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Isolation and Characterization of Eight Tumor Necrosis Factor-Induced Gene Sequences from Human Fibroblasts

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A λ cDNA library was prepared from polyadenylated RNA isolated from quiescent human diploid FS-4 fibroblasts stimulated with tumor necrosis factor for 3 h. Differential screening was used to isolate cDNA sequences that are stimulated by tumor necrosis factor. Eight distinct tumor necrosis factor-stimulated gene sequences (designated TSG-1, -6, -8, -12, -14, -21, -27, and -37) were partially sequenced and compared with known sequences from GenBank. TSG-1 was identical to the gene for interleukin-8. TSG-8 corresponded to the gene for monocyte chemotactic and activating factor. TSG-21 and -27 were identical to the genes for collagenase and stromelysin, respectively. The other four sequences showed no homologies with known genes. Patterns of induction of mRNAs corresponding to the eight cloned cDNAs by various cytokines, growth factors, and activators of second messenger pathways were analyzed in FS-4 cells.

Tumor necrosis factor α (TNF) is a potent pleiotropic cytokine that is important in host defenses against infectious agents and malignancies (reviewed in references 3, 19, and 30). Most biological actions of TNF can be attributed to the triggering of complex genetic programs in the target cells. A relatively large number of genes activated by TNF have already been identified. Among the genes that are known to be activated by TNF are the proto-oncogenes c-*fos*, c-*myc* (22), and c-*jun* (4), transcription factors NF-κB (23, 31) and IRF-1 (8a), the cytokines interleukin-6 (IL-6) (14), IL-1 (29), IL-8 (27), and many others. It is likely that many more TNF-induced genes await identification and characterization.

The goal of our present study was to isolate and characterize DNAs complementary to mRNAs whose steady-state levels are enhanced in human fibroblasts treated with TNF. We chose the FS-4 line of human diploid foreskin fibroblasts for our experiments because extensive data already exist on the actions of TNF and other cytokines in human fibroblasts, including the FS-4 cell line (4, 6, 8a, 14, 18, 22, 32, 41). A cDNA library was prepared from quiescent FS-4 cells incubated for 3 h in the presence of TNF. This library was screened for TNF-inducible gene sequences by differential hybridization with cDNA prepared from control or TNF-treated FS-4 cells. After screening 30,000 clones, we isolated over 40 TNF-inducible recombinant clones that hybridized more strongly with the cDNA from TNF-treated cells. From these, we identified eight non-cross-hybridizing, unique cDNA sequences. Partial sequence analysis revealed that four of these cDNAs were identical to known genes, whereas the other four appeared to represent hitherto unidentified genetic sequences. We describe the induction characteristics of mRNAs corresponding to these eight distinct cDNAs. The availability of a set of cDNAs corresponding to TNF-inducible mRNAs should be useful in the analysis of the molecular pathways of gene regulation by TNF. In addition, characterization of the products of newly identified TNF-induced genes may shed more light on the biological actions of TNF.

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MATERIALS AND METHODS

Materials. *Escherichia coli*-derived recombinant human TNF (specific activity, 3×10^7 U/mg) was supplied by M. Tsujimoto of the Suntory Institute for Biomedical Research, Osaka, Japan. *E. coli*-derived recombinant human IL-1α (specific activity, 1×10^9 U/mg) was a gift of Alvin Stern and Peter Lomedico, Hoffmann-LaRoche, Inc., Nutley, N.J. *E. coli*-derived recombinant gamma interferon (IFN-γ; specific activity, 2.1×10^7 U/mg) was provided by Biogen, Cambridge, Mass. *E. coli*-derived human IFN-β (Betaseron; specific activity, 2×10^8 U/mg) was obtained from Triton Biosciences, Alameda, Calif. Epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and transforming growth factor β were purchased from Collaborative Research, Inc., Bedford, Mass. Poly(I)-poly(C) was from P-L Biochemicals, Inc., Milwaukee, Wis. N^6-2′-*O*-dibutyladenosine cyclic 3′,5′-monophosphate, cycloheximide, forskolin, 12-*O*-tetradecanoylphorbol 13-acetate (TPA), the calcium ionophore A23187, and isobutylmethylxanthine were purchased from Sigma Chemical Co., St. Louis, Mo. The pHe7 plasmid, used as a source of internal reference cDNA (12), was supplied by P. B. Sehgal, Rockefeller University, New York, N.Y.

