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Mini-Review

The role of tumor necrosis factor in viral disease

Christine W. Czarniecki*

ICOS Corporation, 22021 20th Avenue SE, Seattle, Bothell, Washington 98021, USA

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Summary

Tumor Necrosis Factor (TNF) is one of the many cytokines that comprise a complex intertwined network of biological response modifiers that takes on extreme significance as the host response to infectious diseases. Soluble factors such as Interleukin-2 and Interferon-γ released by T cells and Interleukin-1, Interleukin-6 and TNF released by monocytes have been shown to play key roles in proliferation, activation and differentiation of immune cells. It has also become evident that development of treatment modalities for infectious diseases is complicated by the complexity of this cytokine network. In the last decade numerous reports have presented data, often conflicting, which clearly demonstrate a role for TNF in the response to infections caused by viruses. This review summarizes this rapidly growing volume of data, discussing consistencies and discrepancies as appropriate. By better understanding the role of TNF in the host immune response, it may be possible to modulate this complex network for the benefit of the host in its battle against viral infection.

Tumor necrosis factor; Cytokines; Biological response modifier; Host response

Introduction

The host immune response against infection involves the initial recognition of foreign antigens, processing by antigen-presenting cells such as macrophages and dendritic cells and presentation of antigen to lymphocytes. This immune

*Corresponding author.

recognition is followed by the elaboration of a wide array of nonspecific soluble factors termed "cytokines" which exert biological activities on a wide variety of cell types and interact with each other in a complex manner to regulate the host immune system.

In the last decade, recombinant DNA technology has had an enormous impact on our understanding of this complex cascade system by allowing the identification and characterization of cytokines such as tumor necrosis factor (TNF-α), lymphotoxin (TNF-β), interferons (IFNs), various interleukins and others. Assigning identities to factors within biologically active cell supernatants has led to the discovery that, at times, diverse biological activities can be attributed to a single factor. Thus, "cachectin", a molecule initially implicated in the pathogenesis of wasting in chronic disease (Beutler et al., 1985b), was found to be identical to TNF-α, the factor induced in the serum of endotoxin-treated mice and rabbits that had been sensitized with bacillus Calmette-Guerin (BCG) and injected with endotoxin which, when injected into mice, induced hemorrhagic necrosis of transplanted sarcomas (Carswell et al., 1975; Haranaka et al., 1984). The cloning and sequencing of the genes encoding these two factors confirmed that cachectin and TNF-α, molecules which were being studied independently in terms of their different biological activities, were indeed identical (Beutler et al., 1985a; Caput et al., 1986; Fransen et al., 1985; Pennica et al., 1984; 1985). With the widespread availability of purified recombinant TNF-α came the realization that many other biological activities, in addition to the well-described cytotoxic activity, could be attributed to this molecule, and that TNF-α might be a mediator of host pathology including septic shock, cachexia and inflammation. The identification of different forms of TNF-α ("secretory TNF" and "transmembrane TNF") has led to the hypothesis that different physiological responses are mediated by each of these forms (Kriegler et al., 1988; Aversa et al., 1993). Thus, TNF-induced cytotoxicity requiring cell-to-cell contact is mediated by transmembrane TNF and is a localized phenomena. In contrast, septic shock and cachexia, more systemic phenomena, may be mediated by the secretory TNF component.

The studies summarized in this review demonstrate that in addition to the aforementioned biological activities, TNFs (α and β) also play a significant role in viral disease. Increasing our understanding of how to modulate the biological activities of this key cytokine may lead to development of novel approaches to the treatment of viral disease.

Induction of TNF-α and -β by viruses in vitro

TNF-α and TNF-β (lymphotoxin) are two related cytotoxic polypeptides which are encoded by closely linked genes located within the major histocompatibility complex (Spies et al., 1986). These two proteins which exhibit 30% homology in amino acid sequence (Pennica et al., 1984) share many biological activities and compete for a common receptor, at least on some

cell targets (Aggarwal et al., 1985).

The induction of TNF-α mRNA, protein and cytotoxic activity has been studied using various cell types responding to different inducers. While the predominant cellular sources of TNF-α and TNF-β have been identified as the macrophage/monocyte and T lymphocyte, respectively, other cell types have been shown to induce these cytokines in response to various stimuli. Thus, TNF-α gene expression is induced in macrophage/monocytes in response to pathogens (whole or lysates) such as *Legionella pneumophila* (Blanchard et al., 1988; 1989), *Plasmodium yoelii* or *Plasmodium berghei* (Bate et al., 1988), Trypanosomes (Beutler and Cerami, 1988) and viruses (Table 1); human peripheral blood lymphocytes, depleted of contaminating monocytes, produce TNF-α and TNF-β mRNA and protein in response to phorbol diester, calcium ionophore or PHA (Cuturi et al., 1987); B cell lines representing different stages of differentiation induce TNF-α mRNA and protein constitutively or in response to PMA (Sung et al., 1988); tonsillar B cells produce TNF-β after induction by Staphylococcus aureus Cowan I strain (SAC) (Bersani et al., 1987) or TNF-α in response to B cell mitogens (Sung et al., 1988); natural killer cells (Beutler and Cerami, 1988; Peters et al., 1986), mast cells and smooth muscle cells have been shown to produce TNF-α mRNA and/or protein (Beutler and Cerami, 1986). In general, however, cells of the monocyte/macrophage lineage (of pulmonary, hepatic, peritoneal or bone marrow origin) remain quantitatively the most significant source of these cytokines. Additionally, it is important to note that while many cell types may induce mRNA for these factors in response to stimuli, secretion of protein does not always follow mRNA induction (Beutler and Cerami, 1986).

