Antiviral Research, 11 (1989) 77–88
Elsevier

AVR 00338

The in vitro antiviral activity of tumor necrosis factor (TNF) in WISH cells is mediated by IFN-β induction

Vito Ruggiero[1], Guido Antonelli[1], Giancarlo Conciatori[1], Massimo Gentile[1], Jo Van Damme[2] and Ferdinando Dianzani[1]

[1]*Institute of Virology, 'La Sapienza' University of Rome, Rome, Italy and* [2]*Rega Institute, Leuven, Belgium*

(Received 26 October 1988; accepted 14 December 1988)

Summary

We present evidence showing that TNF is capable of inducing an antiviral state in WISH cells thereby protecting them from the cytopathic effect of vesicular stomatitis virus. Establishment of the antiviral state requires pretreatment with TNF. Such pretreatment not only protects the cells in a dose-dependent manner, but it markedly reduces virus yield as well. Kinetic studies have shown that a pretreatment period as short as 4 h at 37°C is effective in conferring protection. The antiviral activity of TNF could be attributed to the induction of IFN-β. In fact, polyclonal antibodies to IFN-β completely neutralized the antiviral state elicited by TNF. 2–5A synthetase activity was significantly enhanced when the cells were treated with doses of TNF that afforded antiviral protection. Finally, addition of specific antibodies to IFN-β_2 (IL-6) during TNF pretreatment failed to abolish the antiviral state, thus suggesting that IFN-β_2 is not involved in the TNF-induced antiviral state. Also, a homogeneous IFN-β_2 preparation failed to exert antiviral activity in our cell system.

TNF; IFN-β_2; IL-6; 2-5A synthetase

Introduction

Tumor necrosis factor (TNF) is a protein originally found in the serum of mice infected with bacillus Calmette-Guérin and subsequently injected with endotoxins

Correspondence to: V. Ruggiero, Institute of Virology, 'La Sapienza' University of Rome, Rome, Italy.

(Carswell et al., 1975). Although TNF was initially recognized because of its cytotoxic and antitumor properties (Old, 1985), the recent availability of large amounts of purified recombinant murine (Fransen et al., 1985) and human TNF (Pennica et al., 1984) has allowed to perform a number of studies which have revealed that TNF possesses a much broader spectrum of biologic activities than originally presumed (Nathan, 1987). Additionally, Kohase et al. (1986) showed that TNF exerts an antiviral effect on 'aged' cultures of human fibroblasts. Mestan et al. (1986) reported an antiviral activity of TNF in three out of six human cell lines. Similar antiviral effects of TNF were also observed by others (Wong and Goeddel, 1986; Van Damme et al., 1987; Arakawa et al., 1987; Gessani et al., 1988). These reports indicate that TNF is able to induce an antiviral activity and, therefore, may play some role as an antiviral agent. However, the mechanism whereby TNF elicits this antiviral activity is still controversial. Data obtained by Kohase et al. (1986) suggest that the antiviral activity of TNF may be ascribable to induction of IFN-β_2. However, Wong and Goeddel (1986) found no correlation between the antiviral activity of TNF and its ability to induce IFN-β_2 mRNA. Gessani et al. (1988) found that addition of antisera neutralizing IFN-α or IFN-β did not reduce the antiviral activity of TNF in HeLa cells. Conversely, Ito and O'Malley (1987) showed that the antiviral action of TNF was completely abrogated by anti-IFN-β serum, but not by anti-IFN-α or anti-IFN-γ antibodies. However, recent data by Van Damme et al. (1987) indicate that the antiviral activity of TNF may be attributed, at least in part, to induction of IFN-β_1 rather than of IFN-β_2.

In an attempt to further study the antiviral effects of TNF and to gain some new insight into the mechanism(s) underlying its effects, we used a line of human amniotic cells (WISH) highly sensitive to IFN. Our results show that TNF is able to elicit an antiviral state in WISH cells. This antiviral state is mediated by the induction of IFN-β, since polyclonal antibodies to IFN-β completely neutralized the antiviral effects of TNF. We also found that 2–5A synthetase levels are significantly enhanced following treatment of the cells with doses of TNF that confer antiviral protection.

Furthermore, we observed that polyclonal antibodies to IFN-β abolish the TNF-induced increase in 2–5A synthetase activity. Finally, we present evidence that in our cell system classical IFN-β, rather than IFN-β_2, is responsible for the antiviral activity of TNF.

