Tumour necrosis factor enhances induction by β-interferon of a ubiquitin cross-reactive protein

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Tumour necrosis factor α (TNF-α) elicited an antiviral response in some cell lines (MG-63 and HEP-2) but not in others (MDBK). Cell lines that generated an antiviral response to TNF-α also showed induction of a 15K protein which shared sequence homology with ubiquitin and reacted with an antibody to ubiquitin. This ubiquitin cross-reactive protein (UCRP) had been demonstrated previously to be induced by interferon. The TNF-α induction of UCRP occurred at the level of transcription. TNF-α induction of both the antiviral state and the 15K protein was blocked by either monoclonal or polyclonal anti-β-interferon (IFN-β) antibody. However no measurable increase in the mRNA specific for IFN-β was detected after TNF-α treatment. Nonetheless, in supernatants from cell cultures, the presence of an antiviral activity inhibitable by anti-IFN-β antibody indicates that these cells are making IFN-β already. We conclude that the TNF-α induction of antiviral activity and UCRP in cells is dependent upon the presence of constitutive low levels of IFN-β in the responding cells. Furthermore TNF functions to enhance the existing IFN-β activity.

Introduction

Tumour necrosis factor alpha (TNF-α) is a protein produced by monocytes/macrophages that causes the necrosis of some tumours (Carswell et al., 1975). This 17K protein has been shown to be identical to cachectin, the protein secreted by macrophages, that induces the wasting associated with many disease states (Beutler & Cerami, 1986). TNF-α has been purified to homogeneity (Aggarwal et al., 1985) and recombinant TNF-α can be produced in Escherichia coli (Pennica et al., 1985). Experiments using purified TNF-α have revealed that this lymphokine has a wide variety of effects on responding cells. Among its diverse biological activities are the induction of differentiation of myeloid cells along the monocye/macrophage pathway (Takeda et al., 1986), the stimulation of proliferation of normal and transformed cells (Sugarman et al., 1985), the induction of phospholipase A2 activity in endothelial cells (Clark et al., 1988), and the inhibition of MYC expression in HL-60 cells (Kronke et al., 1987).

In addition to these varied biological functions, TNF-α has been shown to have a potent antiviral activity in several different cell lines and against a variety of viruses (Mestan et al., 1986; Wong & Goeddel, 1986; Arakawa et al., 1987; Ruggiero et al., 1989). Recent evidence indicates that TNF-α acts synergistically with β-interferon (IFN-β) to establish an antiviral state (Mestan et al., 1988; Reis et al., 1988). We demonstrate here that TNF-α induces a 15K protein that previously has been shown to be inducible by IFN-β and homologous to ubiquitin (Korant et al., 1984; Haas et al., 1987; Knight et al., 1988) since antibody directed against ubiquitin reacts with this 15K protein, we have named it 'ubiquitin cross-reactive protein' (UCRP). The evidence presented here indicates that the presence of IFN-β is required for the induction of UCRP by TNF-α. Furthermore, the endogenous levels of IFN-β that are detectable in cells responding to TNF-α are sufficient for TNF-α to enhance the IFN-β induction of an antiviral state and the synthesis of UCRP mRNA and protein.

Methods

Cell culture. The cell lines used in this study were A-549, a human lung carcinoma cell line; MG-63, a human osteogenic sarcoma cell line; HEP-2, a human laryngeal carcinoma line, L29, a mouse fibroblast cell line and MDBK, a bovine kidney cell line. All cells were obtained from the American Type Culture Collection. Cell lines were maintained in the appropriate culture medium as follows: Dulbecco's modified MEM, supplemented with 10% foetal bovine serum (FBS) for A-549 cells; MEM with Earle's salts supplemented with non-essential amino acids and 10% FBS for MG-63 cells; MEM with Earle's salts supplemented with 10% FBS for HEP-2 cells; F-12 supplemented with 10% FBS for MDBK cells; and, MEM with Hanks' salts supplemented with 10% FBS for L929 cells. Cells were maintained at 37 °C. For
measuring TNF-α activity, confluent monolayers were used 24 h after seeding for all cell types except HEp-2 cells. HEp-2 cells were seeded at a low density 4 or 5 days prior to testing and had become confluent monolayers by the time they were used experimentally.

