J. gen. Virol. (1988), **69**, 3113–3120. *Printed in Great Britain*

Key words: *oligo-2′,5′-adenylate synthetase/interferon (IFN-β, -γ)/tumour necrosis factor*

Antiviral Activity of Tumour Necrosis Factor. Synergism with Interferons and Induction of Oligo-2′,5′-adenylate Synthetase

By JÜRGEN MESTAN,[1] MANFRED BROCKHAUS,[2] HOLGER KIRCHNER[1] AND HELMUT JACOBSEN[1]*

[1]*Institute of Virus Research, German Cancer Research Center, D-6900 Heidelberg, F.R.G. and* [2]*Hoffmann-LaRoche AG, Basel, Switzerland*

(*Accepted 15 August 1988*)

SUMMARY

Tumour necrosis factor (TNF) induces antiviral activity in HEp-2 cells. Virus yield reduction assays with vesicular stomatitis virus as challenging virus demonstrated that the antiviral state was more pronounced in confluent cultures under low serum conditions. A significant enhancement of the antiviral state was obtained by combining TNF with low concentrations of either interferon (IFN)-β1 or IFN-γ. The reduction in virus yield was significantly higher than that expected from summation of the independent antiviral activities of either substance alone, i.e. TNF and IFN acted synergistically as antiviral agents. Synergism of TNF with IFN-β or IFN-γ appeared to be mediated by different pathways, since different requirements for pretreatment and different effects on oligo-2′,5′-adenylate synthetase (2–5AS) induction were observed. Induction of 2–5AS by TNF could be shown to be an indirect event that was sensitive to an antiserum against natural IFN-β1.

Tumour necrosis factor (TNF) was first described by Carswell *et al.* (1975) as a serum factor of mice treated sequentially with BCG and lipopolysaccharide. It was shown to have antitumoural activity *in vivo* as well as cytotoxic activity on transformed cells *in vitro*. Since the cloning of human and mouse TNF genes (Pennica *et al.*, 1984; Shirai *et al.*, 1985; Wang *et al.*, 1985; Fransen *et al.*, 1985; Marmenout *et al.*, 1985) pure recombinant TNF (rTNF) has become available and evidence has accumulated that TNF has an additional variety of biological effects. TNF was shown to have a growth factor-like effect on human diploid fibroblasts (Sugarman *et al.*, 1985; Vilcek *et al.*, 1986), to stimulate production of interleukin 1 in endothelial cells or monocytes (Dinarello *et al.*, 1986; Nawroth *et al.*, 1986) and to modulate functions of the immune system, e.g. to enhance the activities of immunocompetent cells such as neutrophils (Shalaby *et al.*, 1985; Klebanoff *et al.*, 1986) or eosinophils (Silberstein & David, 1986). In a recent review, Old (1985) suggested that TNF may have evolved as a factor with a role in defence against infectious diseases. In accordance with this theory we and others reported that TNF induced an antiviral state in several cell lines (HEp-2, WI-38, HEL) leading to a reduction of virus yields in cells infected with vesicular stomatitis virus (VSV) (Mestan *et al.*, 1986; Kohase *et al.*, 1986; Wong & Goeddel, 1986). In this report we discuss the effects of culture conditions and combination with interferons on the antiviral activity of TNF.

Pretreatment of HEp-2 cells with human rTNF leads to a dose-dependent inhibition of VSV replication, which is observed in cell protection assays as well as in virus yield assays. Dose dependence is affected both by cell confluence and serum concentration in growth medium and is more pronounced in aged, fully confluent monolayers and at low serum conditions. The latter point is demonstrated in Fig. 1, where confluent HEp-2 cells were pretreated with increasing amounts of rTNF in the presence of 1% or 10% calf serum (CS) and virus yield was assayed. Low serum conditions resulted in a 10- to 60-fold increase in virus inhibition. These results are in