Materials and Methods

Cell culture

Human WISH cells, a cell line originally derived from human amnion tissue, were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (Flow Laboratories, Irvine, Scotland). The cells were maintained in a 5% CO_2 incubator and were subcultured twice a week.

Cytokines and antisera

Recombinant human tumor necrosis factor (TNF), lot No. H9087A, and mouse monoclonal antibodies (IgG_1) to TNF, lot No. 3314–3316, were a kind gift of Dr. G.R. Adolf of the Ernst Boehringer Institute, Vienna, Austria. The specific activity of TNF was 6×10^7 units/mg, while the neutralizing capacity of anti-TNF antibodies was 6000 units TNF/μg. Purified natural IFN-β, specific activity of 1.3×10^8 IU/mg, was obtained from Serono, Rome, Italy. Polyclonal antibodies raised to partially purified IFN-β (specific activity: 10^7 IU/mg) were from Sclavo, Siena, Italy, and had a neutralizing titer of 1:200000. Homogeneously purified natural IFN-β_2 (IL-6), with a hybridoma growth factor (HGF) specific activity of 10^8 U/mg, and rabbit antibodies raised to this IFN-β_2, and having a neutralizing titer of 1:100000, were produced as described previously (Van Damme et al., 1987).

Assay for antiproliferative and cytotoxic activity of TNF

Growth inhibition was measured by seeding 10^4 cells/well in microtiter plates; the cells were stained with crystal violet after 4 days of treatment with TNF (Ruggiero et al., 1986). Cytotoxicity was measured by treating 6 to 8×10^4 cells/well in microtiter plates for 18 h with TNF and 0.05 μg/ml of actinomycin D (Ruff and Gifford, 1979). Then, the monolayers were washed three times with 0.9% NaCl to remove dead cells, and were stained with 0.2% crystal violet in 15% ethanol. This dye was eluted with 33% acetic acid, and the absorbance at 540 nm was measured in a microdensitometer. The assays were carried out in triplicate and gave a standard error of 6%.

Antiviral and virus yield assays

The antiviral assay was a slight modification of the method of Beresini et al. (1988). Briefly, confluent cell monolayers in microtiter plates were incubated for 24 h with serial dilutions of the cytokines in RPMI 1640 with 0.5% of fetal bovine serum. Subsequently, the cells were challenged with vesicular stomatitis virus (VSV) for 1 h (MOI=0.1 to 0.3). After infection, the cell monolayers were washed three times, and then incubated with culture medium containing 2% of fetal bovine serum. Following a further incubation for 24 h, the medium was collected for determination of virus yield (see below), and the cells were washed three times with 0.9% NaCl and stained with 0.2% crystal violet in 15% ethanol. The incorporated dye was then eluted with 33% acetic acid and its absorbance was measured in a microdensitometer at 540 nm. Dye uptake in each well allowed the degree of inhibition of the virus-induced cytopathic effect to be quantitated spectrophotometrically. The data are presented as cell protection as a percent of that of untreated, unchallenged controls. Virus yield was determined by a plaque assay. Briefly, the supernatants from the antiviral assays were serially diluted and added (50 μl/well) to confluent monolayers of murine L929 cells in microtiter plates. After 1 h incubation, the cells were overlayed with 100 μl/well of overlay medium (MEM,

1% methyl-cellulose, 3% fetal calf serum). Then, 24 h later, the cells were stained with crystal violet (see above) and the plaques were counted to determine plaque-forming units (PFU) per ml.

Assay of 2–5A synthetase activity

Five μl of cell extracts (about 80–100 μg of proteins) were incubated for 1.5 h at 30°C in 20 μl of the incubation mixture described by Minks et al. (1979) (i.e. 20 mM Hepes KOH pH 7.4, 15 mM KCl, 25 mM Mg $(OAc)_2$, 1 mM dithiothreitol, 5 mM ATP, 4 mM fructose, 1,6-diphosphate and 20 μg/ml poly(rI)-poly(rC), and 0.8 μCi of $(2,8^{-3}H)$ATP). The radioactive 2–5A oligomers synthesized from $(2,8^{-3}H)$ATP in the S-10 extracts were isolated by chromatography on DEAE-cellulose, according to Minks et al. (1979) and analyzed by liquid scintillation.