**Lymphokines and antibodies.** Human recombinant TNF-α was purchased from Amgen Biologicals. When measured in the standard TNF-α cytolytic assay on mouse L929 cells (Aggarwal et al., 1985), a concentration of 1 ng/ml TNF-α routinely yielded 50% lysis in the absence of added actinomycin D or mitomycin C. Human recombinant IFN-β with a specific activity of 1 x 10⁹ units/mg protein was purchased from Triton Biosciences. Polyvalent anti-TNF-α antibody at a concentration of 10000 neutralizing units (NU)/ml was from Endogen. Polyvalent anti-IFN-β antibody from Lee Biomolecular had an initial concentration of 20000 NU/ml. Monoclonal anti-IFN-β antibody at a concentration of 10000 NU/ml was from the Green Cross Corporation.

**Antiviral assays.** Antiviral activity was measured by virus yield reduction. After treatment with or without lymphokine, confluent monolayers of cells were challenged with virus at an m.o.i. > 10. Virus yield was determined 7 to 18 h after infection. Vesicular stomatitis virus (VSV) was determined by microplate assay on MDBK cell monolayers (Langford et al., 1981). Encephalomyocarditis (EMC) virus was measured by haemagglutination (Jameson et al., 1977).

Detection of UCRP. Confluent cultures treated as described were rinsed twice with phosphate-buffered saline (PBS) then harvested into SDS sample buffer and boiled. Aliquots of samples were subjected to SDS-PAGE on 11 to 15% (w/v) polyacrylamide gels and electrophoretically transferred on Immobilon (Millipore). The Immobilon blots were immunostained with affinity-purified polyclonal antibody directed against ubiquitin. The ubiquitin-specific antibody was generously donated by A. Haas who has previously described its specificity (Haas & Bright, 1985). Following incubation with ¹²⁵I-labelled Protein A, blots were visualized by autoradiography using Kodak XAR-5 film.

Detection of IFN-β- and UCRP-specific mRNAs. After the treatment indicated in the figure legends, cells were washed twice with cold PBS and homogenized in cold lysis buffer (3 M-LiCl, 6 M-urea). The cell lysates were kept overnight at 4 °C before centrifugation at 10000 r.p.m. Total RNA was extracted twice with phenol-chloroform, once with chloroform and then precipitated with ethanol.

For Northern blot analysis, 25 μg of total RNA was denatured, mixed with ethidium bromide (60 ng/ml) and fractionated in formaldehyde gels according to Maniatis et al. (1982). At the end of the electrophoresis, the gel was illuminated under u.v. light (312 nm) to ensure that all lanes contained equal amounts of RNA. RNA was then transferred onto a Hybond-N nylon membrane (Amersham). After u.v. fixation, the RNAs were hybridized with ³²P-labelled probes. The human IFN-β gene was a generous gift from W. Fiers (Tavernier et al., 1982) to a specific activity > 10⁹ c.p.m./μg. The oligonucleotide probe for 15K protein-specific mRNA was synthesized on a DNA synthesizer and ³²P-labelled at its 5' end by kination (Maniatis et al., 1982) to a specific activity of approximately 2 x 10⁸ c.p.m./μg. The sequence of this oligonucleotide was 5' GTCCACCACGACGACGACGTGC 3'. Hybridization using 5 x 10⁶ c.p.m./ml of ³²P-labelled probe was performed at 42 °C in 50% (v/v) formamide, 5 x SSPE (900 mM-NaCl, 5 mM-EDTA, 50 mM-sodium phosphate, pH 7.4, 5 x Denhardt's solution) for 2 h or 18 h for oligonucleotide or cDNA probes respectively. The blots were then washed stringently for 30 min in 0.1 x SSC, 0.1% SDS at 65°C. Kodak XAR-5 films were used for autoradiography.