Cell culture. The human diploid FS-4 foreskin fibroblast line (40) was used at passage level 15 in all experiments. FS-4 cells were grown in Eagle minimum essential medium supplemented with 6 mM *N*-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 3 mM Tricine, 50 μg of gentamicin per ml, and 5% heat-inactivated (56°C, 30 min) fetal bovine serum (GIBCO Laboratories, Grand Island, N.Y.). For experiments, 4×10^6 cells were seeded in 175-cm^2 Falcon flasks (Becton Dickinson Labware, Oxnard, Calif.), incubated at 37°C, and allowed to grow to confluence over 6 days. The confluent monolayers were washed once with phosphate-buffered saline and replenished with Eagle minimum essential medium containing 0.25% fetal bovine serum. The cultures were incubated in this medium for another 72 h at 37°C to let the cells become quiescent and then treated with the appropriate agents as specified below.

Preparation of cDNA and construction of cDNA library. Total cytoplasmic RNA was isolated from quiescent FS-4 cells treated for 3 h with TNF (10 ng/ml) as described

previously (22). Poly(A)$^+$ RNA was selected by one cycle of binding to oligo(dT)-cellulose (type 7; P-L Biochemicals). Double-stranded cDNA was made from 10 μg of poly(A)$^+$ RNA by using the cDNA synthesis system of Bethesda Research Laboratories, Inc., Gaithersburg, Md. The double-stranded cDNA was methylated with *Eco*RI methylase and made blunt-ended with T4 DNA polymerase. *Eco*RI linkers were ligated onto the cDNA, which was then restricted with *Eco*RI. The resulting cDNA greater than 600 base pairs in size was fractionated and separated from the linker fragments by Sepharose CL4B column chromatography and ligated into the *Eco*RI site of λgt10. The library was packed in vitro with Gigapack packaging extract (Stratagene).

Differential screening of the cDNA library. The λgt10 cDNA library was plated on *E. coli* LE392 at a density of 1,000 PFU per 150-mm-diameter petri dish. Nitrocellulose filters were used to prepare duplicate plaque lifts of each plate. Prehybridization and hybridization of filters with ^{32}P-labeled single-stranded cDNA probe were performed as described elsewhere (21). Probes were synthesized by using the Bethesda Research Laboratories cDNA synthesis system with 10 μg of poly(A)$^+$ RNA. The first-strand synthesis reaction was adjusted to contain 0.5 mM each of dATP, dGTP, and dTTP, 0.1 mM dCTP, 100 μg of dactinomycin per ml, and 200 μCi of [α-^{32}P]dCTP (3,000 Ci/mmol; ICN Pharmaceuticals, Inc., Irvine, Calif.). After synthesis of the cDNA, the RNA was removed by incubation in 0.2 M NaOH at 70°C for 20 min. The reaction was neutralized with HCl, and the cDNA was ethanol precipitated in the presence of 2 M ammonium acetate. The pellet was suspended in 200 μl of TE (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA) and added to the hybridization solution and filters. One of two probes was used to hybridize to each of the two replica filters; one was made from untreated FS-4 cells, and the other was made from FS-4 cells treated for 3 h with TNF (10 ng/ml). After hybridization, the filters were washed in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at room temperature for 30 min and then washed in 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C for 1 h with one or two changes. Filters were exposed to Kodak XAR-5 film for 1 to 2 days with an intensifying screen at −70°C. Plaques that showed different intensities of the hybridization signal with the two probes were selected. These clones were subjected to one further round of differential screening, and the plaques were purified.