Results

Antiviral activity of TNF on WISH cells

The cells were pretreated overnight with increasing amounts of TNF or IFN-β and then challenged with VSV. As shown in Fig. 1, TNF was capable of protecting the cells, but its antiviral activity was much less pronounced than that of IFN-β. In fact, 50% of the cell monolayer was protected by 0.3 ng/ml of TNF and 7.5 pg/ml

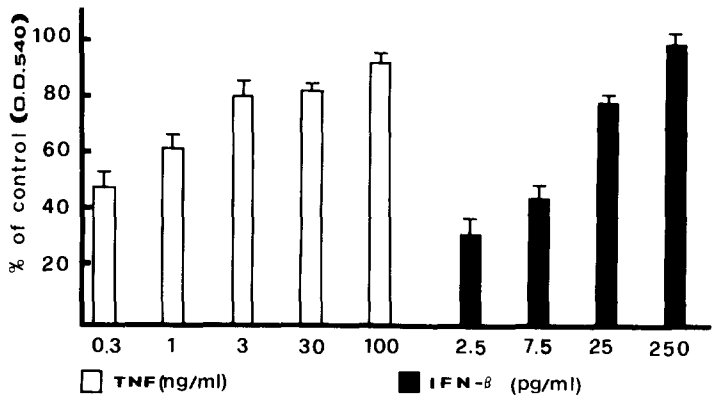

Fig. 1. Antiviral effects of TNF and IFN-beta on WISH cells. Cells were grown to confluence in microtiter plates. After treatment for 24 h with different concentrations of TNF or IFN-β the cells were challenged with VSV for 1 h. Based on the IFN-β specific antiviral activity of 1.3×10^8 IU/mg, 2.5, 7.5, 25, and 250 pg/ml of IFN-β approximately correspond to 0.3, 1, 3 and 30 IU/ml, respectively. After infection, the cells were washed 3 times and fed with fresh medium. After incubation for 24 h, the cells were stained with crystal violet; then, the dye was eluted and its absorbance at 540 nm was quantitated spectrophotometrically. Dye uptake in each well gave a quantitative measure of cell protection from viral CPE. Data (means ± SE of 5 individual wells) are presented as cell protection as percent of that of untreated uninfected cell control (100%).

of IFN-β (corresponding to about 1 IU/ml). At the highest concentration tested, i.e. 100 ng/ml, TNF protected about 90% of cells from the cytopathic effect (CPE) of VSV, whilst a 400-fold lower concentration of IFN-β was sufficient to obtain complete protection. This result shows that TNF is effective in protecting the cells against viral CPE, although, on a molar basis, it possesses a relatively limited antiviral activity compared to that of IFN-β.

Effect of TNF on WISH cell growth

After assessing the antiviral effect of TNF, we examined whether a prolonged treatment with doses of TNF that afforded antiviral protection could have any antiproliferative effects on uninfected WISH cells. As it can be seen from Fig. 2 (panel B), after 4 days of treatment with different concentrations of TNF, the cells were not significantly inhibited in their growth. The following experiment was aimed at establishing whether these cells were sensitive to TNF cytotoxicity. Confluent cultures were treated for 18 h with different concentrations of TNF and 0.05 μg/ml of actinomycin D, which enhances TNF cytotoxicity (Ruff and Gifford, 1979; Kull and Cuatrecasas, 1981; Ruggiero et al., 1987). As shown in Fig. 2 (panel A), WISH cells turned out to be quite sensitive to the toxic effect of TNF in the presence of this inhibitor, the LD_{50} being 0.3 ng/ml of TNF. These results showed that treatment with doses of TNF active in antiviral protection did not exert any negative effect in the antiproliferative assay while being, on the contrary, quite effective in the cytotoxic assay.