**Results**

**Antiviral activity of TNF-α.**

The ability of TNF-α to elicit an antiviral response was tested using several cell lines. Fig. 1 illustrates that TNF-α induced antiviral activity in a dose-dependent manner in three of the cell lines tested (MG-63, HEp-2 and A-549). The ability of TNF-α to produce an antiviral state in responding cells was compared to that of IFN-β. If one
comparably enhances the concentration of lymphokine required to bring about a 50% reduction in viral yield, the potency of TNF-α is similar to that of IFN-β (1.5 × 10^{-12} M compared to 3 × 10^{-13} M) on MG-63 cells. Both HEp-2 and A-549 cells require much more TNF-α (approximately 10^{-11} M) than IFN-β (approximately 10^{-12} M) to produce the equivalent antiviral response. In contrast, MDBK cells showed no antiviral activity in response to the highest concentration of TNF-α used although these cells do give an antiviral response to human α-interferon. None of the cell lines showed a significant reduction in cell viability during TNF-α treatment, as determined in the cytolytic assay. Therefore the cytocidal activity of TNF-α did not appear to be influential in the production of the antiviral state in these cells.

**Induction of UCRP by TNF-α**

Cells were treated for 24 h with concentrations of either IFN-β or TNF-α sufficient to give a 90% reduction in viral yield. Cellular proteins were separated by SDS-PAGE. Blots were immunostained using affinity-purified antibodies against ubiquitin. In blots from untreated control cells, this polyclonal antibody recognizes both the free ubiquitin (M, 8500) as well as ubiquitin that is covalently conjugated to proteins of higher M, (Fig. 2). After treatment with either IFN-β or TNF-α, an additional 15K band that is present in the control was clearly enhanced in both treated samples (Fig. 2). The increase in this UCRP was observed in cells that developed an antiviral state in response to TNF-α treatment (MG-63, HEp-2 and A-549) but was not seen after TNF-α treatment of cells that failed to show an antiviral response to the lymphokine (MDBK) or cells that responded to the cytotoxic activity of TNF-α (L929).

**TNF-α induction of mRNA for UCRP**

To determine whether the increase in UCRP was initiated at the transcriptional or translational level of control, RNA samples from control and TNF-α-treated cells were subjected to electrophoretic separation, blotted onto a nylon membrane and probed with an oligonucleotide that recognizes the 15K mRNA. Although there was some 15K mRNA present in control cells, there was a pronounced increase in this mRNA after treatment with as little as 5 ng/ml TNF-α (Fig. 3). Cells treated with 50 ng/ml TNF-α for various periods of time exhibited an increase in UCRP mRNA with time of treatment. A strong signal was observed 3 to 6 h after initiating TNF-α treatment (Fig. 3). Clearly there was an increase in both UCRP mRNA and protein following TNF-α treatment.

**Cooperation between TNF-α and IFN-β in induction of both antiviral activity and UCRP**

During the course of this work several publications indicated that TNF-α and IFN-β could act synergisti-
Fig. 4. Antiviral activity of TNF-α blocked by anti-IFN-β antibody. (a) MG-63 cells were exposed to control medium or medium supplemented with either 0.1 ng/ml TNF-α (△) or 2 units/ml IFN-β (○) in the presence or absence of various concentrations of polyclonal anti-IFN-β antibody. After 24 h, cells were challenged with VSV. The VSV yield in untreated control medium was 1.2 × 10⁷ p.f.u./ml. (b) HeP-2 cells were exposed to control medium or medium supplemented with either 10 ng/ml TNF-α (△) or 10 units/ml IFN-β (○) in the presence or absence of various concentrations of polyclonal anti-IFN-β antibody. After 24 h, cells were challenged with EMC virus. The EMC virus yield in untreated control medium was 64 HAU.