Subcloning of the cDNA inserts and cross-hybridization studies. *E. coli* LE392 cells in soft 0.7% agarose were poured into 150-mm plates. The λ clones were then spotted on the plates in a grid array. Four nitrocellulose filters were lifted from each plate, processed, and stored until use. To prepare cDNA inserts from plaque-purified recombinant clones, 10 ml of liquid lysate was clarified and digested with 2 μg of DNase I per ml to remove contaminated chromosomal DNA. Then 2 ml of 2.5% SDS–0.5 M Tris hydrochloride (pH 9.5)–0.25 M EDTA was added, and plates were incubated at 65°C for 15 min to lyse the phages. The solution was then cooled to room temperature before 2.0 ml of 10 M ammonium acetate was added. The sample was chilled on ice for 20 min and centrifuged at 15,000 × *g* for 15 min. To the supernatant, 0.6 volume of isopropanol was added, and the solution was centrifuged at 15,000 × *g* at 4°C for 10 min to obtain the DNA pellet. The pellet was suspended in 100 μl of TE buffer containing 2 μg of RNase A (Boehringer) per ml and cut with the restriction enzyme *Eco*RI. The cDNA insert was isolated and subcloned into the *Eco*RI site of an M13mp19 vector. The cDNA inserts to be used as probes for cross-hybridization and Northern (RNA) blot experiments were prepared from the recombinant M13 clones by restriction with *Eco*RI to minimize background. The probes were ^{32}P labeled by nick translation and hybridized onto the filters prepared earlier. The hybridization conditions were essentially as described above for the differential screening experiments.

Northern blot analysis. Cytoplasmic RNA was fractionated on a 1% agarose gel containing formaldehyde and blotted onto Zeta-probe blotting membranes (Bio-Rad Laboratories, Richmond, Calif.) (22). Cytoplasmic RNA was loaded at 10 μg per lane. Prehybridization and hybridization of the Northern blots were performed as described previously (22). Filters were probed with ^{32}P-labeled cDNA insert from recombinant M13 clones and/or with ^{32}P-labeled internal reference pHe7 insert. Northern blots were quantified with a laser densitometer.

Sequence analysis. Single-stranded DNA templates from recombinant M13 clones were prepared, and several hundred nucleotides from each end of the cDNA were determined by the dideoxynucleotide-chain termination method (35). The partial nucleotide sequences were compared with sequences entered in GenBank (release 60.0).

RESULTS

Preparation and screening of cDNA library. A recombinant cDNA library was prepared from poly(A)$^+$ RNA from TNF-treated quiescent FS-4 cells. This library contained 2 × 10^6 recombinant clones and was screened for TNF-inducible gene sequences by differential hybridization. Two replica nitrocellulose filters, prepared from the cDNA library plate, were each probed with one of two single-stranded ^{32}P-labeled cDNA probes prepared from the poly(A)$^+$ RNA of either untreated FS-4 cells or cells exposed to TNF for 3 h. The hybridization signal of the two probes to each plaque was then compared. The plaques that hybridized preferentially with the cDNA probe from TNF-treated cells (as indicated by a stronger hybridization signal) were picked as presumptive TNF-inducible genes. Approximately 30,000 plaques were screened; 44 of the plaques consistently gave a much stronger hybridization signal to the cDNA probe from TNF-treated cells after two rounds of screening, suggesting that the corresponding mRNAs are induced by TNF.

Identification of cDNA clones containing distinct TNF-inducible sequences. To determine the number of distinct mRNA species represented among the 44 cDNA clones selected by differential screening, the cDNA inserts were tested for sequence homology to one another by cross-hybridization. To minimize background levels, the cDNA inserts to be used as probes were isolated from recombinant phage DNA, subcloned into a M13mp19 vector, labeled by nick translation, and tested against all of the phages. The recombinant clones cross-hybridizing to each other were considered to be derived from identical mRNAs. This procedure led to the identification of eight distinct TNF-stimulated gene sequences (designated TSG-1, -6, -8, -12, -14, -21, -27, and -37). TSG-8 and -14 were isolated with a high frequency (11 and 13 clones, respectively, out of 44), whereas the others were less abundant (1 to 6 out of 44). Table 1 gives a summary of the abundance and the approximate sizes of mRNAs corresponding to the eight distinct TSG cDNAs.