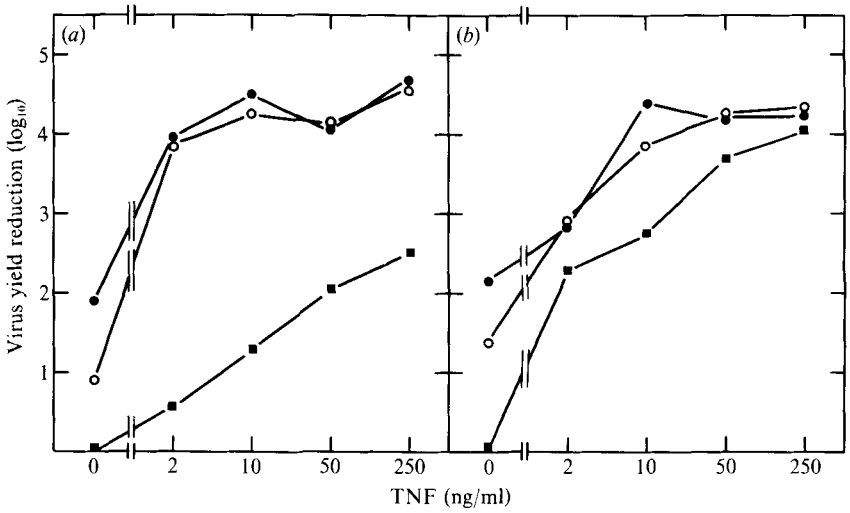

Fig. 1. Effects of serum and interferon on the antiviral activity of TNF. HEp-2 cells were grown in microtitre plates with modified Eagle's medium (MEM) containing 10% CS. Confluent cells were incubated for 20 h with different doses of TNF alone (■) or in the presence of 10 IU/ml IFN-γ (○) or IFN-β1 (●) added in triplicate in the presence of MEM with 10% CS (*a*) or MEM with 1% CS (*b*). Subsequently all monolayers were washed and infected with VSV in MEM at an m.o.i. of 0·2. After 1 h cells were washed twice and 200 µl of fresh MEM, 10% CS was added followed by incubation at 37 °C, 5% CO_2 for 24 h. Virus yields were determined in a plaque assay on confluent RITA cell (monkey kidney cells, RC-37 RITA; Italdiagnostics, Rome, Italy) monolayers in six-well plates. After freeze–thawing (−70 °C) virus-containing supernatants were diluted with basal medium Eagle (BME) in 10-fold dilution steps. RITA cell monolayers were infected with 200 µl virus suspension per well and incubated for 1 h. Subsequently overlay medium (BME, 1% carboxymethyl cellulose, 5% foetal CS) was added. After incubation for 24 h cells were fixed with 3% formaldehyde, stained with gentian violet and plaques were counted to determine the virus titre (p.f.u./ml). Reduction of virus yields is expressed in $\log_{10}$ of difference in virus titres compared to virus controls ($1{\cdot}86 \times 10^8$ p.f.u./ml for *a*; $2{\cdot}04 \times 10^8$ p.f.u./ml for *b*). The TNF was recombinant material with a specific activity of $2{\cdot}9 \times 10^7$ U/mg measured in a L-929 cytotoxicity assay. Recombinant IFN-β and IFN-γ (specific activities 3×10^8 and 4×10^7 IU/mg protein, respectively) were a gift of Bioferon GmbH, Laupheim, F.R.G.

agreement with observations of Kohase *et al.* (1986) obtained in TNF-treated cultures of human foreskin fibroblasts.

The cytotoxic activity of TNF is greatly enhanced by combination with IFN-γ and similar findings have been reported for its antiviral activity on A549 cells (Wong & Goeddel, 1986). We have analysed the effect of combinations of TNF with IFN-γ or IFN-β1 on VSV replication in HEp-2 cells. Fig. 1 compares the antiviral activities of various concentrations of TNF alone with those of TNF in combination with 10 international units (IU)/ml of IFN-γ or IFN-β1. In 10% serum in the absence of IFNs the maximum virus yield reduction is still not obtained with doses of TNF as high as 100 ng/ml (Fig. 1*a*). Treatment with concentrations of IFN-β or IFN-γ which by themselves lead to a 1 to $1{\cdot}8$ $\log_{10}$ reduction in virus yield greatly enhance the antiviral effect of TNF, resulting in maximal virus inhibition with TNF concentrations of less than 10 ng/ml. Both IFNs lead to a comparable enhancement of the antiviral activity of TNF. Especially at lower TNF doses the enhancement is 10- to 100-fold above the activity calculated by summation of the individual activities, thus fulfilling criteria for a synergistic effect. Enhanced antiviral activity was not due to an increased cytotoxic activity of the combined treatment. This was demonstrated by cell protection assays comparing intact cell monolayers after virus infection to untreated, virus-infected controls (data not shown). These results were obtained with cells grown in 10% serum. Treatment with TNF at a low serum concentration (1%) potentiated its activity to such an extent that enhancement by IFN was less significant (Fig. 1*b*). The following experiments were thus performed at high serum concentrations (10%) allowing optimal synergism.