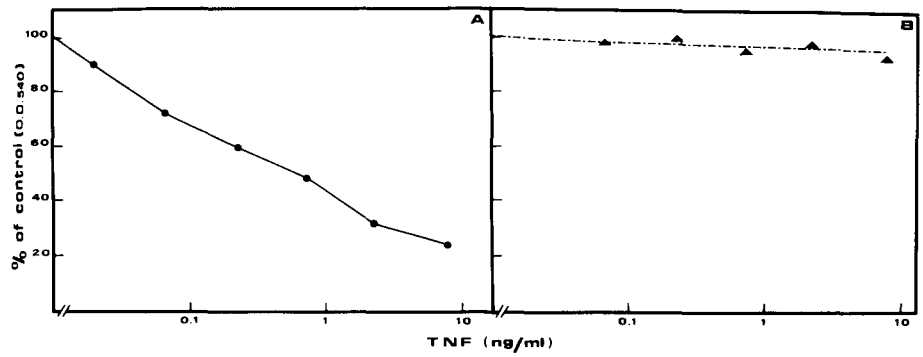

Fig. 2. Antiproliferative (panel B) and cytotoxic activity in the presence of actinomycin D (panel A) of TNF. WISH cells were seeded in microtiter plates and were treated either for 4 days (B) or for 18 h (A) with the indicated concentrations of TNF; 0.05 μg/ml of actinomycin D was added in the cytotoxic assay. In the antiproliferative assay, the cell control is given by untreated cells, while in the cytotoxic assay the cell control is represented by cells not treated with TNF but treated with 0.05 μg/ml of actinomycin D. Viable cells were measured as reported in Materials and Methods. The viability of cells treated only with actinomycin D was about 85% of that of cell control.

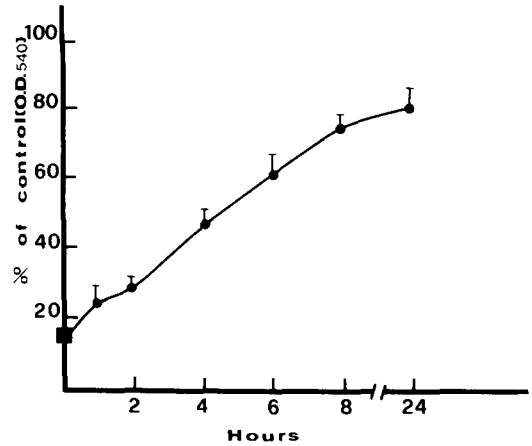

Fig. 3. Kinetics of induction of the antiviral state against VSV in WISH cells treated with TNF. The cells were treated for various lengths of time with culture medium supplemented with 10 ng/ml of TNF and 20 mM Hepes, pH 7.4, in a water bath set at 37°C. Infection with VSV and determination of cell protection was performed as described in Fig. 1. Data represent means ± S.E. of 5 individual wells.

Kinetics of establishment of the antiviral state by TNF

The kinetics of induction of the antiviral state by TNF in WISH cells was evaluated in an experiment where the cells were treated with 10 ng/ml of TNF for varying periods of time prior to VSV challenge. Treatment of WISH cells was carried out in a water bath set at 37°C and by using prewarmed (37°C) TNF with the objective to shorten the time required for temperature adjustment as much as possible. In these conditions, preincubation with TNF for a period as short as 4 h gave significant protection, while 8 h pretreatment with TNF achieved almost maximum protection (Fig. 3). These findings indicated that TNF causes a fairly rapid induction of antiviral activity. Furthermore, the kinetics of induction of the antiviral state closely resembled that of poly(I)-poly(C), a well known inducer of IFN-β.

TABLE 1

VSV yield reduction by TNF; effects of anti-TNF and anti-IFN-β antibodies

Experimental conditions	VSV yield (log PFU/ml ± S.E.)	Yield reduction
VSV control	8.2 ± 0.1	
TNF	6.7 ± 0.1	1.5
TNF + anti-TNF	8.3 ± 0.3	0.1
TNF + anti-IFN-β	8.0 ± 0.2	0.2
IFN-β	5.9 ± 0.1	2.3
IFN-β + anti-IFN-β	8.1 ± 0.2	0.1
IFN-β + anti-TNF	6.1 ± 0.2	2.1

TNF and IFN-β concentrations were 10 ng/ml and 250 pg/ml, respectively. Sufficiently diluted antibodies were added to each assay to neutralize 50 ng of TNF and 1250 pg of IFN-β.

Effect of TNF on VSV yield and its reversal by anti-TNF and anti-IFN antibodies

In our earlier experiment (Fig. 1) we showed that TNF exerted detectable antiviral protection on WISH cells. To confirm the actual decrease in virus production in TNF-treated cells as well as to investigate the role (if any) of IFN-β in this process, we carried out a VSV-yield reduction assay. As shown in Table 1, 10 ng/ml of TNF caused a significant inhibition (1.5 log) of viral replication. Monoclonal antibodies to TNF completely neutralized its antiviral activity, but, interestingly, polyclonal antibodies to IFN-β turned out to be effective in abolishing the antiviral effect of TNF as well (Table 1), while neither anti-IFN-α nor anti-IFN-γ antibodies affected it at all (data not shown).