Several groups have described the ability of different classes of viruses to induce TNF-α and TNF-β. A summary of these is presented in Table 1. In general, peripheral blood leukocytes, monocytes and B cells (normal and virus transformed) have been shown to induce TNF-α and TNF-β in response to RNA or DNA viruses. The major cytotoxic protein induced by Sendai virus in human peripheral blood mononuclear cells and the human monocytic cell line U937 was characterized as TNF-α by neutralization of protein activity with anti-TNF-α antibody (Aderka et al., 1986; Berent et al., 1986) and nucleic acid hybridization studies of the induced mRNAs (Berent et al., 1986). Similarly, Wong and Goeddel (1986) showed that TNF-α and TNF-β mRNA were induced in human peripheral blood leukocytes induced by poly(I)·poly(C), VSV or HSV-2. This latter report (Wong and Goeddel, 1986) also demonstrated the production of cytotoxic activity from HL-60 (human promyelomonocytic) cells and RPMI-1788 (human B lymphoblastoid) cells in response to EMCV, VSV, HSV-2, Adenovirus-2 or poly(I)·poly(C) and inferred that the activity was due to TNF-α (from HL-60 cells) and TNF-β (from RPMI-1788 cells). In a later report, Fukuda et al. (1988) showed that both TNF-α and TNF-β (identified by amino acid sequence analysis and the nucleotide sequence of the cDNA) could be simultaneously induced by human B-cell lymphoblastoid (BALL-1) cells in response to infection with Hemagglu-

tinating virus of Japan (HVJ, also referred to as Sendai virus), emphasizing a need for caution in identifying a cytotoxic activity as TNF-α or β by its cell source alone.

Goldfeld and Maniatis (1989) reported studies of Sendai virus-induced gene expression of human TNF-α and IFN-β in different cell types. They found that the inhibition of protein synthesis with cycloheximide had no effect on mRNA expression of either gene in human monocyte (U937) cells but inhibited the viral induction of both genes in Namalwa cells (a Burkitt's lymphoma B-cell line). In JY cells (an Epstein Barr virus-transformed B-cell line) cycloheximide treatment blocked virus-induced expression of IFN-β but not TNF-α mRNA. These results demonstrated that these two human genes, IFN-β and TNF-α can be co-induced in the same cell by Sendai virus infection and that the mechanisms by which these genes are induced can be distinct.

Macrophages are also susceptible to Influenza A virus infection and as shown by Nain et al. (1990), in human monocytes, rat alveolar macrophages and a murine macrophage cell line infected with Influenza A virus, TNF-α mRNA levels increased but only low levels of TNF-α protein were produced. These protein levels were greatly increased in the presence of low levels of LPS.

It is interesting to note that induction of TNA-α by viruses is not a universal phenomenon. As observed by Gosselin et al. (1992), HSV-1 infection of human PBMC induced significant levels of TNF-α mRNA and protein; however, EBV infection of these cells actually reduced TNF-α transcription and protein synthesis.

Induction of TNF in vivo: role of TNF in AIDS

The detection of elevated serum levels of TNF have been associated with disease states such as parasitic infections (Scuderi et al., 1986), cancer (Balkwill et al., 1987), meningococcal disease (Waage et al., 1987) and virus infections such as cytomegalovirus infection in liver transplant recipients (Tilg et al., 1991) and acquired immunodeficiency syndrome (AIDS) as described below.

The etiological agent of AIDS has been identified as the human immunodeficiency virus (HIV) and the pathogenesis of HIV infection involves infection of CD4+ lymphocytes as well as cells of the monocyte/macrophage lineage. While infection of CD4+ lymphocytes by HIV results in cytopathic effect, monocytes/macrophages, including bone marrow precursor cells, can be infected by HIV in vitro and are relatively resistant to the cytopathic effects. Human monocytes infected in vitro with HIV produce low levels of infectious virus budding from the cell surface. These cells are thought to play a major role in the pathogenesis of AIDS, serving as a reservoir of virus and allowing for persistent infection. Additionally, the depletion of the CD4+ helper/inducer subset of T lymphocytes occurring in AIDS patients leads to a major defect in the host immune response leaving these patients susceptible to neoplasms and a wide variety of opportunistic infections. AIDS patients are thus exposed to

many stimuli which can act as potential TNF inducers.

The results of studies of the role of TNF in disease states associated with AIDS have been conflicting. The ability of peripheral blood mononuclear cells of AIDS patients to secrete TNF-α or TNF-β has been shown to be decreased (Ammann et al., 1987), increased (Wright et al., 1988, Roux-Lombard et al., 1989) or equal to control cells from healthy individuals (Haas et al., 1987). Amman et al. (1987) isolated peripheral blood mononuclear cells from patients with AIDS or AIDS-related complex (ARC) and measured TNF-α and TNF-β in cell supernatants after stimulation with PHA/PMA. These investigators found that patients with AIDS or ARC produced significantly less TNF-α and TNF-β than control subjects and the response of cells from AIDS patients was not significantly different from that of ARC patients. In contrast, Haas et al. (1987) reported that the levels of TNF detected in LPS-induced cells from a similar patient population were similar to those of similarly induced cells from normal individuals. The cells used by Wright et al. (1988) were obtained from AIDS patients with Kaposi sarcoma. These investigators utilized highly enriched adherent monocytes stimulated with IFN-γ and found significantly higher levels of TNF-α secreted by cells from AIDS patients compared to control cells. Similarly, Roux-Lombard et al. (1989) showed that purified monocytes from HIV-1 seropositive patients, in the absence of in vitro stimulation or in response to IFN-γ plus LPS, produced higher levels of TNF-α and IL-1β as compared to cells from normal individuals. Subsequent studies with alveolar macrophages reported increased TNF production from alveolar macrophages isolated from HIV-seropositive patients with no lung infections as compared to cells from control individuals (Israel-Bief et al., 1991).

Patient sera has also been examined for the presence of TNF as a means of studying the role of this cytokine in disease states. Lahdevirta et al. (1988) measured TNF levels by radioimmunoassay in the sera of 39 HIV-seropositive patients. The TNF levels for the asymptomatic HIV-infected patients and for the patients with lymphadenopathy were within the reference range of the control values (<30 ng/ml), while all of the patients with AIDS and 5 of 9 patients with ARC had elevated levels of TNF. The highest levels were observed in patients who had both secondary infections and weight loss (cachexia). However, the levels did not appear to correlate with the degree of weight loss. Reddy et al. (1988) reported elevated TNF levels in the serum of HIV-seropositive intravenous drug abusers, homosexuals and patients with lymphadenopathy and AIDS. Similarly, Mintz et al. (1989) found elevated serum levels of TNF-α in children with AIDS which correlated with progressive encephalopathy, but similar to the findings of Lahdevirta et al. (1988), levels did not correlate with the degree of cachexia observed.

Patients with AIDS may have multiple infections caused by several different classes of microorganisms and it is possible that the increased levels of TNF found in these patients is a response to HIV and/or these microbes. To address this issue, a recent report (Jones et al., 1992) described the results of a study in which AIDS patients with concurrent secondary infections and intravenous

drug users were excluded. These data indicated that TNF concentrations were not elevated in the patients included in the study.