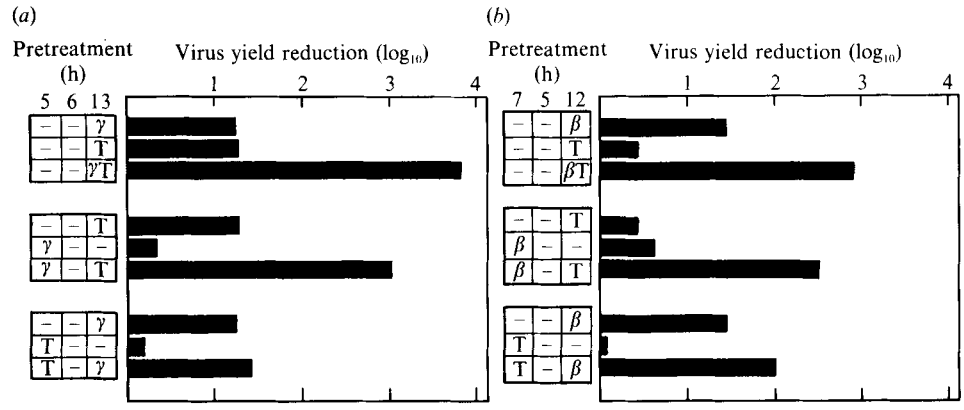

Fig. 2. Synergism of the antiviral activity of TNF and IFN, and its dependence on the sequence of pretreatment. HEp-2 cells, grown to confluence in microtitre plates were incubated in triplicate with medium alone (MEM, 10% CS), TNF (T) (20 ng/ml) and 10 IU/ml IFN-γ (γ) (*a*) or IFN-β1 (β) (*b*) as indicated. Cell monolayers were washed twice after each treatment. Infection with VSV and determination of virus titres were performed as described in the legend of Fig. 1.

We next investigated whether synergism requires pretreatment with both agents simultaneously or whether the induction of the antiviral state could be dissected by sequential treatment with each substance alone. To this end, cells were either treated with both substances together or sequentially treated with IFN and TNF in various orders (Fig. 2, see legend for details). Fig. 2(*a*) gives the results for an experiment with TNF and IFN-γ. The upper three bars indicate the reductions in virus yield in cultures which were either treated with each substance alone or together for 13 h before infection. More than 10-fold enhancement in virus inhibition was obtained by simultaneous treatment. The middle three bars summarize an experiment where treatment with IFN-γ preceded treatment with TNF (plus two controls with each substance alone to compare virus inhibitions). Sequential treatment with both agents potentiates reduction in virus yield although at any given time of pretreatment cells were only subjected to treatment with each agent alone. The lower three bars give the result of a similar experiment with TNF treatment preceding IFN-γ. Under these experimental conditions, however, no synergistic effect is observed since virus inhibition in cultures treated with both agents is no more than the sum of the inhibitions observed after pretreatment with either substance alone.

In Fig. 2(*b*) this experiment is repeated with TNF and IFN-β1 instead of IFN-γ. Simultaneous treatment with both substances leads to the expected synergism (upper bars) as does IFN-β1 treatment preceding that with TNF (middle bars). Interestingly, however, conditions where TNF precedes IFN-β1 also lead to virus inhibition exceeding that expected for treatment with either substance alone. Thus, IFN-γ differs from IFN-β1 in its capability to synergize with TNF as an antiviral factor in that it is more restricted in its requirements for induction of the antiviral state.

Since IFN-γ reportedly enhances the expression of TNF-specific binding sites on several cell lines including HeLa or L929 cells (Tsujimoto *et al.*, 1986) we assayed the binding activity of HEp-2 cells. In Fig. 3 a Scatchard plot analysis of ^{125}I-labelled TNF binding to control and IFN-γ-pretreated cells is shown. Even at high concentrations (500 U/ml) IFN-γ causes only a 30 to 50% increase in TNF-binding sites starting from a basal level of 1500 to 2500 per cell in different experiments. Binding affinities of TNF to IFN-γ-treated cells were either unaltered or even slightly decreased when compared with control cells (Fig. 3). IFN-β1 pretreatment of HEp-2 cells was without effect on TNF binding (data not shown). Hence, we conclude that modulation of TNF binding by IFNs is not a sufficient prerequisite for synergistic action.