The results of Table 1 also show that, in control experiments, IFN-β activity was not affected by the anti-TNF antibodies while being totally annihilated by the corresponding anti-IFN-β antibodies. These results indicate (i) that TNF is actually decreasing the viral yield, and (ii) that the establishment of the antiviral state by TNF is actually mediated through the induction of IFN-β.

Role of 2–5A synthetase in the TNF-induced antiviral state

To further assess the role of IFN in the TNF-induced antiviral state, we assayed the activity of 2–5A synthetase, an enzyme known to be indicative of the presence of IFN. As shown in Table 2, TNF proved to be effective in increasing the activity of 2–5A synthetase up to about 70% of that induced by IFN-β. Interestingly, the induction of 2–5A synthetase activity was neutralized not only by monoclonal antibodies to TNF, but also by polyclonal antibodies directed against IFN-β. These results suggest that, at least in our cell system, TNF did not have any intrinsic antiviral activity, but rather it acted by virtue of its ability to induce IFN-β. Furthermore, 2-5A synthetase appeared to be the ultimate mediator of the TNF-induced antiviral protection since antibodies of IFN-β not only abolished this enzymatic activity but also completely abrogated the antiviral state induced by TNF.

TABLE 2

2–5A synthetase induction by TNF; effects of anti-TNF and anti-IFN-β antibodies

Experimental conditions	2–5A synthetase activity (cpm/μg of proteins)
Cell control	260 ± 20
TNF	589 ± 50
TNF + anti-TNF	290 ± 30
TNF + anti-IFN-β	338 ± 40
IFN-β	847 ± 60
IFN-β + anti-IFN-β	415 ± 60

Cytokine concentrations and antibody dilutions were the same as in Table 1.

Lack of a role of IFN-β_2 in the antiviral action of TNF in WISH cells

After establishing the role of IFN-β in the antiviral state elicited by TNF, we next performed experiments aimed at investigating whether the molecular species of IFN-β involved was either IFN-β_1 or IFN-β_2, or both of these, and we used highly specific antibodies to IFN-β_2 and tested whether they could neutralize the antiviral activity induced by TNF. The results (Fig. 4) show that while both anti-TNF monoclonal and anti-IFN-β polyclonal antibodies abolished the antiviral activity of TNF, polyclonal antibodies to IFN-β_2, were completely ineffective. These findings suggest that IFN-β_2 does not play a significant role as a mediator of the TNF-induced antiviral activity in our cell system. Additional support to this conclusion was provided by the inability to detect antiviral protection by using a homogeneously purified preparation of IFN-β_2 in a typical antiviral protection assay (Fig. 5).

Discussion

We have examined the effects of TNF on viral replication in a line of human amniotic cells (WISH) highly sensitive to IFN. We found that in this cell system TNF exerts an antiviral activity, as shown by its ability to protect the cell monolayers from the cytopathic effects of VSV, and reduces viral yield as well. We also observed similar effects of TNF in WISH cells infected with Sindbis virus (data not shown), thus suggesting that the antiviral activity of TNF is not just limited against VSV, but is effective against other viruses as well. TNF alone did not affect cell

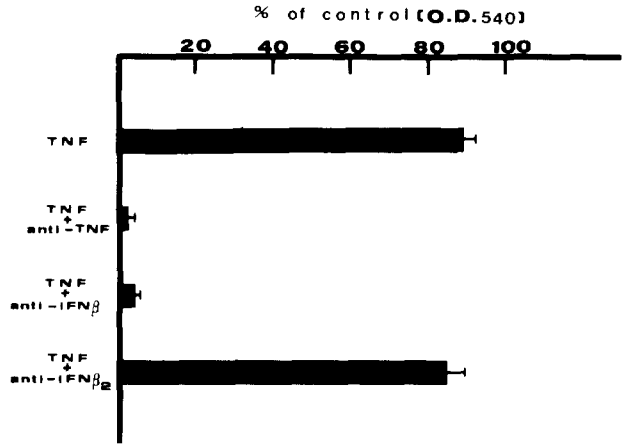

Fig. 4. Failure of antibodies to IFN-β_2 to neutralize the antiviral activity of TNF. Confluent cells in microtiter plates were incubated with TNF alone (10 ng/ml) or in the presence of diluted antibodies to neutralize 50 ng of TNF, 1250 pg of IFN-β, and 100 ng of IFN-β_2. After treatment for 24 h, the cells were challenged with VSV and further incubated for 24 h. Staining of cells and determination of cell protection was performed as described in Fig. 1. Data represent means ± SE of 3 individual wells.