Results from in vitro studies are equally complex. To examine the effects of HIV infection on TNF-α and IL-1β production, Molina et al. (1989) chose the human monocytic cell line THP-1 as the target cell since the monocyte/macrophage acts as a major target for HIV infection and may serve as a reservoir for latent virus infection. These studies distinguished between "acutely-infected" THP-1 cells and "chronically-infected" cells. Cells harvested on days 8–10 after infection, prior to differentiation of the cells, were termed "acutely-infected". These cells were $>90\%$ CD4+ and exhibited peak reverse transcriptase (RT) activity and viral antigen expression (measured by indirect immunofluorescence). The "chronically-infected" cells were those harvested after 10–13 days of culture and these were characterized by being CD4-, and having decreased RT activity and viral antigen expression. These studies showed that HIV infection alone was not sufficient for induction of TNF-α or IL-1β; no detectable protein or mRNA was observed. Stimulation with LPS did, however, result in induction of both cytokines in both infected and uninfected THP-1 cells and this induction was enhanced by IFN-γ. While the levels of TNF-α and IL-1β produced by chronically infected and uninfected cells were similar, the levels of these two induced cytokines (protein and mRNA) were higher in acutely-infected cells. These authors speculated that the acutely-infected THP-1 cells which exhibited phenotypic changes suggestive of differentiation acted as monocytes which have been shown to release increased levels of TNF-α and IL-1β in response to LPS after they are primed by maturation and/or activation. Similar results were obtained with cultured human peripheral blood macrophages (Molina et al., 1989) and fresh PBMC (Molina et al., 1990) infected with HIV-1.

Munis et al. (1990) examined the effect of HIV infection on production of TNF-α in macrophages using highly purified populations of human macrophages isolated from blood monocytes. The virus used in these studies was the macrophage-tropic HIV-1 strain HTLV-III$_{\text{Ba-L/85}}$ Similar to the results of Molina et al. (1989), they found that the virus HTLV-III$_{\text{Ba-L/85}}$ established a productive and cytopathic infection in macrophages without the release of TNF protein or induction of TNF-α mRNA (as measured utilizing the sensitive polymerase chain reaction (PCR) amplification technique). However, in contrast to the results of Molina and co-workers described above, Munis and co-workers found that the production of TNF-α in response to LPS was similar in infected and uninfected purified human macrophages in terms of kinetics and dose-response curves of LPS stimulation. These data suggest that the circulating levels of TNF detected in AIDS patients are produced by macrophages in response to signals other than HIV or alternatively, that the TNF is produced by cells other than macrophages. Consistent with the hypotheses of alternate signals for TNF production, Smith et al. (1992) reported that an isolate of human cytomegalovirus (HCMV) obtained from a patient with AIDS induced human monocytes to express TNF-α mRNA and

TABLE 1

Induction of TNF in human cells by viruses[d]

Cell Line	Virus	TNF detected TNF-α	TNF-β	Detection method	References
PBL (peripheral blood leukocytes)	Sendai	+	ND[a]	Bio[b] Bio/Ab[c] mRNA	Berent et al. (1986)
PBL	Sendai	+	–	Bio/Ab	Aderka et al. (1986)
PBL	EMCV	+	+	Bio	Wong & Goeddel (1986)
	VSV	+	+	Bio	Wong & Goeddel (1986)
	HSV-2	+	+	Bio	Wong & Goeddel (1986)
	Adenovirus-2	+	+	Bio	Wong & Goeddel (1986)
PBL	VSV	+	+	mRNA	Wong & Goeddel (1986)
	HSV-2	+	+	mRNA	Wong & Goeddel (1986)
PBMC	Coxsackie	+	ND	Bio mRNA	Henke et al. (1992)
PBMC	HHV-6	+	ND	mRNA, ELISA	Flamand et al. (1991)
Alveolar macrophage	RSV	+	ND	mRNA Bio ELISA/RIA	Becker et al. (1991)
Monocytes	Influenza A	+	ND	mRNA ELISA	Nain et al. (1990)
HL-60 (promyelomonocyte)	EMCV	+	ND	Bio	Wong & Goeddel (1986)
	HSV-2	+	ND	Bio	Wong & Goeddel (1986)
	Adenovirus-2	+	ND	Bio	Wong & Goeddel (1986)
U937 (monocyte)	Sendai	+	–	Bio/Ab	Aderka et al. (1986)
U937	Sendai	+	ND	mRNA	Goldfeld Maniatis (1989)
BALL-1 (lymphoblastoid B cell)	HVJ (Sendai)	+	+	AA sequence	Fukuda et al. (1988)
RPMI-1788 (lympho-blastoid B cell)	EMCV		+	Bio	Wong & Goeddel (1986)
	VSV		+	Bio	Wong & Goeddel (1986)
	HSV-2		+	Bio	Wong & Goeddel (1986)
	Adenovirus-2		+	Bio	Wong & Goeddel (1986)
Namalwa (Burkitt lymphoma B cell)	Sendai	+	ND	mRNA	Goldfeld & Maniatis (1989)
JY (Epstein-Barr virus transformed cell)	Sendai	+	ND	mRNA	Goldfeld & Maniatis (1989)

[a]ND: Not done. [b]Bio: Bioassay measuring cytotoxic activity. [c]Bio/Ab: Ab neutralization of bioactivity. [d]Excluding HIV.

primed these cells to release increased levels of TNF-α. Similarly, Peterson et al. (1992) showed that HCMV and also varicella zoster virus (VZV) induced TNF release from PBMC and stimulated HIV replication.

What is the role of TNF in AIDS or in any of the disease states in which its induction is observed? The above studies have demonstrated the presence of this cytokine; however, further studies are necessary to determine if TNF: (i) plays a role in the pathogenesis of disease; (ii) is a component of the host's protective response to disease; or (iii) acts as an innocent bystander, a marker indicative of the disease state.