It has been shown that TNF treatment leads to an increase in oligo-2′,5′-adenylate synthetase (2–5AS) levels (Mestan *et al.*, 1986; Wong & Goeddel, 1986). Since this enzyme may be involved

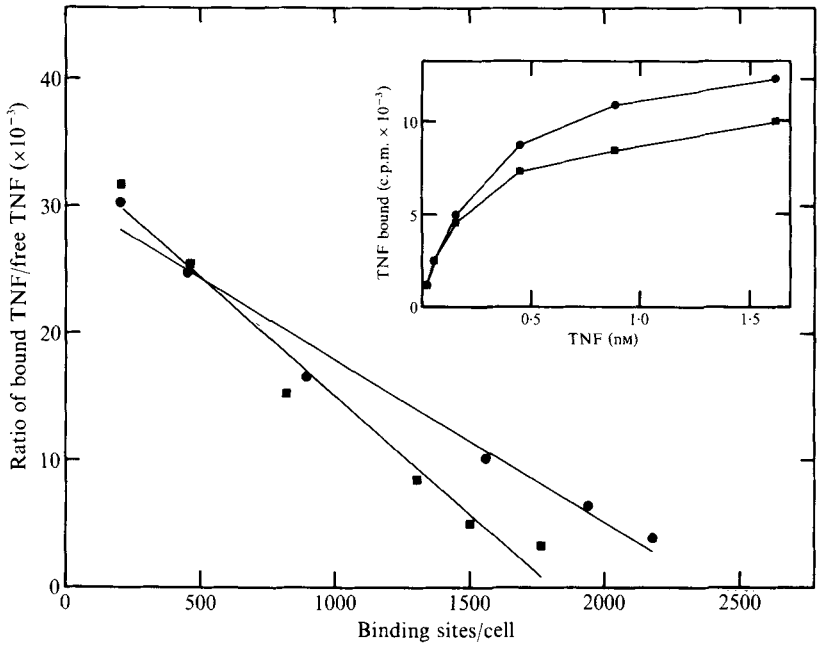

Fig. 3. Scatchard plot analysis of labelled TNF binding to control (■) and IFN-γ-pretreated (●) cells. TNF was iodinated with Na^{125}I and Iodo-gen (Pierce, Rockford, Ill., U.S.A.) (Fraker & Speck, 1978). The product was 99% acid-precipitable after gel filtration through a PD10 column (Pharmacia) and had a specific radioactivity of approx. 600 Ci/mmol protein retaining full cytotoxic activity. HEp-2 cells were seeded into C-6 plates (Costar) 2 days before the binding assay. IFN-γ was added 16 h before the experiment at 500 U/ml. Cells were preincubated at 37 °C for 15 min with complete medium containing 0·1% sodium azide in order to prevent internalization of bound ligand. The cells ($3·5 \times 10^6$) were incubated with ^{125}I-labelled TNF for 90 min at 37 °C in the same medium. Control wells with 1000 times the concentration of unlabelled TNF were incubated in parallel to enable determination of non-specific binding. The adherent cells were washed five times with cold phosphate-buffered saline, lysed with 2% Triton X-100 and counted. All data are the mean of duplicate determinations showing less than 5% deviation from each other.

in the antiviral state, we decided to assay its activity in TNF- or TNF/IFN-treated HEp-2 cells (Fig. 4) in addition to virus yield assays as a possible marker for synergism. Pretreatment with either TNF or IFN increased 2–5AS activity. Combined treatment with both TNF and IFN-β1 enhanced the induction more than twofold. In contrast, treatment with TNF and IFN-γ resulted in enzyme activities not higher than those observed with IFN-γ or TNF alone. Oligo-2′,5′-adenylate synthetase induction by IFN-β1 is a primary response, i.e. gene activation and mRNA accumulation occur in the presence of inhibitors of protein synthesis whereas induction by IFN-γ has been reported to be sensitive to such inhibitors (Faltynek *et al.*, 1985). We have found that in HEp-2 cells the level of synthetase-specific mRNA is much reduced when TNF treatment occurs in the presence of cycloheximide (data not shown) pointing to an indirect induction pathway. Since so far only IFNs have been described as specific inducers of this activity, it was of interest to determine whether its induction was due to an IFN-like intermediate. We and others have reported previously that the induction of the antiviral state can be blocked to a variable extent by the addition of an antiserum to natural IFN-β1 (Mestan *et al.*, 1986; Van Damme *et al.*, 1987). In Fig. 5(*a*) an experiment is shown where cultures were treated with TNF in the presence or absence of an antiserum to IFN-β1 and subsequently assayed for 2–5AS activity. Included is a control experiment in which cells were treated with the dsRNA poly(I)·poly(C), a potent inducer of IFN-β in fibroblast cell lines. Induction of 2–5AS is greatly reduced in those cultures which were treated with the neutralizing antiserum. The specificity of this effect is underlined by the results of a Northern blot analysis for 2–5AS-