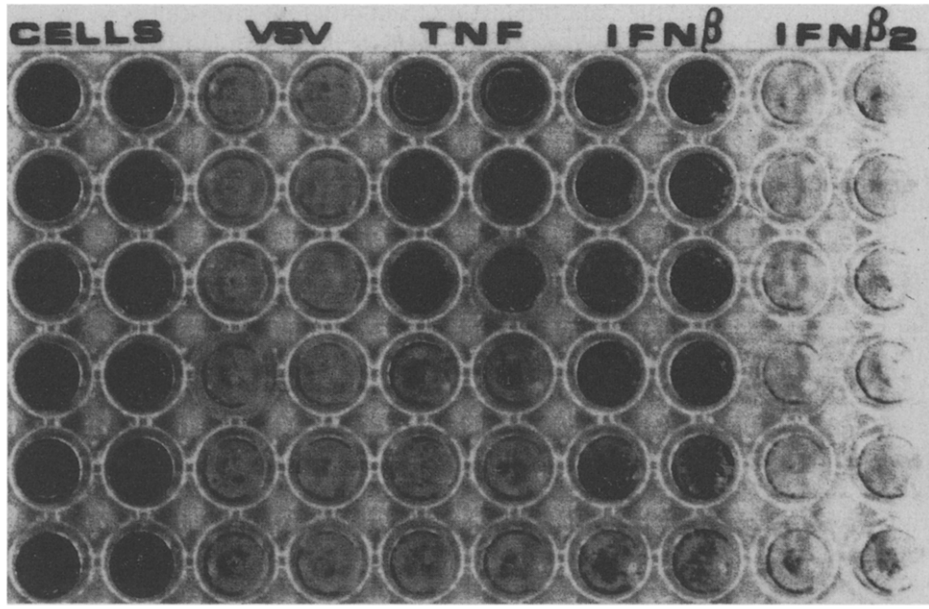

Fig. 5. Lack of antiviral protection by IFN-β_2. Cells grown in microtiter plates were incubated for 24 hr with serial 0.5 log dilutions of TNF, IFN-β, and IFN-β_2. Initial concentrations of TNF, IFN-β, and IFN-β_2 were 10 ng/ml, 250 pg/ml, and 30 ng/ml, respectively. Infection with VSV and staining of the cells was carried out as described in Fig. 1.

proliferation over 4 days of treatment with doses that conferred antiviral protection. Conversely, TNF turned out to be quite toxic to the cells in the presence of actinomycin D. For the cells to be protected from VSV, preincubation with TNF for 4 h was sufficient, while 8 h of pretreatment were necessary to achieve almost maximum protection. The failure of TNF to induce an antiviral activity in the presence of antibodies against IFN-β was suggestive of an involvement of IFN-β as a mediator of the antiviral state elicited by TNF. To clarify whether the antiviral activity of TNF could partially or completely be attributed to the induction of IFN, we assayed the activity of 2–5A synthetase, an enzyme known to be a characteristic marker of IFN activity. We observed that treatment with TNF significantly enhanced 2–5A synthetase levels, and that this enzymatic activity could be abolished not only by antibodies to TNF, but also by antibodies against IFN-β. This result demonstrated that, in our cell system, the activation of 2–5A synthetase was actually dependent upon induction of IFN-β. These experiments also revealed that, at least in our cell system, TNF is devoid of an intrinsic antiviral activity, but exerts its antiviral effect solely via induction of IFN-β. These findings, while in agreement with some previous studies (Mestan et al., 1986; Van Damme et al., 1987; Arakawa et al., 1987), differ from two others (Wong, 1986; Gessani, 1988), showing a direct and intrinsic antiviral activity of TNF. However, our results are not necessarily at variance with the latter observations, since it is not pos-

sible to strictly compare the data originating from different virus-cell systems.