The biological activities of TNF are mediated through cell surface receptors and circulating soluble receptors which bind TNF-α and -β have been observed in response to infections and cancer (Aderka et al., 1991; Godfried et al., 1993). In addition, the cloning of the gene encoding a receptor for TNF has allowed for the establishment of identity of this protein with one of the urinary TNF-binding proteins (Heller et al., 1990). Further studies with specific disease states are necessary to determine if the presence of these circulating receptors serves to limit infection by blocking TNF activity (Howard et al., 1993) or to promote infection by binding to TNF and prolonging its biological activity by stabilizing its structure (Aderka et al., 1992).

Effect of TNF on virus-infected cells

Enhanced cytotoxicity

Macrophages are found widely distributed within host organs. Their ability to phagocytize and destroy pathogens, including virus particles, allows them to act as an important line of defense against initiation and dissemination of viral infections.

Prior to the identification of soluble factors produced by activated macrophages which exert cytotoxic activity, several reports, examining the role of macrophages in in vivo models of infection demonstrated that infection with virus resulted in the in vivo production of activated macrophages which exhibited cytotoxicity for infected, but not uninfected target cells. Chapes and Tompkins (1979) showed that after immunization of hamsters with infectious vaccinia virus, macrophages harvested from the peritoneal cavity were cytotoxic for vaccinia or HSV-infected target cells, but not for uninfected cells. Similarly, Mak et al. (1982) showed that macrophages from mice infected with infectious, but not non-infectious influenza or Sendai virus could kill homologous or heterologous virus-infected but not uninfected target cells. Stanwick et al. (1982) showed that human monocyte/macrophages mediated both spontaneous cytotoxicity and antibody-dependent cell-mediated cytotoxicity against human fibroblasts infected with type 1 herpes simplex virus (HSV-1). While IFN-α was induced during the reaction, this IFN was not involved in the spontaneous cytoxicity, however, exogenously added IFN-α or poly (I) poly (C) (an inducer of IFN-β) effectively augmented the spontaneous cytotoxicity.

This preferential ability of "cytotoxic factors" to kill virus-infected cells over normal, uninfected cells was studied by several groups. Aderka et al. (1985) examined the cytotoxic activity of semi-purified "lymphotoxin" (from human peripheral blood lymphocytes induced with phytohemagglutinin-P (PHA)) on cells apparently insensitive to cell killing by this factor (which they termed LT). in the absence of virus infection, viability of HeLa, WISH, Vero and SV-80 cells was uneffected by LT treatment. However, addition of LT 2 h after infection of these cell lines with VSV dramatically enhanced their vulnerability to cell destruction. Since it had been shown previously (Wallach, 1984) that treatment of cells with inhibitors of RNA or protein synthesis enhanced cell sensitivity to LT cytotoxicity, it was hypothesized that the shut-off of cellular RNA and protein synthesis accompanying VSV infection might play a role in the virus-induced sensitization of cells to LT cytotoxicity.

In similar studies, incubation of mouse embryo 10E2 cells infected with HSV-1 or HSV-2 with recombinant human TNF-α (rHuTNF-α) (added 2 h after virus infection) resulted in cytolysis, as measured in ^{51}Cr-release cytotoxicity assays (Koff and Fann, 1986). In contrast, uninfected 10E2 cells were not effected. The other cytokines evaluated in that study, recombinant human interleukin-1 (rHuIL-1), interleukin-2 (IL-2), IFN-αA/D, IFN-γ and recombinant murine IFN-γ (rMuIFN-γ exhibited no cytotoxic activities. In the same report, these investigators showed that pre-incubation of the supernatants from LPS-activated human monocytes with monoclonal TNF-α antibodies significantly reduced the cytotoxic activity against HSV-2-infected cells. Similar results were observed with A549 cells, which are resistant to the cytotoxic effects of TNF-α and TNF-β and were rendered susceptible to TNF-mediated lysis after infection with VSV or Adenovirus-2 (Wong and Goeddel, 1986). Taken together, the studies from these independent laboratories strongly supported the hypothesis that TNF-α could be one of the soluble mediators of cytotoxicity against virus-infected cells induced by macrophages activated by in vivo virus replication.

Since TNF enhances the cytotoxicity of viruses, one might speculate that TNF might function in the host response during persistent virus infections, in which the virus causes little cytotoxicity to the host cell. Enhancing cytotoxicity of virus-infected cells would thus eliminate the reservoir for the virus and limit the spread of the virus infection. Bielefeldt Ohmann and Babiuk (1988) addressed this possibility with the bovine system and showed that, while recombinant bovine TNF-α (rBoTNF-α) exhibited no antiviral activity against bovine viral diarrhoea virus (BVDV) replication in MDBK cells (a cytopathic infection), rBoTNF-α treatment of cell cultures infected with a non-cytopathic strain of BVDV resulted in cytopathic effect which was morphologically similar to that induced by infection with the cytopathic strain of BVDV.

Inhibition of virus production

Both TNF-α and TNF-β have been shown to effectively establish an in vitro antiviral state in various cell lines and to protect them from infection with