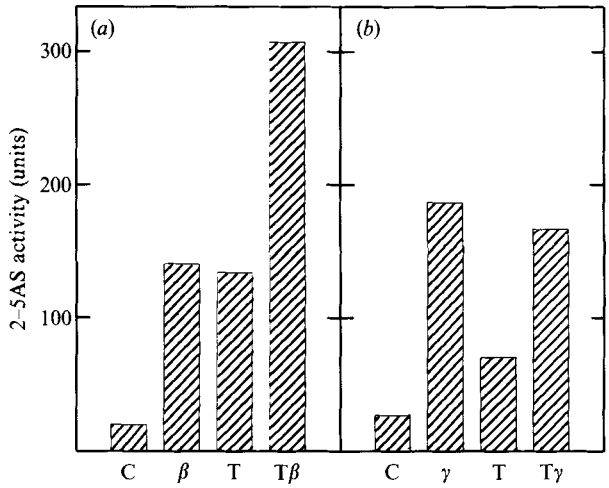

Fig. 4. Induction of 2–5AS activity in TNF- or IFN/TNF-treated HEp-2 cells. HEp-2 cells were grown to confluence in 14 cm Petri dishes with MEM 10% CS and incubated with TNF (T), IFN-β1 (β), IFN-γ (γ) or combinations of TNF and IFN at 37 °C, 5% CO_2 for 20 h. Untreated cells were used as a control (C). The assay for measuring 2–5AS activity was performed as described elsewhere (Knight *et al.*, 1980; Silverman *et al.*, 1982). Briefly, after harvesting and washing cells were lysed in NP40-containing lysis buffer and centrifuged at 12000 *g* for 10 min at 4 °C. The protein content of the supernatant was determined using a Bio-Rad protein assay kit with bovine serum albumin as the standard. Protein (200 µg) from each supernatant was added to 20 µl poly(I)·poly(C)–agarose (Pharmacia) followed by incubation for 1 h at room temperature and three washes to remove unbound material with DBG buffer (50 mM-KCl, 1·5 mM-magnesium acetate, 20% v/v glycerol, 7 mM-2-mercaptoethanol, 10 mM-HEPES pH 7·5). DBG (15 µl) containing 15 mM-$MgCl_2$ and 8 mM-ATP as substrate for 2–5AS was added and incubated for 3 h at 37 °C. Subsequently the poly(I)·poly(C)–agarose was removed by centrifugation and serial dilutions of the supernatant were mixed with 10 µl L-929 cell extract containing 200 µg protein and with 5 µl ppp(A2′p)$_3$A3′[^{32}P]pCp (1000 c.p.m./µl) radiolabelled according to Knight *et al.* (1980). After incubation for 1 h at 4 °C the reaction mixture was transferred to small pieces of nitrocellulose sheet and washed twice in distilled water to remove unbound material. The radioactivity bound to the nitrocellulose sheets was measured in a liquid scintillation counter. The concentration of the synthesized 2′–5′A_n was evaluated using a standard competition curve with serial dilutions of unlabelled 2′–5′A_4 used to provide a standard. Activity of 2–5AS is expressed in units such that 1 unit is defined as pmol 2′–5′A_n produced/mg protein/h.

specific transcripts (Fig. 5*b*). Whereas there is a large increase in mRNA in TNF-treated cells, this increase is largely cancelled when an antiserum to IFN-β is included. Together these data prove that 2–5AS induction by TNF is indirect and mediated by a TNF-induced, IFN-β-like activity.

However, induction of IFN-β1 by TNF has not been directly demonstrated, either by Northern blot analysis or by detection of IFN activity in supernatants from TNF-treated cultures. This may either reflect the induction of a mediator different from IFN-β1, which is not detected by the latter methods, or the production of very small amounts of IFN-β1. Since our experiments demonstrate that small amounts of exogenous IFN-β1 are sufficient to potentiate the activity of TNF by more than an order of magnitude, it seems possible that endogenous TNF-induced IFN-β1 below the level of detection could play a decisive role in the TNF-mediated antiviral state, e.g. as inducer of 2–5AS. This raises the question of whether the sole role of TNF in the establishment of the antiviral state is that of an inducer of an IFN-β activity with no antiviral activity on its own. Such a model, however, would not explain the synergism in antiviral activity observed after treatment of HEp-2 cells with TNF and exogenous IFN-β1 but would rather predict an additive effect. Our observations therefore favour a model where TNF and some induced IFN-β-like activity combine to cause antiviral activity.