The mechanism underlying the antiviral effects of TNF may not be the same in different cell lines, and different cells may also differ in sensitivity to TNF (Ruggiero et al., 1987). We also addressed the question whether the antiviral effect of TNF was due to induction of either IFN-β_1 or IFN-β_2. The findings that neutralization of TNF-induced antiviral activity occurred in the presence of anti-IFN-β antibodies but did not take place in the presence of specific antibodies against IFN-β_2 support the role of IFN-β_1, but not of IFN-β_2, as mediator of the antiviral activity of TNF.

The possibility that the synthesis of IFN-β_2 may still take place following treatment with TNF cannot be completely ruled out by our results. Even so, however, it would appear that WISH cells are simply not sensitive to the antiviral effects of IFN-β_2. This conclusion is supported by the failure of a homogeneously purified preparation of IFN-β_2 to exert any antiviral effects in our cell system. Therefore, although the data presented here do not directly demonstrate IFN-β_1 in supernatants from TNF-treated WISH cells, and cannot be considered an absolute proof for the sole induction of IFN-β_1, they do favor a major role of this cytokine in the antiviral protection elicited by TNF in our cell system. There has been considerable controversy about the antiviral activity of IFN-β_2 (Kohase et al., 1988). It is even questionable that it is an IFN at all (Billiau, 1987). In fact, a few researchers found IFN-β_2 to be antivirally active (Zilberstein et al., 1986; Kohase et al., 1986), while others did not (Van Damme, 1987; Poupart et al., 1987; Reis et al., 1988).

When expressed in CHO cells (Zilberstein et al., 1986), IFN-β_2 seems to be antivirally active. However, when it is expressed in *Xenopus* oocytes, the resulting product shows no antiviral activity at all (Poupart et al., 1987). Although the primary sequence of the molecule is the same regardless of the expression system, it has recently been postulated that the variable antiviral activity of IFN-β_2 may be attributed to differently phosphorylated forms of IFN-β_2, some of which might show antiviral activity (May et al., 1988).

Finally, a few studies have suggested a clear separation between the antiviral and cytotoxic activities of TNF (Ito and O'Malley, 1987; Mestan et al., 1986). However, Gessani et al. (1988) have recently found that HeLa cell variants selected for resistance to the cytotoxic activity of TNF are insensitive to its antiviral activity, thus suggesting that the antiviral effect of TNF is related to its cytotoxic activity. Our present findings show antiviral activity of TNF in a cell system that is also sensitive to the cytotoxic effects of this cytokine. This provides a convenient system to directly investigate whether the antiviral and the cytotoxic effects of TNF are separate activities or share some common metabolic steps.

Acknowledgements

This work was supported by grants from CNR (Progetto Finalizzato Biotecnologie e Biostrumentazione) and Associazione Italiana per la Ricerca sul Cancro awarded to F.D. The authors wish to thank Dr. Adolf from the Ernst Boehringer

Institute, Vienna, Austria, and Dr. Leproux from Boehringer Ingelheim, Florence, Italy for the very generous supply of TNF and its monoclonal antibodies. The skilful secretarial assistance of E. Di Francesco is also gratefully acknowledged.

References

Arakawa, T., Hsu, Y.R., Toth, E. and Stebbing, N. (1987) The antiviral activity of recombinant human tumor necrosis factor-alpha. J. Interferon Res. 7, 103–105.

Beresini, M.H., Lempert, M.J. and Epstein, L.B. (1988) Overlapping polypeptide induction in human fibroblasts in response to treatment with interferon-α, interferon-γ, interleukin 1-α, interleukin 1-β and tumor necrosis factor. J. Immun. 2, 485–493.

Billiau, A. (1987) Redefining interferon: the interferon-like antiviral effects of certain cytokines (interleukin-1, interferon-β_2, interferon-γ) may be indirect or side effects. Antiviral Res. 8, 55–70.

Carswell, E.A., Old, L.J., Kassel, R.L., Green, S., Fiore, N. and Williamson, B. (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl. Acad. Sci. USA 72, 3666–3670.