Fig. 5. Induction of UCRP by TNF-α blocked by anti-IFN-β antibody. (a) MG-63 cells were exposed to control medium (lanes 1 and 2) or medium supplemented with either 1000 units/ml IFN-β (lanes 3 and 4) or 1 ng/ml TNF-α (lanes 5 and 6) in the absence (lanes 1, 3 and 5) or presence (lanes 2, 4 and 6) of 500 NU/ml polyclonal anti-IFN-β antibody. Western blots of cell lysates separated by SDS-PAGE were immunostained with anti-ubiquitin antibody. (b) HeP-2 cells were exposed to control medium (lanes 1 and 2) or medium supplemented with either 100 ng/ml TNF-α (lanes 3 and 4) or 500 units/ml IFN-β (lanes 5 and 6) in the absence (lanes 1, 3 and 5) or presence (lanes 2, 4 and 6) of 500 NU/ml polyclonal anti-IFN-β antibody. Western blots of both MG-63 samples (a) and HeP-2 samples (b) were immunostained with anti-ubiquitin antibody. The arrows indicate UCRP.

cally in evoking an antiviral response in cells (Wong & Goeddel, 1986; Mestan et al., 1988; Reis et al., 1988). Since MG-63 cells are known to be able to synthesize interferon and in fact have been used for interferon production, it seemed possible that interferon might be involved in the TNF-α action in our system. Either MG-63 or HeP-2 cells were treated with 1 to 10 antiviral units/ml IFN-β or an amount of TNF-α that gave comparable reduction in virus yield. Increasing amounts of polyclonal antisera specific for IFN-β were added to cultures during the overnight lymphokine treatment. Cells were then challenged with either VSV (MG-63) or EMC virus (HeP-2). The anti-IFN-β antibody completely blocked the antiviral action of either IFN-β or TNF-α in both cell types (Fig. 4). Surprisingly, a relatively high concentration of antibody (500 to 1000 NU/ml) was required in order completely to block the action of this relatively low concentration of TNF-α (approximately 1 ng/ml). Similar results were obtained when the experiment was repeated using a monoclonal antibody specific for IFN-β.

In addition, MG-63 or HeP-2 cells were treated for 24 h with fresh medium or medium supplemented with...
concentrations of TNF-α or IFN-β sufficient to reduce viral yields to less than 10% of the virus control. To parallel cultures of both control and lymphokine-treated cells, 500 NU/ml anti-IFN-β polyclonal antibody was added. Cellular proteins were separated by SDS-PAGE and visualized on immunoblots using the anti-ubiquitin antibody described above. UCRP was increased markedly over the control level after treatment with either TNF-α or IFN-β. This increase was blocked by anti-IFN-β in both IFN-β- and TNF-α-treated cells (Fig. 5). The possibility that the anti-IFN-β antibody was cross-reacting with TNF-α appears unlikely since the inclusion of anti-IFN-β during the standard assay of TNF-α cytolytic activity on L929 cells did not block TNF-α activity.

Lack of detectable IFN-β induction by TNF-α

The relatively large amount of anti-IFN-β needed to block the TNF-α activity suggested that TNF-α was inducing IFN-β to a significant extent in these cells. RNA from control and TNF-α-treated MG-63 cells were probed to detect IFN-β mRNA. To determine whether some other TNF-α-induced protein intermediate might be responsible for induction of IFN-β during TNF-α treatment, cycloheximide was included in a parallel set of control and TNF-α-treated cells. The Northern blot revealed no measurable increase in IFN-β mRNA after TNF-α treatment (Fig. 6). Although the cycloheximide had no effect on the level of IFN-β mRNA in TNF-α-treated cells, there was a marked increase in cells treated with cycloheximide alone (Fig. 6). The accumulation of IFN-β mRNA during the 6 h cycloheximide treatment was also observed in cultures to which IFN-β had been added. This increase in IFN-β mRNA appears to be due solely to the cycloheximide treatment since treatment with IFN-β alone caused no change over that of the control value. The failure to detect increased IFN-β mRNA after combined treatment with both cycloheximide and TNF-α may indicate that the combination of these compounds is toxic to these cells. Indeed cycloheximide alone causes a reduction in both cell survival and [3H]uridine incorporation in MG-63 cells (data not shown). The addition of TNF-α plus cycloheximide decreases both these parameters further.