It has been demonstrated that TNF induced IFN-β2 (interleukin 6, 26K protein) activity in various cells (Kohase *et al.*, 1986; DeFilippi *et al.*, 1987). We have also measured increased levels

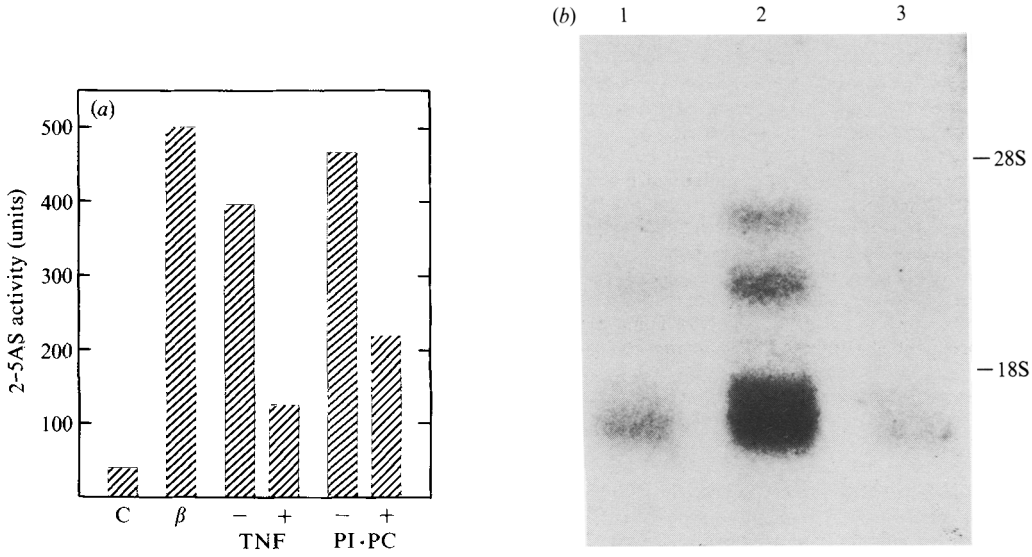

Fig. 5. Effect of IFN-β antiserum on the induction of 2–5AS by TNF in HEp-2 cells. (*a*) Effect on enzyme activity. HEp-2 cells grown to confluence in 14 cm Petri dishes with MEM, 10% CS were incubated for 14 h with medium alone (control; C), 100 IU/ml IFN-β as a positive control (β), 100 ng/ml TNF in the absence (−) or presence (+) of polyclonal IFN-β antibodies (Paesel, Frankfurt, F.R.G.) or 100 μg/ml poly(I)·poly(C) (PI·PC) in the absence or presence of the antibodies. The final concentration of antibodies (previously confirmed to inhibit the antiviral activity of IFN-β) was 2500 neutralizing units (NU)/ml. Evaluation of 2–5AS induction was performed as described in legend of Fig. 4. (*b*) Effect on induction of mRNA. Cells were incubated for 12 h with medium alone (control; lane 3) or with 500 ng/ml TNF in the presence (lane 1) or absence (lane 2) of 2500 NU/ml IFN-β antibodies. Total RNA was isolated by the guanidinium thiocyanate method (Chirgwin *et al.*, 1979) and purified through a CsCl cushion. It was separated on a denaturing formaldehyde gel (20 μg per lane) and blotted onto nylon membrane and hybridized essentially as described by Maniatis *et al.* (1982). Hybridization was for 18 h at 42 °C in 50% formamide, 5 × SSPE, 5 × Denhardt's solution, 1% SDS and 200 μg/ml of yeast RNA containing $1{\cdot}5 \times 10^6$ c.p.m./ml of a cDNA of the human 2–5AS gene which was labelled to 1×10^9 to 2×10^9 c.p.m./μg by the incorporation of [^{32}P]dCTP in a random priming reaction (Feinberg & Vogelstein, 1983). Stringent washing was for 60 min at 68 °C in 2 × SSC, 0·1% SDS. Radioactively labelled bands were visualized by autoradiography on Kodak X-AR film.

of IFN-β2 mRNA in TNF-treated HEp-2 cells as well as a B cell-stimulating activity in the supernatants of such cultures (data not shown). Although its role in this system remains to be established using either purified IFN-β2 or monospecific antiserum our failure to detect antiviral activity in supernatants of TNF-treated cultures argues against a role of IFN-β2 as an independent mediator of the antiviral activity of TNF.