TABLE 2

In vitro antiviral effects of TNF on normal and transformed human cells

Cell line	TNF-α	TNF-β	IFN-γ + TNF-α potentiation	References
Normal human cell				
HEL (embryonic lung)	+ VSV			Mestan et al. (1986)
WI-38 (embryonic lung)	+ VSV			Mestan et al. (1986)
WISH (amnion)	− VSV			Mestan et al. (1986)
	+ VSV			Ruggiero et al. (1989a); (1989b)
	+ VSV			van Damme et al. (1987)
	+ Sindbis			Ruggerio et al. (1989a)
FS-4 (foreskin fibroblast)	+ EMCV			Reis et al. (1988); Kohase et al. (1986)
	+ HSV-1			Reis et al. (1988)
MLD (diploid foreskin fibroblast)	+ VSV		+ VSV	Ito & O'Malley (1987)
	+ HSV-1;			Ito & O'Malley (1987)
	+ HSV-2			Ito & O'Malley (1987)
	+ CMV		+ CMV	Ito & O'Malley (1987)
BG-9 (diploid foreskin fibroblast)	+ VZ; + VSV			Ito & O'Malley (1987)
	+ ECMV			Ito & O'Malley (1987)
E_6SM (diploid fibroblast)	+ VSV			Van Damme et al. (1987)
BUD-8 (skin fibroblast)	− VSV			Mestan et al. (1986)
Human fibroblast	+ VSV			Leeuwenberg et al. (1987)
HUV (umbilical vein endothelium)	+ VSV			Leeuwenberg et al. (1987)
Transformed human cells				
HEp-2 (laryngeal carcinoma)	+ EMCV; + VSV			Mestan et al. (1986)
	+ HSV			Mestan et al. (1986)
	+ VSV		+ VSV	Mestan et al. (1988)
	− HSV-1		+ HSV-1	Feduchi et al. (1989)
MG-63 (osteosarcoma)	+ VSV			van Damme et al. (1987)
U87MG (glioblastoma)	+ VSV	+ VSV	+ VSV	Wong & Goeddel (1986)
RPMI 8226 (myeloma)	+ VSV	+ VSV	+ VSV	Wong & Goeddel (1986)
7860 (renal carcinoma)	+ VSV	+ VSV	+ VSV	Wong & Goeddel (1986)
	+ EMCV	+ EMCV		Wong & Goeddel (1986)
	+ Ad-2	+ Ad-2		Wong & Goeddel (1986)
	+ HSV-2	+ HSV-2		Wong & Goeddel (1986)
HT-29 (colon adenocarcinoma)	+ VSV			Ito & O'Malley (1987)
RT-4 (urinary bladder ca)	+ VSV			Ito & O'Malley (1987)
5637 (urinary bladder ca)	− VSV			Ito & O'Malley (1987)
SK-BR-3 (breast carcinoma)	− VSV			Ito & O'Malley (1987)
PC-13 (lung large cell ca)	− VSV			Ito & O'Malley (1987)
A549 (lung carcinoma)	− VSV			Ito & O'Malley (1987)
	− VSV	− VSV	+ VSV	Wong & Goeddel (1986)
	− ECMV	− ECMV	+ ECMV	Wong & Goeddel (1986)
	− Ad-2	− Ad-2	+ Ad-2	Wong & Goeddel (1986)
	− HSV-2	− HSV-2	+ HSV-2	Wong & Goeddel (1986)
T24 (bladder carcinoma)	− VSV	− VSV	+ VSV	Wong & Goeddel (1986)
HT-1080 (lung fibrosarcoma)			+ VSV	Wong & Goeddel (1986)
ST-486 (Burkitt's lymphoma)			+ VSV	Wong & Goeddel (1986)
HT-29 (colon carcinoma)			+ VSV	Wong & Goeddel (1986)
HeLa (cervix carcinoma)	− VSV			Mestan et al. (1986)
	+ ECMV			Arakawa et al. (1987)
	± EMCV			Gessani et al. (1988)
			+ VSV	Wong & Goeddel (1986)

RNA- or DNA-containing viruses. A summary of reports from the scientific literature is presented in Table 2. From the list it is evident that human cell lines of normal (HEL, WI-38, FS-4, MLD, BG-9, E_6SM, HUVE) as well as transformed (HEp-2, MG-63, U87MG, RPMI 8226, 7860, RT-4, HT-29) phenotype responded to the antiviral effects of TNF. However, these reports also demonstrate that several normal (BUD-8, WISH) and transformed (5637, PC-13, A549, SK-BR-3, T24) cells were not protected from virus infection by TNF treatment. Additionally, two urinary bladder carcinoma cell lines exhibited differential sensitivities to the anti-VSV effect of TNF-α (Ito and O'Malley, 1987).

In very few instances have the same cell line and virus been utilized in studies by different groups and in those few instances, both discrepancies and consistencies were noted: Reiss et al. (1988) observed TNF-α induced inhibition of EMCV replication in FS-4 cells similar to that which was reported by Kohase et al. (1986); two groups reported the inhibition of VSV replication in HEp-2 cells (Mestan et al., 1986; Feduchi et al., 1989); the inability of TNF-α to protect A549 cells from VSV replication was reported by Wong and Goeddel (1986) and Ito and O'Malley (1987). However, differing results were observed for VSV replication in TNF-treated WISH cells (Mestan et al., 1986; Ruggiero et al., 1989a; 1989b; van Damme et al., 1987) and HSV infection of TNF treated HEp-2 cells (Mestan et al., 1986; Feduchi et al., 1989). In the latter set of studies using HSV infection, Feduchi and co-workers utilized HSV-1 whereas Mestan and co-workers did not specify the strain of HSV used.

The many years of study of the antiviral state induced by IFNs has led to the understanding that the establishment of the antiviral state may be dependent upon many factors including the antiviral agent, the target cell, the infecting virus, as well as experimental conditions such as confluency of the cells and serum concentration. Additionally, the IFN-induced antiviral state can be established through multiple mechanisms, evidenced by dissociation of IFN-induced inhibition of viruses classified within different families (Allen et al., 1976; Nilsen et al., 1980; Czarniecki et al., 1981; Samuel and Knutsen, 1981; Tomita et al., 1982), as well as within the same family (Czarniecki and Allen, 1984). The in vitro antiviral state established by TNF may be effected by the same variables that act upon the IFN-induced antiviral state and differences in responses may occur with variations in experimental conditions. It is interesting to note that in HeLa cells treated with rHuTNF-α, EMCV replication was shown to be inhibited (Arakawa et al., 1987; Gessani et al., 1988), while VSV infection was uneffected (Mestan et al., 1986). While it is tempting to speculate that this may be an example of a dissociated antiviral state, confirmation of this differential response to TNF in side-by-side comparative assays is necessary, especially in light of the known variability of cultured cells from various sources (laboratories).

Since human TNF-α has been shown to exert many of its pleiotropic effects (cytotoxicity/cytostasis for transformed cells, modulation of lipid metabolism, induction of cell surface antigens, activation of neutrophils (reviewed in Le and