Characterization of TSG mRNA induction kinetics and partial sequencing of TSG cDNAs. To ascertain that the eight distinct TSG cDNAs isolated indeed corresponded to

TABLE 1. Abundance of individual TSG cDNAs among 44 TNF-specific cDNA clones

cDNA	Abundance[a]	Approximate size of corresponding mRNA (kilobases)
TSG-1	6	1.6
TSG-6	6	1.5
TSG-8	11	1.1
TSG-12	3	4.5
TSG-14	13	2.3
TSG-21	1	2.4
TSG-27	2	2.4
TSG-37	2	0.8

[a] Each of the 44 cDNA inserts was isolated from the λgt10 vector and subcloned into the M13mp19 vector. Inserts from the M13 vector were ^{32}P labeled by nick translation and hybridized with each of the 44 λ cDNA clones. Cross-hybridization was taken as evidence that the cDNAs were divided from the same mRNA species. Numbers indicate the frequency with which individual TSG cDNAs were represented among the 44 cDNA clones.

mRNAs whose levels were upregulated in FS-4 cells by TNF treatment, quiescent FS-4 cultures were treated with TNF (20 ng/ml) for different intervals ranging from 0.5 to 16 h, cytoplasmic RNA was isolated, and mRNA levels corresponding to each of the eight cDNAs were quantitated by Northern blot analysis (Fig. 1) and densitometric scanning of the autoradiograms (Fig. 2). The increase in mRNA levels ranged from about 3-fold (TSG-21) to over 100-fold (TSG-6 and -8). The kinetics of mRNA stimulation fell into three distinct patterns. The first pattern was characterized by an increase to peak levels by 2 to 4 h, followed by a gradual decrease in mRNA levels (TSG-1 and -6). The second pattern was a rapid increase of mRNA levels to a maximum by 1.5 to 4 h, followed by a plateau maintained for at least 16 h (TSG-8, -12, -14, and -37). The third pattern was characterized by a possible initial decrease, followed by a slow gradual increase in mRNA levels throughout the 16-h observation period (TSG-21 and -27).

To determine whether the isolated cDNAs were homologous to previously identified genes, all eight cDNAs were partially sequenced (300 to 400 base pairs) and the sequences determined were checked against sequences available in GenBank (release 60.0). Sequences of four cDNAs (TSG-1, -8, -21, and -27) were found to be identical to earlier identified genes. Of these, TSG-1, corresponded to the gene for beta-thromboglobulin-like protein 3-10C (36) (also known as IL-8 [15, 25]). TSG-8 was identical to the recently cloned monocyte chemotactic and activating factor termed MCAF or MCP-1 (9, 43). TSG-21 and -27 were found to be identical to the collagenase and stromelysin genes (8), respectively. The other four partial cDNA sequences showed no significant homologies with known genes, and they may represent previously unidentified gene sequences. (Nucleotide sequence data of TSG-6, -12, -14, and -37 cDNAs will appear in the EMBL, GenBank, and DDBJ nucleotide sequence databases under the accession numbers M31165, M31163, M31166, and M31164, respectively.)