The accumulation of IFN-β mRNA during cycloheximide treatment suggests that the gene encoding IFN-β mRNA is available for reading in these cells. Perhaps the IFN-β gene is being transcribed at low levels normally in these cells and the degradation of IFN-β mRNA is blocked in the presence of cycloheximide thereby permitting the accumulation of a sufficient amount of mRNA to be detected during analysis of Northern blots. If this were the case, these low levels of IFN-β might be detectable in supernatants of these cells. Medium was conditioned by exposure to MG-63 monolayers in the presence or absence of TNF-α. Fresh medium and conditioned medium were compared for their ability to support virus production on new MG-63 monolayers (Fig. 7). The conditioned medium containing residual TNF-α reduced virus yield in comparison to either the fresh medium or control conditioned medium. However treatment with anti-TNF-α blocked this antiviral activity. Addition of anti-IFN-β to any of the media consistently increased the virus yield by approximately 10-fold over that of the fresh medium control. This suggests that some factor recognized by the monoclonal antibody directed against IFN-β is responsible for reducing the virus yield even in cells tested in fresh medium. The antiviral activity of conditioned medium from MG-63 cells was titrated on new MG-63 monolayers using the limiting dilution method and an internal control value. VSV yield was determined by plaque assay and expressed as the percentage of virus yield in fresh medium without additions. The graph presents the mean ± S.E.M. from three separate experiments.

Fig. 6. Absence of detectable IFN-β-specific mRNA after TNF-α treatment. MG-63 cells were treated with control medium (lanes 1 and 2) or medium supplemented with either 100 ng/ml TNF-α (lanes 3 and 4) or 1000 units/ml IFN-β (lanes 5 and 6) in the absence (lanes 1, 3 and 5) or presence (lanes 2, 4 and 6) of 50 μg/ml cycloheximide for 6 h. A Northern blot of cellular RNA was probed for the presence of IFN-β-specific mRNA.

Fig. 7. IFN-β detectable in cell supernatants. MG-63 cells were exposed to either fresh medium or medium that had been conditioned in the presence or absence of 1 ng/ml TNF-α for 24 h over MG-63 monolayers. Treatment of new MG-63 monolayers with these media was performed as a control (open bars) or after supplementing with 50 NU/ml anti-TNF-α polyclonal antibody (diagonal-filled bars), 50 to 250 NU/ml anti-IFN-β monoclonal antibody (cross-hatched bars). VSV yield was determined by plaque assay and expressed as the percentage of virus yield in fresh medium without additions. The graph presents the mean ± S.E.M. from three separate experiments.
Table 1. Titration of MG-63 conditioned medium

<table>
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<th>Sample dilution (log2)</th>
<th>VSV Yield (p.f.u./ml)</th>
<th>VSV Yield (p.f.u./ml) with anti-IFN-β (250 NU/ml)</th>
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<tr>
<td>1</td>
<td>$6 \times 10^4$</td>
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<tr>
<td>2</td>
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<td>4</td>
<td>$1.4 \times 10^6$</td>
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Laboratory standard for IFN-β. Antiviral activity was measured in comparison to samples to which 250 NU/ml anti-IFN-β had been added (Table 1). The conditioned medium from MG-63 cells contained no more than 2 units/ml antiviral activity.