TABLE 3

In vitro antiviral effects of TNF on non-human cells

Cell line	TNF-α	TNF-β	IFN-γ + TNF-α potentiation	References
C127 (mouse epithelial)	+ VSV	+ VSV	+ VSV	Wong & Goeddel (1986)
MEF (mouse embryo fibroblast)	+ VSV			Mestan et al. (1986)
C3H 10T½ (murine cell)	+ VSV			Ito & O'Malley (1987)
L929 (mouse fibroblast)	– VSV			Mestan et al. (1986)
L-M (murine cell)	– VSV			Ito & O'Malley (1987)
L929 W (murine cell)	– VSV			Ito & O'Malley (1987)
L929 WR (TNF resistant murine cell)	– VSV			Ito & O'Malley (1987)
NIH Swiss 3T3 (murine fibroblast)	– VSV			Ito & O'Malley (1987)
RAW 26 (mouse macrophage)			+ VSV	Wong & Goeddel (1986)
Rat-1 (fibroblast)	+ VSV	+ VSV	+ VSV	Wong & Goeddel (1986)
REF (rat embryo fibroblast)	– VSV			Mestan et al. (1986)
NRK (rat kidney)	– VSV			Ito & O'Malley (1987)
SIRC (rabbit cornea)	– VSV			Ito & O'Malley (1987)
ML (mink lung)	– VSV			Ito & O'Malley (1987)
CV-1 (green monkey kidney)	– VSV			Ito & O'Malley (1987)
BGM (green monkey kidney)	– VSV		– VSV	Ito & O'Malley (1987)
RITA (monkey kidney)	– VSV			Mestan et al. (1986)
MDBK (bovine kidney)			+ VSV	Wong & Goeddel (1986)
AM (bovine kidney)	– VSV		– VSV	Ito & O'Malley (1987)
MDBK (bovine kidney)	– BVDV[a]		– BVDV[b]	Bieldfeld-Ohmann & Babiuk (1988)
GBK (bovine kidney)	– BVDV[a]		– BVDV[b]	Bieldfeld-Ohmann & Babiuk (1988)

[a]BoTNF-α. [b]BoTNF-α + BoIFN-γ.

Vilcek, 1987) on cell lines of heterologous species, it has been characterized as having a broad host range. Thus, several groups have examined the ability of human TNF-α to establish an antiviral state in heterologous cell lines and these reports are summarized in Table 3. Similar to results with the human cell lines, VSV replication was inhibited in several rodent cell lines (Wong and Goeddel, 1986; Ito and O'Malley, 1987; Mestan et al., 1986). However, many cell lines were not protected from infection by TNF-treatment (Ito and O'Malley, 1987; Mestan et al., 1986). With the exception of Bielefeldt-Ohmann and Babiuk (1988) who treated bovine kidney cells with rBoTNF-α, these non-human "resistant" cell lines were treated with human TNF but not the appropriate homologous TNF. Thus, it is not possible to ascertain if these cell lines do not respond to the human cytokine or if they are not capable of establishing an antiviral state in response to any TNF.

Enhanced antiviral effects in combination with other cytokines

Previous studies of antiviral effects induced in vitro by treatment of cells with IFNs have demonstrated that enhancement of inhibition of virus replication can be obtained when cells are treated with IFN-γ in combination with either IFN-α or IFN-β (Fleishman et al., 1979; Czarniecki et al., 1984; Oleszak and Stewart, 1985). Synergistic effects observed in such combination treatments

implies that the two agents exert their activities through different mechanistic pathways.

As shown in Tables 2 and 3, potentiation of antiviral activities has been observed after treatment of cells with IFN-γ plus TNF-α. Potentiation was observed in cell lines (MLD, HEp-2, U87MG, RPMI 8226, 7860) in which VSV replication was inhibited by TNF-α alone (Wong and Goeddel, 1986; Ito and O'Malley, 1987) and also in cell lines in which VSV (in A549, or T-24 cells) or HSV (in Hep-2 cells) replication was not inhibited by TNF-α alone (Wong and Goeddel, 1986; Feduchi et al., 1989). In several cells lines (HT-1080, ST-486, HT-29), potentiation of anti-VSV activity occurred with combination IFN-γ and TNF-α treatment (Wong and Goeddel, 1986); however, in this report, the response of the cells to TNF-α alone was not described.

Mestan et al. (1988) examined the effects of sequential treatment of HEp-2 cells with TNF-α and IFN-γ or IFN-β. They reported that treatment of cells with TNF-α plus IFN-γ simultaneously led to a 10-fold reduction in VSV yield. Synergistic enhancement of antiviral effects was also observed when IFN-γ treatment preceded TNF-α; however, no synergistic effect was observed when TNF-α preceded IFN-γ. In contrast, replacing IFN-γ with IFN-β in the above studies led to a synergistic inhibition of virus replication under all conditions. These data demonstrated that IFN-γ and IFN-β synergize with TNF-α (in tends of antiviral effects) through different mechanisms. Modulation of binding of TNF-α by IFNs did not play a role in this cell system since pretreatment of these cells with either IFN-γ or IFN-β had no effect on binding of ^{125}I-labeled TNF-α.

Mechanism of action: role of IFN-β

Most reports describing antiviral activities induced by TNF have addressed the possibility that this activity of TNF is an indirect effect and inhibition of virus replication is actually caused by an autocrine IFN-β which is induced by TNF treatment. Earlier studies demonstrated spontaneous IFN production during various experimental conditions and implicated autocrine IFN-β production in the regulation of cell cycle progression and cell differentiation (Friedman-Einat et al., 1982; Yarden et al., 1984; Creasey et al., 1983). These studies led several groups to study the effects of varying cell confluency and serum concentrations on the antiviral effects of TNF. Kohase et al. (1986) found that the establishment of the antiviral state in human foreskin (FS-4 or FS-7) cells by rHuTNF-α required "aged" cultures; protection of FS-4 cells from EMCV or HSV infection occurred only when cultures were exposed to TNF-α after 6 days in culture and protection was more pronounced in cultures aged for 8 days. Mestan et al. (1986) compared HEp-2 cell cultures at different stages of confluency and found that fully confluent cell cultures were most responsive to the rHuTNF-α-induced antiviral effects. In addition, the TNF-α induced antiviral activity was greater in cultures exposed to 1% calf serum compared to those grown in 10% calf serum (Mestan et al., 1988). These results are consistent with the report that the establishment of the TNF-induced

antiviral state in FS-4 cell required "aged", nutrient-starved cultures (Kohase et al., 1986).

The hypothesis that induction of an autocrine IFN-β might play a role in the TNF induced antiviral state was complicated by the discovery that human fibroblasts which were stimulated to produce IFN-β simultaneously produced another distinct factor thought to exert antiviral activity, originally termed IFN-β_2 and now referred to as IL-6. The numerous studies of identification and characterization of IL-6 have been reviewed in Billiau (1988). For the purpose of the present discussion, it is important to note that while the gene products of the two distinct mRNA populations from stimulated fibroblasts are precipitable with polyclonal antisera raised against semi-purified IFN-β, highly specific polyclonal antibodies that distinguish between these two proteins are now available. Additionally, the amino acid sequence of IL-6 is as different from IFN-β as that of IFN-α is from IFN-γ (Billiau, 1988).