Patterns of TSG mRNA induction by different cytokines and

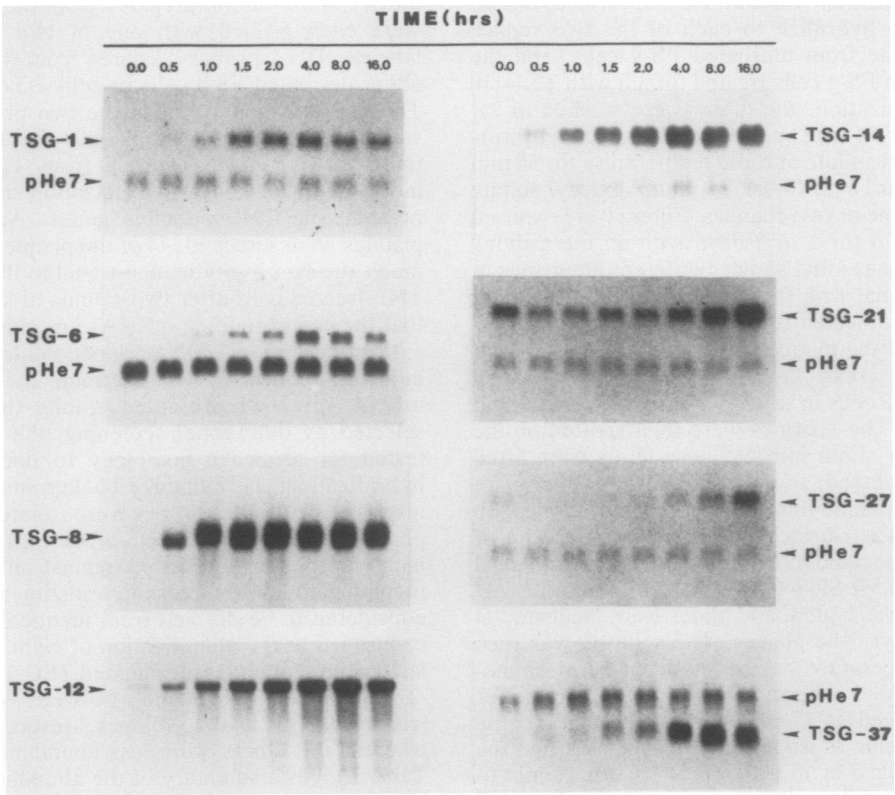

FIG. 1. Induction of mRNAs corresponding to eight TSG cDNAs in FS-4 cells treated with TNF. Growth-arrested FS-4 cells were exposed to TNF (20 ng/ml) at 0 h. At different intervals thereafter, total cell RNA was isolated, fractionated on formaldehyde-agarose gels, transferred to Zeta-probe blotting membranes, and hybridized separately to each of the ^{32}P-labeled TSG cDNA inserts. To ascertain whether equal amounts of RNA were loaded in each lane, most blots were also probed with a ^{32}P-labeled pHe7 internal reference cDNA insert specific for an invariant ≈1.0-kilobase mRNA species.

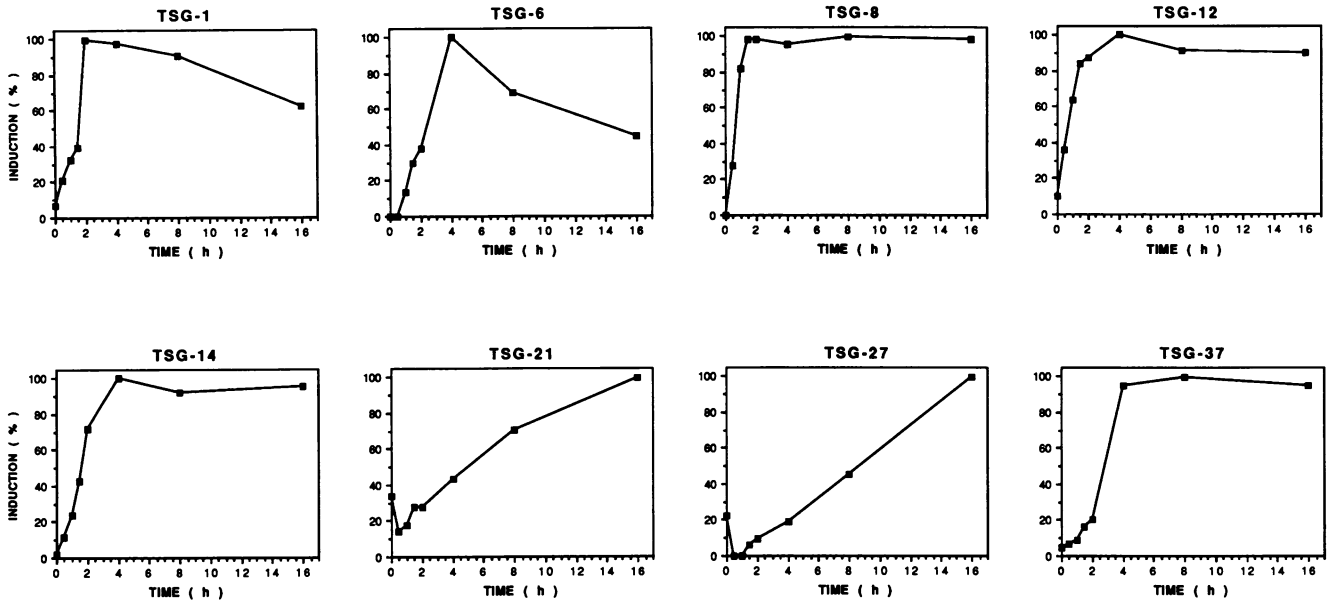

FIG. 2. Densitometric analysis of the kinetics of induction of eight TSG mRNAs by TNF. Autoradiograms of the Northern blots shown in Fig. 1 were scanned by laser densitometry. For each individual mRNA, the highest-density band was normalized to represent 100% induction.