**TNF-α induction of mRNA for UCRP blocked by cycloheximide**

To determine whether protein synthesis was required for the induction of the mRNA for UCRP by TNF-α, cells were treated with TNF-α in the presence or absence of cycloheximide and their cellular RNA was analysed in Northern blots by probing with an oligonucleotide that recognizes the 15K mRNA. TNF-α-treated cells exhibited an increase in 15K mRNA over the control. This increase was similar to that seen in cells treated with IFN-β (Fig. 8). The combination of TNF-α and cycloheximide reduced the 15K mRNA to control levels (Fig. 8). The blot revealed that cells treated with cycloheximide alone or in combination with IFN-β exhibited a large accumulation of UCRP mRNA. The absence of UCRP mRNA in cells treated with both TNF-α and cycloheximide may reflect some general cellular deficiency brought about by the combination of drugs.

**TNF-α induction of mRNA for UCRP blocked by anti-IFN-β**

Although the data from experiments using cycloheximide as an inhibitor of translation indicated that protein synthesis was necessary for the TNF-α-stimulated increase in 15K protein mRNA, the potential cytotoxicity of the experimental regime left this inference unresolved. To determine whether the specific presence of IFN-β was required for the TNF-α induction of UCRP-specific mRNA, cells were treated with TNF-α in the presence of anti-IFN-β antibody. The period of TNF-α treatment was extended from the 6 h used previously to 15 h in order to ensure a strong signal on the Northern blots. Clearly there was UCRP mRNA present in the control and this mRNA increased after TNF-α treatment. In both control and TNF-α-treated cells, the accumulation of UCRP mRNA over the 15 h period was blocked if anti-IFN-β were included (Fig. 8). This suggests that IFN-β is present in both control and TNF-α-treated cells and that this IFN-β is responsible for stimulation of UCRP mRNA production in either case.

**Discussion**

Interferon induces a number of proteins including a 15K protein for which the amino acid sequence and gene nucleotide sequence have been determined (Korant et al., 1984; Blomstrom et al., 1986; Reich et al., 1987; Knight et al., 1988). This 15K protein cross-reacts with an antibody raised against ubiquitin (Haas et al., 1987). The explanation for this cross-reactivity is a remarkable sequence homology in a highly conserved region at the carboxyl termini of the 15K protein and ubiquitin (Haas et al., 1987).

In this paper, we report that the IFN-inducible 15K protein with homology to ubiquitin (UCRP) was also induced in cells treated with TNF-α. The ability of TNF-α to stimulate UCRP production was limited to cells that also showed an antiviral response to this lymphokine. Cells responsive to the anti-cellular activity of TNF-α (i.e. L929) did not show increased levels of UCRP
although these same cells increased UCRP in response to interferon treatment (Haas et al., 1987). Whether UCRP is responsible for the antiviral action of TNF-α remains undetermined.

Control of TNF-α induction of UCRP occurred at the level of mRNA accumulation. There was a marked increase in mRNA coding for the 15K protein after treatment with as little as 5 ng/ml TNF-α and this increase was dependent upon the duration of the TNF-α treatment (Fig. 3).