Both IFN-β1 and IFN-γ potentiate the antiviral activity of TNF. However, our induction experiments point to different mechanisms underlying this synergism. IFN-γ may increase the responsiveness of cells to TNF and therefore require concomitant treatment for extended periods or IFN-γ treatment preceding that with TNF. Our binding data suggest that modulation of TNF-binding sites by IFN-γ is of only minor importance for this effect. For IFN-β1 a different mode of action has to be assumed, since its interaction with TNF is unaffected by the sequence of treatment. Together with previous reports on synergisms of IFN-α with either IFN-β or IFN-γ (Czarniecki *et al.*, 1984; Oleszak & Stewart, 1985) our results suggest a network of multiple interactions through which these cytokines exert their activities leading to the induction of an antiviral state.

The authors thank Mrs V. Feussner for expert technical assistance and Dr M. Rutherford (Toronto) for a human 2–5AS cDNA clone. Recombinant TNF was generously provided by Knoll AG, Ludwigshafen, F.R.G.

REFERENCES

CARSWELL, E. A., OLD, L. J., KASSEL, R. L., GREEN, S., FIORE, N. & WILLIAMSON, B. (1975). An endotoxin-induced serum factor that causes necrosis of tumors. *Proceedings of the National Academy of Sciences, U.S.A.* **72**, 3666–3670.

CHIRGWIN, J. M., PRZYBYLA, A. E., MACDONALD, R. J. & RUTTER, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294–5299.

CZARNIECKI, C. W., FENNIE, C. W., POWERS, D. P. & ESTELL, D. A. (1984). Synergistic antiviral and antiproliferative activities of *Escherichia coli*-derived human alpha, beta, and gamma interferons. *Journal of Virology* **49**, 490–496.

DEFILIPPI, P., POUPART, P., TAVERNIER, J., FIERS, W. & CONTENT, J. (1987). Induction and regulation of mRNA encoding 26-kDa protein in human cell lines treated with recombinant human tumor necrosis factor. *Proceedings of the National Academy of Sciences, U.S.A.* **84**, 4557–4561.

DINARELLO, C. A., CANNON, J. G., WOLFF, S. M., BERNHEIM, H. A., BEUTLER, B., CERAMI, A., FIGARI, I. S., PALLADINO, M. A., JR & O'CONNOR, J. V. (1986). Tumor necrosis factor (cachectin) is an endogeneous pyrogen and induces production of interleukin 1. *Journal of Experimental Medicine* **163**, 1433–1450.

FALTYNEK, C. R., McCANDLESS, S., CHEBATH, J. & BAGLIONI, C. (1985). Different mechanisms for activation of gene transcription by interferons β and γ. *Virology* **144**, 173–180.

FEINBERG, A. P. & VOGELSTEIN, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132**, 6–13.

FRAKER, P. J. & SPECK, J. C. (1978). Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrochloro-3α,6α-diphenylglycoluril. *Biochemical and Biophysical Research Communications* **80**, 849–857.

FRANSEN, L., MÜLLER, R., MARMENOUT, A., TAVERNIER, J., VAN DER HEYDEN, J., KAWASHIMA, E., CHOLLET, A., TIZARD, R., VAN HEUWERSWYN, H., VAN VLIET, A., RUYSSCHAERT, M. & FIERS, W. (1985). Molecular cloning of mouse tumour necrosis factor cDNA and its eukaryotic expression. *Nucleic Acids Research* **13**, 4417–4429.

KLEBANOFF, S. J., VADAS, M. A., HARLAN, J. M., SPARKS, L. H., GAMBLE, J. R., AGOSTI, J. M. & WALTERSDORPH, A. M. (1986). Stimulation of neutrophils by tumor necrosis factor. *Journal of Immunology* **136**, 4220–4225.

KNIGHT, M., CAYLEY, P. J., SILVERMAN, R. H., WRESCHNER, D. H., GILBERT, C. S., BROWN, R. E. & KERR, I. M. (1980). Radioimmune, radiobinding and HPLC analysis of 2-5 A and related oligonucleotides from intact cells. *Nature, London* **288**, 189–192.

KOHASE, M., HENRIKSEN-DE STEFANO, D., MAY, L. T., VILCEK, J. & SEHGAL, P. B. (1986). Induction of β_2-interferon by tumor necrosis factor: a homeostatic mechanism in the control of cell proliferation. *Cell* **45**, 659–666.

MANIATIS, T., FRITSCH, E. F. & SAMBROOK, J. (1982). *Molecular Cloning: A Laboratory Manual.* New York: Cold Spring Harbor Laboratory.