other agents. Table 2 provides a summary of a large number of experiments in which levels of mRNAs corresponding to the eight distinct TSG cDNAs in FS-4 cells exposed to various agents were determined by Northern blot analysis. With the exception of TSG-21 (collagenase) and TSG-27 (stromelysin), all mRNA levels were determined 2 h after the initiation of the treatments. Three of the mRNAs (corresponding to TSG-8 [MCAF], TSG-12, and -14) were inducible by the protein synthesis inhibitor cycloheximide. In some cases the addition of cycloheximide either did not block (TSG-12 and -14) or increased (TSG-1 [IL-8], TSG-6, and TSG-8 [MCAF]) mRNA inducibility by TNF, suggesting that the increase in these mRNA levels is the result of a direct action of TNF that did not require a protein interme-

TABLE 2. Effect of various treatments on TSG mRNA levels in FS-4 cells

Treatment[a]	Relative increase in mRNA level							
	TSG-1 (IL-8)	TSG-6	TSG-8 (MCAF)	TSG-12	TSG-14	TSG-21 (collagenase)	TSG-27 (stromelysin)	TSG-37
TNF	+++	+++	+++	++++	++++	++	++	+++
Cycloheximide	0	0	+	+++	+	0	0	0
TNF + cycloheximide	++++	++++	++++	++++	++++	0	0	+
IFN-β	0	0	++	+	0	++	++	+++
IFN-γ	0	0	++	+	0	0	0	0
TNF + IFN-β	+	++++	+++	++++	++++	++	++	++++
TNF + IFN-γ	+	+++	++++	++++	++++	++	++	+++
IL-1	+	+	++	++++	++++	++	++	++
EGF	0	0	+	+	+	++	++	+
PDGF	0	0	+	+	+	0	0	0
TGF-β	0	0	0/+	0	0	0	0	0
Poly(I)-poly(C)	+	+	+++	++	++	++	++	+
TPA	+	+	++	++	+	++++	++++	+
A23187	+	+	++	++	0	ND	ND	0
Forskolin	0	0/+	0	0	0	0	0	0
dBcAMP	0	0	+	+	0	ND	ND	0/+
IBMX	0	0/+	+	+	0	0	0	0

[a] Growth-arrested FS-4 cells were treated with the following agents: TNF, 20 ng/ml; cycloheximide, 10 μg/ml; IFN-β, 500 U/ml; IFN-γ, 100 U/ml; IL-1-α, 1 ng/ml; EGF, 25 ng/ml; PDGF, 5 ng/ml; transforming growth factor β (TGF-β), 2 ng/ml; poly(I)-poly(C), 50 μg/ml; TPA, 100 ng/ml; A23187, 1 μM; forskolin, 10 μM; N^6-2′-*O*-dibutyladenosine cyclic 3′,5′-monophosphate (dBcAMP), 100 μM; or isobutylmethylxanthine (IBMX), 100 μM. All treatments were for 2 h, except that TSG-21 and −27 mRNA levels were determined after 16 h of treatment. (Since longer periods of treatment with a mixture of TNF and cycloheximide were toxic, TSG-21 and −27 mRNA levels in the groups treated with TNF and cycloheximide were determined at 4 h after the onset of treatment.) Total cellular RNA was isolated and fractionated on a 1% agarose gel containing formaldehyde; the RNA was then subjected to Northern blot analysis as described in Materials and Methods. Relative increases in each of the TSG mRNA levels were quantitated by densitometric scanning of the autoradiograms: 0, No increase in the mRNA level compared with the control mRNA level (no treatment); +, ++, +++, and ++++, relative increase in the mRNA level after treatment as compared with the untreated control. For each of the individual TSG mRNA species, the highest-density band(s) was assigned a value of ++++, and the relative densities of other mRNAs bands in the same experiment were then scored accordingly. ND, Not determined.