To determine whether the synthesis of an intermediary protein was required for the induction of UCRP by TNF-α, cycloheximide was included during TNF-α treatment to inhibit protein synthesis. Indeed the induction of the 15K protein mRNA was prevented by the inclusion of cycloheximide during TNF-α treatment. However treatment with cycloheximide alone caused a pronounced accumulation of mRNAs for both the 15K protein and IFN-β (Fig. 6 and 8) but this augmentation was obliterated by TNF-α treatment. The most plausible explanation for these results is that the combined treatment with both TNF-α and cycloheximide is toxic for these cells. Toxicity of combined TNF-α and cycloheximide treatment has been described by Kirstein & Baglioni (1986) and Reid et al. (1989). Although the results from combined treatment with cycloheximide and TNF-α are difficult to interpret, the ability of anti-IFN-β to block the action of TNF-α was unequivocal. Anti-IFN-β inhibited the antiviral activity of TNF-α (Fig. 5) and blocked the accumulation of both the 15K protein and its mRNA normally seen after TNF-α treatment (Fig. 4 and 8). These data reveal an absolute requirement for IFN-β in the events leading to TNF-α induction of both the antiviral state and the elevation of 15K protein and its mRNA. This conclusion agrees with results demonstrating a synergism between TNF-α and IFN-β in producing an antiviral state (Mestan et al., 1988; Reis et al., 1988). Furthermore there was no detectable increase in synthesis of mRNA specific for IFN-β after TNF-α treatment (Fig. 6) suggesting that induction of IFN-β is not the mechanism responsible for the TNF-α activities measured. Other laboratories have also failed to demonstrate TNF-α induction of IFN-β mRNA by analysis of Northern blots from TNF-α-treated cells. However, using a highly sensitive assay for the presence of mRNA (i.e. polymerase chain reaction after reverse transcription of existing messages) both these groups have demonstrated the existence of a small but measurable increase in IFN-β mRNA in response to TNF-α treatment (Jacobsen et al., 1989; Reis et al., 1989). In both cases the amount of measurable IFN-β produced in response to TNF-α treatment (<1 unit/ml) does not appear to be sufficient to account for the magnitude of the antiviral activity measured in TNF-α-treated cultures when expressed in standard IFN-β activity units (Jacobsen et al., 1989; Reis et al., 1989).

Although TNF-α induction of IFN-β-specific mRNA was not demonstrated in the present study, the accumulation of this mRNA during cycloheximide treatment suggested that the IFN-β gene was accessible for transcription in these cells (Fig. 6). Experiments designed to scrutinize cell supernatants for the presence of IFN-β revealed its existence (Fig. 7). The results indicate that IFN-β is present in these cell cultures even in the absence of TNF-α treatment. Presumably the sensitivity of the VSV plaque assay exceeds the sensitivity of the Northern blot analysis of IFN-β mRNA.

Furthermore there was no evidence for additional IFN-β in conditioned medium from TNF-α-treated cultures. The presence of active TNF-α remaining in this conditioned medium was demonstrated by the increased virus yield after addition of anti-TNF-α (Fig. 7). However this virus yield was comparable to that seen in control conditioned medium. Furthermore both control and TNF-α-conditioned media exhibit the same virus yield after addition of anti-IFN-β. We conclude that these cells normally make a small but detectable amount of IFN-β and that this endogenous IFN-β is sufficient to meet the requirement for IFN-β during TNF-α action. Endogenous IFN-β is also responsible for the 15K protein mRNA observed in untreated MG-63 monolayers since the amount of the product on Northern blots was reduced when anti-IFN-β was added (Fig. 8). Apparently these cells are continually responding to low level IFN-β stimulation.

The mechanism for TNF-α enhancement of IFN-β action is not yet understood. TNF-α does not induce IFN-β in quantities sufficiently large to account for the observed antiviral activity. Yet since all the antiviral activity can be blocked by addition of anti-IFN-β, there is no reason to assume that TNF-α itself or some unidentified TNF-α-induced protein is primarily responsible for the antiviral state. Perhaps TNF-α augments the intracellular signal which is normally initiated when IFN-β interacts with its cell surface receptor. The nature of this second signal is unknown at this time. Indeed, interferon-induced nuclear factors that bind to promoter elements of interferon-stimulated genes have been described (Kessler et al., 1988; Raj et al., 1989). It is possible that some nuclear factor induced by TNF-α can interact with the promoter region of IFN-β-stimulated genes. As an alternative, it is possible that some portion of the interferon produced constitutively by these cells remains in a cryptic form. TNF-α treatment might make this cryptic IFN-β accessible for interaction with IFN-β receptors. In any case, TNF-α enhances the activity of IFN-β even when the IFN-β is present in barely detectable amounts. The induction of UCRP and an
antiviral state by TNF-α is dependent upon the presence of IFN-β.

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