MARMENOUT, A., FRANSEN, L., TAVERNIER, J., VAN DER HEYDEN, J., TIZARD, R., KAWASHIMA, E., SHAW, A., JOHNSON, M., SEMON, D., MÜLLER, R., RUYSSCHAERT, M., VAN VLIET, A. & FIERS, W. (1985). Molecular cloning and expression of human tumor necrosis factor and comparison with mouse tumor necrosis factor. *European Journal of Biochemistry* **152**, 515–522.

MESTAN, J., DIGEL, W., MITTNACHT, S., HILLEN, H., BLOHM, D., MÖLLER, A., JACOBSEN, H. & KIRCHNER, H. (1986). Antiviral effects of recombinant tumour necrosis factor in vitro. *Nature, London* **323**, 816–819.

NAWROTH, P. P., BANK, I., HANDLEY, D., CASSIMERIS, J., CHESS, L. & STERN, D. (1986). Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin 1. *Journal of Experimental Medicine* **163**, 1363–1375.

OLD, L. J. (1985). Tumor necrosis factor (TNF). *Science* **230**, 630–632.

OLESZAK, E. & STEWART, W. E. (1985). Potentiation of the antiviral and anticellular activities of interferons by mixtures of huIFN-γ and huIFN-α or huIFN-β. *Journal of Interferon Research* **5**, 361–371.

PENNICA, D., NEDWIN, G. E., HAYFLICK, J. S., SEEBURG, P. H., DERYNCK, R., PALLADINO, M. A., KOHR, W. J., AGGARWAL, B. B. & GOEDDEL, D. V. (1984). Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature, London* **312**, 724–729.

SHALABY, M. R., AGGARWAL, B. B., RINDERKNECHT, E., SVEDERSKY, L. P., FINKLE, B. S. & PALLADINO, M. A., JR (1985). Activation of human polymorphonuclear neutrophil functions by interferon-γ and tumor necrosis factors. *Journal of Immunology* **135**, 2069–2073.

SHIRAI, T., YAMAGUCHI, H., ITO, H., TODD, C. W. & WALLACE, R. B. (1985). Cloning and expression in *Escherichia coli* of the gene for human tumour necrosis factor. *Nature, London* **313**, 803–806.

SILBERSTEIN, D. S. & DAVID, J. R. (1986). Tumor necrosis factor enhances eosinophil toxicity to *Schistosoma mansoni* larvae. *Proceedings of the National Academy of Sciences, U.S.A.* **83**, 1055–1059.

SILVERMAN, R. H., WATLING, D., BALKWILL, F. R., TROWSDALE, J. & KERR, I. M. (1982). The ppp(A2′p)$_n$A and protein kinase systems in wild-type and interferon-resistant Daudi cells. *European Journal of Biochemistry* **126**, 333–341.

SUGARMAN, B. J., AGGARWAL, B. B., HASS, P. E., FIGARI, I. S., PALLADINO, M. A., JR & SHEPARD, H. M. (1985). Recombinant human tumor necrosis factor-α: effects on proliferation of normal and transformed cells in vitro. *Science* **230**, 943–945.

TSUJIMOTO, M., YIP, Y. K. & VILCEK, J. (1986). Interferon-γ enhances expression of cellular receptors for tumor necrosis factor. *Journal of Immunology* **136**, 2441–2444.

VAN DAMME, J., DE LEY, M., VAN SNICK, J., DINARELLO, C. A. & BILLIAU, A. (1987). The role of IFN-β_1 and the 26-kDa protein (interferon-β_2) as mediators of the antiviral effect of interleukin 1 and tumor necrosis factor. *Journal of Immunology* **139**, 1867–1872.

VILCEK, J., PALOMBELLA, V. J., HENRYKSEN-DESTEFANO, D., SWENSON, C., FEINMAN, R., HIRAI, M. & TSUJIMOTO, M. (1986). Fibroblast growth enhancing activity of tumor necrosis factor and its relationship to other polypeptide growth factors. *Journal of Experimental Medicine* **163**, 632–643.

WANG, A. M., CREASEY, A. A., LADNER, M. B., LIN, L. S., STRICKLER, J., VAN ARSDELL, J. N., YAMAMOTO, R. & MARK, D. F. (1985). Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science* **228**, 149–228.

WONG, G. H. W. & GOEDDEL, D. V. (1986). Tumour necrosis factors α and β inhibit virus replication and synergize with interferons. *Nature, London* **323**, 819–822.

(*Received 4 May 1988*)