diate. In contrast, induction of TSG-21 (collagenase) and TSG-27 (stromelysin) mRNAs by TNF was inhibited in the presence of cycloheximide, suggesting that they are not the products of genes directly responsive to TNF. Five of the mRNAs were inducible by IFN-β, but only two of these responded to IFN-γ. Simultaneous treatment with IFN-β reduced inducibility by TNF of TSG-1 (IL-8) mRNA but appeared to increase somewhat the inducibility of TSG-6 and -37 mRNAs. All mRNAs were inducible by IL-1 and also by the double-stranded RNA poly(I)-poly(C) or the phorbol ester TPA, but the efficiency of induction by these agents varied. Whereas the mRNA levels produced in response to IL-1 and TNF were generally quite similar (except that TNF was much more effective in inducing TSG-1 [IL-8] and TSG-6), the efficiency with which TPA or poly(I)-poly(C) increased levels of the individual mRNA species did not correlate with the actions of TNF. EGF was moderately efficient in increasing mRNA levels for TSG-21 (collagenase) and TSG-27 (stromelysin) mRNAs and weakly stimulated several other mRNAs. PDGF and transforming growth factor β were at best only weakly effective, as were N^6-2′-*O*-dibutyladenosine cyclic 3′,5′-monophosphate and the phosphodiesterase inhibitor isobutylmethylxanthine. The weak stimulatory actions of EGF and PDGF were not due to an absence of receptors, because both EGF and PDGF are potent mitogens for FS-4 cells, and both are synergistic with TNF in their mitogenic action (32).

In comparing the induction patterns summarized in Table 2, it is apparent that none of the mRNAs responded exclusively to TNF. However, at least two of the mRNAs (TSG-1 [IL-8] and TSG-6) were inducible significantly better by TNF than by any other stimulus. It is interesting that TSG-1 (IL-8) and TSG-6 mRNAs had very similar patterns of inducibility (except for an apparent difference in the actions of IFN-β and IFN-γ on their induction by TNF). It is also apparent that the patterns of inducibility of TSG-8 and -12 mRNAs were very similar to each other, as were those of TSG-21 (collagenase) and -27 (stromelysin) mRNAs. Finally, it seems noteworthy that TSG-37 mRNA was strongly inducible by both TNF and IFN-β.

DISCUSSION

As a step toward a more complete understanding of the molecular actions of TNF, we prepared a cDNA library from TNF-treated human FS-4 fibroblasts and used differential hybridization to identify cDNA clones corresponding to mRNAs enriched in TNF-treated cells. Our experimental approach was partly drawn from studies in which serum (1, 17), PDGF (5), and nerve growth factor (21) were examined for their ability to activate specific genes. Although TNF is mitogenic in human (39, 41) and murine (33) fibroblasts, our goal is not restricted to the identification of genes mediating the mitogenic action.

In quiescent FS-4 cells TNF induces an increase in the level of some mRNAs within 20 to 30 min. Some of these immediate-early response mRNAs are elevated only transiently for about 30 to 120 min, e.g., c-*fos* and c-*myc* (22) or the transcription factor IRF-1 (8a). Such immediate-early gene products may be important for the activation of other genes, but their transient induction suggests that they are not the actual effector molecules responsible for the phenotypic changes induced by TNF. We chose a 3-h incubation with TNF because we were seeking cDNAs corresponding to messages that are more stably elevated after TNF treatment. Indeed, the results shown in Fig. 1 and 2 indicate that all of the mRNAs corresponding to the eight TSG cDNAs isolated remained significantly elevated after 16 h of continuous treatment with TNF, and their kinetics of induction were clearly different from those of the immediate-early response mRNAs such as c-*fos*, c-*myc*, or IRF-1. Nevertheless, only the induction of TSG-21 (collagenase) and TSG-27 (stromelysin) mRNAs was completely inhibited by cycloheximide, and the induction of TSG-37 was reduced in the presence of this inhibitor of protein synthesis (Table 2). Induction of the other five TSG mRNAs by TNF was completely resistant to cycloheximide, suggesting that no protein intermediate is needed for the upregulation of these mRNAs.