Several years of research carried out independently by groups studying apparently unrelated biological activities identified IL-6 as yet another biological response modifier with pleiotropic activities. Similar to the establishment of identity between TNF-α and cachectin, IL-6 has been shown to be indistinguishable, at least in the human system, from B-cell differentiation factor (BSF-2) and hybridoma/plasmacytoma growth factor (HGF or HPGF).

The role of IFN-β and/or IL-6 in TNF-α or -β induced activities has been studied by measuring induction of specific mRNAs for these two gene products and by antibody neutralization of these biological activities. The results of studies reported in the literature are summarized in Table 4. Using polyclonal antisera that recognized both IFN-β and IL-6 Kohase et al. (1986) observed complete neutralization of rHuTNF-α induced antiviral (EMCV) activity in FS-4 cells. This type of antisera only partially neutralized the rHuTNF-α induced antiviral effect in HEp-2 cells (Mestan et al., 1986) and had no inhibitory effect on the anti-VSV effect in 7860 cells (Wong and Goeddel, 1986) or anti-EMCV effect in HeLa cells (Gessani et al., 1988) treated with rHuTNF-α. In terms of induction of specific mRNA encoding IFN-β or IL-6, Kohase et al. (1986) detected induced IL-6 mRNA but no IFN-β mRNA in TNF-sensitive FS-4 cells, and concluded that IL-6 must play a role in the TNF-induced antiviral, state. However, other reports found no correlation between induction of IL-6 mRNA and antiviral effects. T24 cells (Wong and Goeddel, 1986) and TNF-resistant HeLa cells (Gessani et al., 1988) were not protected from virus infection by TNF treatment in spite of IL-6 mRNA induction while 7860 cells (Wong and Goeddel, 1986) were sensitive to the antiviral effects of TNF with no detectable IL-6 mRNA induction.

The availability of purified IL-6 and antisera with specificity against IFN-β or IL-6 as well as the development of improved technology to detect low levels of mRNA have dramatically affected our understanding of TNF effects on virus-infected cells. As shown in Table 4, antisera with specificity for IFN-β completely neutralized the antiviral effect of TNF-α in FS-4, E_6SM, WISH, HEp-2, and MU-63 cells, while antisera with specificity for IL-6 had no effect

TABLE 4

Role of IFN-β in the TNF-induced antiviral effect on human cells

Method of detection	Cell line	Virus	Involvement of IFN-β	IL-6	References
Antibody neutralization[a]					
	FS-4 (foreskin fibroblast)	+ EMCV	+		Kohase et al. (1986)
	HUVE (umbilical vein endothelium)	+ VSV	+		Leeuwenberg et al. (1987)
	Human fibroblast	+ VSV	+		Leeuwenberg et al. (1987)
	MLD (diploid foreskin fibroblast)	+ ECMV	+		Ito & O'Malley (1987)
		+ VSV	+		Ito & O'Malley (1987)
		+ HSV-1	+		Ito & O'Malley (1987)
		+ HSV-2	+		Ito & O'Malley (1987)
		+ CMV	+		Ito & O'Malley (1987)
		+ VZ	+		Ito & O'Malley (1987)
	7860 (renal carcinoma)	+ VSV	–		Wong & Goeddel (1986)
	HeLa (cervix carcinoma)	± EMCV	–		Gessani et al. (1988)
	HEp-2 (laryngeal carcinoma)	+ VSV	±		Mestan et al. (1986)
Antibody neutralization[b]					
	FS-4 (foreskin fibroblast)	+ EMCV	+	–	Reis et al. (1988)
	E_6SM (diploid fibroblasts)	+ VSV	+	–	van Damme et al. (1987)
	WISH (amnion)	+ VSV	+	–	Ruggiero et al. (1989a); (1989b); van Damme et al. (1987)
	HEp-2 (laryngeal carcinoma)	+ VSV	+	–	van Damme et al. (1987); Feduchi & Carrasco (1991)
	MG-63 (osteosarcoma)	+ VSV	+	–	van Damme et al. (1987)
mRNA analysis					
	FS-4 (foreskin fibroblast)	+ EMCV	–	+	Kohase et al. (1986)
	7860 (renal carcinoma)	+ VSV	ND[c]	–	Wong & Goeddel (1986)
	A549 (lung carcinoma)	– VSV	–	–	Wong & Goeddel (1986)
	T24 (bladder carcinoma)	– VSV	ND	+	Wong & Goeddel (1986)
	HeLa (cervix carcinoma)	± EMCV	–[d]	ND	Jacobsen et al. (1989)
	HeLa (TNF resistant)	– EMCV	ND	+	Gessani et al. (1988)
	HEp-2 (laryngeal carcinoma)	+ VSV	+[d]	+[d]	Jacobsen et al. (1989)

[a]Polyclonal antibody used in these studies neutralized biological activities of both IFN-β and IL-6. [b]Antibodies used in these studies distinguished between IFN-β and IL-6. [c]Not done. [d]PCR amplification.

in these cell systems. These data plus the demonstration that purified natural IL-6 had no inhibitory effect on VSV replication in WISH cells (Ruggerio et al., 1989a) and recombinant IL-6 had no effect on EMC virus in FS-4 cells (Reis et al., 1988) and no effect on VSV or HSV-1 infection of HEp-2 cells (Feduchi and Carrasco, 1991), are consistent with the hypothesis that TNF-α might exert its antiviral activity in some cell lines through induction of IFN-β but not IL-6.

Subsequently, utilizing polymerase chain reaction (PCR) IFN-β mRNA induction was detected in TNF-α treated HEp-2 cells and induction of this mRNA correlated with the TNF-α induced antiviral state (Jacobsen et al., 1989). In this report, using PCR and nuclear run-on analysis with nuclei from TNF-treated cell lines, the transcriptional activation of IFN-β and IL-6 genes

was analyzed. In HEp-2 cells which were protected by TNF from virus infection, both IFN-β and IL-6 mRNAs were induced. While transcription of the IL-6 gene increased shortly (15 min) after onset of TNF treatment and remained at an elevated level, activation of transcription of IFN-β, MHC class I and $(2'–5'A)_n$ synthetase genes was not observed until more than 1 h of TNF treatment. These data suggest that the activation of the IL-6 gene may be a primary response to TNF and IFN-β gene expression may be an indirect consequence of TNF exposure. The investigators proposed that the induction of IFN-β may be the intermediate in the induction of $(2'–5'A)_n$ synthetase and MHC class I antigen. Additionally, they found that a strain of HeLa cells which did not respond to the antiviral effects of TNF, induced pronounced levels of IL-6 mRNA but failed to produce a signal in IFN-β specific PCRs. Consistent with earlier reports (Wong and Goeddel, 1986; Gessani et al., 1988) there was no correlation between TNF-α induced antiviral state and induction of IL-6. There was, however, a close correlation between TNF-α antiviral activity and induction of IFN-β mRNA.