Fransen, L., Muller, R., Marmenout, A., Tavenier, J., Van der Heyden, J., Kawashima, E., Chollet, A., Tizard, R., Van Heuverswyn, H., Van Vliet, A., Ruysschaert, M.R. and Fiers, W. (1985) Molecular cloning of mouse tumor necrosis factor and its eukaryotic expression. Nucleic Acids Res. 13, 4417–4429.

Gessani, S., Johnson, S.E., McCandless, S. and Baglioni, C. (1988) The antiviral activity of tumor necrosis factor in HeLa cells is not mediated by interferons. Studies with TNF-resistant variants. J. Biol. Regul. Homeost. Agents 3, 139–144.

Ito, M. and O'Malley, J.A. (1987) Antiviral effects of recombinant tumor necrosis factor. Lymphok. Res. 4, 309–318.

Kohase, M., Henriksen-De Stefano, D., May, L.T., Vilček, J. and Sehgal, P.B. (1986) Induction of β 2-interferon by TNF: a homeostatic mechanism in the control of cell proliferation. Cell 45, 659–666.

Kull, F.C. and Cuatrecasas, P. (1981) Possible requirement of internalization in the mechanism of in vitro cytotoxicity in tumor necrosis serum. Cancer Res. 41, 4885–4890.

May, L.T., Santhanam, V., Tatter, S.B., Bhardwaj, N., Ghrayeb, J. and Sehgal, P.B. (1988) Phosphorylation of secreted forms of human β_2-interferon/hepatocyte stimulating factor/interleukin-6. Biochem. Biophys. Res. Commun. 3, 1144–1150.

Mestan, J., Digel, W., Mittnacht, S., Hillen, H., Blohm, D., Möller, A., Jacobsen, H. and Kirchner, H. (1986) Antiviral effects of recombinant tumour necrosis factor in vitro. Nature (London) 323, 816–819.

Minks, M.A., Benvin, S., Maroney, P.A. and Baglioni, C. (1979) Synthesis of 2′5′-oligo(A) in extracts of interferon treated HeLa cells. J. Biol. Chem. 254, 5058–5064.

Nathan, C.F. (1987) Secretory products of macrophages. J. Clin. Invest. 79, 319–326.

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

Pennica, D., Nedwin, G.E., Hayflick, J.S., Seeburg, P.H., Derynck, R., Palladino, M.A., Kohr, W.J., Aggarwal, B.B. and Goeddel, D.V. (1984) Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. Nature (London) 312, 724–729.

Poupart, P., Vandenabeele, P., Cayphas, S., Van Snick, J., Haegeman, G., Kruys, V., Fiers, W. and Content, J. (1987) B cell growth modulating and differentiating activity of recombinant human 26-kD protein (BSF-2, HuIFN-β 2, HPGF). Eur. Mol. Biol. Organ. J. 6, 1219–1224.

Reis, L.F.L., Le, J., Hirano, T., Kishimoto, T. and Vilcek, J. (1988) Antiviral action of tumor necrosis factor in human fibroblasts is not mediated by B cell stimulatory factor 2/IFN-β 2, and is inhibited by specific antibodies to IFN-β. J. Immun. 5, 1566–1570.

Ruff, M.R. and Gifford, G.E. (1979) Tumor necrosis factor. Lymphokines, vol. 2, 235–272.

Ruggiero, V., Tavernier, J., Fiers, W. and Baglioni, C. (1986) Induction of the synthesis of tumor necrosis factor receptors by interferon-γ. J. Immun. 136, 2445–2450.

Ruggiero, V., Latham, K. and Baglioni, C. (1987) Cytostatic and cytotoxic activity of tumor necrosis factor on human cancer cells. J. Immunol. 138, 2711–2717.

Van Damme, J., De Ley, M., Van Snick, J., Dinarello, C.A. and Billiau, A. (1987) The role of interferon-β_1 and the 26-kDa protein (interferon-β_2) as mediators of the antiviral effect of interleukin 1 and tumor necrosis factor. J. Immunol. 139, 1867–1872.

Wong, G.H.W. and Goeddel, D.V. (1986) Tumour necrosis factors α and β inhibit virus replication and synergize with interferons. Nature (London) 323, 81–822.

Zilberstein, A., Ruggieri, R., Korn, J.H. and Revel, M. (1986) Structure and expression of cDNA and genes for human interferon-β_2, a distinct species inducible by growth stimulatory cytokines. Eur. Mol. Biol. Organ. J. 5, 2529–2537.