise. In a third set of experiments, reverse transcription and subsequent PCR were performed with oligonucleotide primers specific for IFN-γ (Fig. 2C). No IFN-γ signal was observed under any circumstances.

Maximal induction of an antiviral activity by TNF in HEp-2 cells requires a concentration of approximately 100 ng/ml. In a dose-response experiment, concentration-dependent induction of IFN-β_1 was examined (Fig. 3). Some signal was present in untreated cells (Fig. 2). An increase in IFN-β_1 was detected with doses as low as 1 ng/ml, with good induction at 10 ng/ml and higher. Dose requirements for the induction of antiviral activity and IFN-β_1 RNA were thus in close agreement.

A line of HeLa cells was completely unresponsive to the antiviral activity of TNF (18). RNA extracted from TNF-treated HeLa cells failed to give a signal in IFN-β_1-specific PCRs (Fig. 3), again demonstrating an agreement between biological activity and IFN induction.

Transcriptional activation of IFN-β_1 and IFN-β_2 genes. To obtain independent evidence for the induction of IFN-β_1 by TNF and to examine the possible basis for this enhanced expression, a nuclear run-on analysis with nuclei from TNF-treated HEp-2 cells was performed (Fig. 4). Run-on transcripts were hybridized against sequences from genes which have been previously shown to be activated by TNF (IL-6–IFN-β_2, MHC class I, and 2-5A synthetase), against a TNF-unresponsive gene (β-actin), and against IFN-β_1. Total human DNA was included to measure possible variations in the overall transcriptional activity after TNF treatment. Transcription of the IL-6–IFN-β_2 gene increased shortly after onset of TNF treatment (15 min) and stayed at an elevated level. After 2 h of TNF treatment, an IFN-β_1-specific signal appeared. MHC transcription was observed at 2 and 4 h of TNF treatment. There was no consistent change in β-actin transcription, and overall incorporation was largely unaffected, as demonstrated by hybridization to total DNA. The occurrence of IFN-β_1 run-on transcripts was delayed relative to the rapid activation of IL-6–IFN-β_2 transcription and coincided with the appearance of IFN-β_1 RNA in the cytoplasm (Fig. 2A).

The IFN-β_1-directed PCR had demonstrated a differential effect of TNF on IFN-β_1 expression in HEp-2 and HeLa cells. Following the observation of enhanced IL-6–IFN-β_2 transcription in TNF-treated HEp-2 cells, we assayed for steady-state levels of IL-6–IFN-β_2 transcripts in both cell lines (Fig. 5). TNF treatment led to a dose-dependent increase of such transcripts. This increase was even more pronounced in HeLa cells than in HEp-2 cells. Thus, whereas IFN-β_1 expression correlated with TNF-mediated antiviral activity, such correlation could not be found for IL-6–IFN-β_2.

DISCUSSION

The biological activities of TNF partially overlap with those of IFNs, most notably the antiviral activity, the enhancement of MHC expression, and a growth-inhibitory effect. Evidence from neutralization studies with antiserum

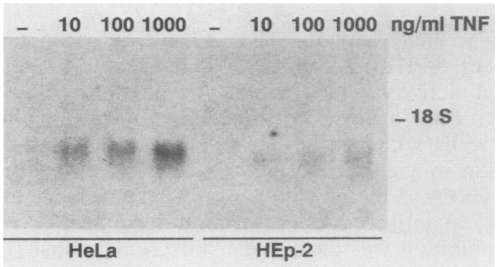

FIG. 5. Northern blot analysis of IL-6–IFN-β_2 expression in HEp-2 and HeLa cells. Confluent monolayers were treated with the indicated amounts of TNF. RNA was isolated after 4 h of treatment. A 20-μg portion of total RNA was applied per lane and analyzed for IL-6 RNA.

against IFN suggested that, indeed, an IFN-β_1-like activity was the mediator of these effects of TNF. Our data provide firm support for a TNF-induced activation of IFN-β_1-expression in TNF-treated HEp-2 cells. Dose-response and kinetics of IFN-β_1 induction, as detected by the modified PCR, were in close agreement with dose requirements for and kinetics of the induction of the antiviral state and 2-5A synthetase activity. Nuclear run-on analysis showed enhanced rates of transcription for the IL-6–IFN-β_2, IFN-β_1, MHC class I, and 2-5A synthetase genes. While activation of IFN-β_2 transcription occurs within a few minutes after TNF addition, transcriptional signals from the three latter genes are not observed until after more than 1 h of TNF treatment. These differential kinetics indicate the likelihood of different activation signals; i.e., activation of the IFN-β_2 gene may be a primary response, while IFN-β_1 expression may be an indirect consequence of TNF exposure. The nuclear run-on data are in line with a model in which intermediate IFN-β_1 production leads to enhanced induction of the 2-5A synthetase and MHC class I antigens. IFN-β_1 may thus be of crucial importance for the antiviral, growth-inhibitory, or immunomodulatory activity of TNF. The antiviral activity of TNF on HEp-2 cells is only partially abolished by the antiserum to IFN-β. This is in contrast to other studies, in which complete reversal in similar experiments was observed (24). The latter results indicate that IFN-β_1 may be the principal and possibly sole inducer of the antiviral state. We maintain our earlier statement that in addition to the IFN-β, there must be a second antiviral agent, possibly TNF itself. Our argument is based on two observations. First, the amounts of the induced IFN-β are extremely small, i.e., not detectable by more common experimental procedures, and it is difficult to conceive that these amounts are sufficient to account for a 10^2 to 10^4 reduction in virus yield. Second, we have recently shown that TNF acts synergistically with IFN-β_1 in its antiviral activity (17). If IFN-β_1 was the sole mediator of the TNF effect, combination of TNF with exogenous IFN-β_1 should yield an additive effect at most. The expression of IFN genes is tightly regulated, and IFN activity is observed only following specific induction. Typical inducers are viruses, double-stranded RNA, and bacterial products. Recently, low-level IFN production has been reported in fibroblast cultures which were activated from G_0-G_1 arrest by growth factor treatment and in macrophages following culture in the presence of colony stimulating factor-1 (19, 29). TNF has a mitogenic activity on normal fibroblasts, and this activity is enhanced by neutralizing antiserum to IFN-β (11). Low-level production of IFN-β_1 may thus be more commonly associated with exposure of cells to growth factors and endogenous IFN-β_1, a physiological antagonist to such agents.

The modified PCR as described here and elsewhere (4, 20) has been proven to be a highly sensitive and reliable method for the detection of rare transcripts. The limits of sensitivity have been investigated. In the system described here, as few as 250 copies of IFN-β_1 mRNA were detected. The amounts of IFN-β_1 mRNA induced by TNF in HEp-2 cells were in the range of 0.5 to 2 mRNA molecules per cell. This method should be valuable for analysis of other systems in which IFN production has been proposed but not proven and should be in its principal design applicable to a multitude of other experimental systems.

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