The fact that the responses to IL-1 most closely resembled TNF actions (Table 2) is not surprising. Although TNF and IL-1 are structurally unrelated and they bind to different receptors (2, 25), they appear to produce the activation of similar second messengers and transcription factors (8a, 45). All of the TNF-inducible mRNAs were also responsive to TPA, an activator of protein kinase C. Although TNF was recently reported to cause activation of protein kinase C in some cell lines (4), it is unlikely that the stimulatory actions of TNF on the eight TSG mRNA species can be ascribed to protein kinase C activation alone, because TPA was more efficient than TNF in inducing collagenase and stromelysin mRNAs but less efficient in stimulating all other TSG mRNAs. However, the possibility cannot be ruled out that TPA and TNF activate different isozymic forms of protein kinase C in FS-4 cells. The finding that poly(I)-poly(C) induced all eight TSG mRNA species (albeit mostly with an efficiency lower than that of TNF) was unexpected. Poly(I)-poly(C) is known to cause activation of a double-stranded RNA-dependent p68 protein kinase (11) and was recently also shown to cause activation of an NF-κB-like protein (42). Which, if any, of these actions contribute to the inducing activity of poly(I)-poly(C) on TSG mRNAs is not known. The phosphodiesterase inhibitor isobutylmethylxanthine and the cyclic AMP analog N^6-2′-*O*-dibutyladenosine cyclic 3′,5′-monophosphate were at best weakly active in elevating levels of some of the mRNAs. This result contrasts with the very pronounced ability of these agents to induce IL-6—another TNF-inducible gene (44). The results summarized in Table 2 suggest that the stimulation of the eight TSG mRNAs species by TNF is probably not due to the activation of a single known major second messenger pathway.

Induction of IL-8 and MCAF by TNF (and IL-1) was recently observed by others (16, 27). IL-8 has been identified as a neutrophil and T-cell chemotactic factor structurally related to several members of a family of inflammatory cytokines that include platelet factor 4, the IFN-γ-inducible protein IP-10, and a growth-regulated gene in transformed cells termed GRO (13, 15, 28). MCAF has been identified as a chemotactic and activating factor for monocytes (26). MCAF belongs to another closely related family of inducible proteins that includes the PDGF-inducible genes JE, LD 78, murine macrophage inflammatory protein, RANTES (28), and TCA-3 (28). Collagenase (TSG-21) was also reported earlier to be TNF inducible in synovial cells and fibroblasts (4, 7). Although, to our knowledge, the induction of stromelysin by TNF has not been reported, transcription of human stromelysin mRNA was recently shown to be inducible by IL-1 (34). The inhibitory effect of cycloheximide on the induction of collagenase and stromelysin mRNAs by TNF seen in our experiments suggests that the inducing effect is indirect. Induction of the stromelysin gene by TPA was also inhibited by cycloheximide (8), as was the induction by EGF of the rat homolog of the stromelysin gene, termed transin

(24). It is very likely that the ability of TNF to induce collagenase and stromelysin is related to the important role of TNF in tissue remodeling during inflammation.

It is significant that all four cDNA we have identified so far by sequencing correspond to genes coding for proteins that are important in inflammatory processes. Recently, we have completed the sequencing of a 1.4-kilobase TSG-6 cDNA insert (T. H. Lee and J. Vilček, unpublished data). Comparison of the amino acid sequence deduced from the TSG-6 cDNA with the protein sequences available in the NBRF protein database (release 18.0) revealed that the N-terminal sequence of the putative TSG-6 gene product has a high degree of homology with the cartilage link protein-proteoglycan core protein-lymphocyte homing receptor CD44 family of proteins (10, 37, 38). Based on the predicted secondary structure of the putative TSG-6 protein, the homologous region appears to be part of an extended loop formed by disulfide bonds that are highly conserved in all of these proteins (10, 37), suggesting that TSG-6 plays a role in cell-cell or cell-matrix interactions.

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