The above data suggest that IFN-β may play a crucial role in the antiviral activity of TNF-α in HEp-2 cells. Additionally, it is possible that low levels of IFN-β may be induced in the other cell lines in which TNFs exert antiviral activity and the techniques used in the studies reported in Table 4 were not sensitive enough to detect this mRNA. However, the TNF-induced IFN-β is probably not the sole mediator of TNF's antiviral activity in all cell systems. While IFN-β is induced by TNF-α in HEp-2 cells, it is evident that the levels of this protein are extremely low, since the mRNA was not detected by conventional Northern blot analysis. It is unlikely that these low levels of IFN-β alone could be responsible for the dramatic reductions in virus yield that have been reported in the studies in Table 2. Additionally, ascribing TNF's antiviral activity to autocrine IFN-β makes it difficult to explain the lack of effect of anti-IFN-β reported by Wong and Goeddel (1986) and Gessani et al. (1988); the partial effect reported by Mestan et al., 1986; and the synergistic antiviral activity observed on HEp-2 cells after treatment with TNF-α and IFN-β (Mestan et al., 1988). If the antiviral effect of TNF-α in HEp-2 cells were mediated solely through induction of IFN-β then treating cells with a combination of TNFα and IFN-β should lead to additive effects, at best, as in FS-4 cells (Kohase et al., 1988). It is likely that, similar to the IFN-induced antiviral state, the induction of an antiviral state by TNF-α may occur through multiple mechanisms, and the triggering of one or more of these mechanisms may be dependent upon the target cell, the infecting virus, and the experimental conditions in a particular study. Thus, antisera which neutralizes IFN-β, might completely abrogate the TNF-induced inhibition of VSV replication in human fibroblasts, FS-4, HUVE, and MLD cells (Leeuwenberg et al., 1987; Kohase et al., 1986; Ito and O'Malley, 1987); only partially abrogate the TNF-induced inhibition of VSV replication in HEp-2 cells (Mestan et al., 1986) and have no effect on the anti-VSV effect in 7860 cells (Wong and Goeddel, 1986) and anti-EMCV effect in HeLa cells (Gessani et al., 1988).

Mechanism of action: inhibition of protein synthesis

In considering the mechanisms by which TNF induces an in vitro antiviral state, once again, comparisons can be drawn with the IFN-induced antiviral state. It is clear that dependent upon the particular virus and cell line examined, restriction of virus growth can occur at many stages of the virus replicative cycle including: penetration, uncoating, mRNA synthesis and methylation, viral translation and assembly (reviewed in Friedman, 1977; Stewart, 1979; Whitaker-Dowling and Youngner, 1987).

While TNF-α has been shown to inhibit replication of many RNA and DNA-containing viruses (Table 1), few studies have been done to determine if the mechanism by which virus yield is inhibited is similar to the mechanisms by which IFNs inhibit a particular virus in a particular cell system. Several reports (Mestan et al., 1986; Ruggiero et al., 1989a; 1989b) have examined VSV protein synthesis in TNF-α treated cells and showed inhibition of VSV protein synthesis similar to that observed in IFN-β-treated cells.

Feduchi and co-workers (Feduchi et al., 1989) examined the effects on HSV-1 translation and DNA synthesis in HEp-2 cells after treatment with TNF-α plus IFN-γ. In their cell system, treatment of cells with TNF-α alone had no inhibitory effects on HSV-1 replication, while treatment with IFN-γ alone led to inhibition of viral translation and transcription, which was overcome by increasing the multiplicity of infection. The treatment of cells with combinations of TNF-α plus IFN-γ resulted in decreased expression of immediate early genes (α22 and TK) similar to the block of immediate early HSV-1 genes in murine macrophages treated with IFN-α/β (Domke et al., 1985; Domke-Opitz et al., 1986).

Mechanism of action: induction of $(2'5'A)_n$ synthetase

For those viruses that appear to be blocked primarily at the stages of viral transcription and translation, two IFN-induced enzymes, the protein P_1/eIF-2α protein kinase and the 2'-5'-oligoadenylate synthetase [$(2'–5'A)_n$ synthetase] are thought to play major modulatory roles in the IFN-induced antiviral effect. Identification and characterization of these two enzyme systems, as well as correlations of their induction and establishment of the IFN-induced antiviral state have been reviewed in detail (Samuel, 1987). The P_1/eIF-2α protein kinase induced by IFN catalyzes the phosphorylation of the α subunit of eIF-2, decreasing its ability to act in the initiation of translation of viral mRNAs. The mechanism by which discrimination between cellular and viral mRNA occurs in these IFN-treated cells is not clear. Induction of $(2'–5'A)_n$ synthetase also leads to inhibition of viral translation. The $(2'–5'A)_n$ synthetase catalyzes the polymerization of ATP into a series of (2'–5') linked oligoadenylate molecules with a 5'-terminal triphosphate [$(2'–5'A)_n$] which in turn activates an endoribunuclease (referred to as RNase L or F) leading to degradation of viral mRNA.

Similar to the IFN-induced antiviral state, the induction of an antiviral state by TNF is also associated with the induction of $(2'–5'A)_n$ synthetase. Treatment

of WISH (Ruggiero et al., 1989a; 1989b), 7860 (Wong and Goeddel, 1896) and HEp-2 (Mestan et al., 1988) cells with TNF-α led to induction of $(2'–5'A)_n$ synthetase which correlated with inhibition of VSV replication and the addition of anti-IFN-β greatly reduced the induction of $(2'–5'A)_n$ synthetase in WISH (Ruggiero et al., 1989a) and HEp-2 cells (Mestan et al., 1988). While the induction of $(2'–5'A)_n$ synthetase by TNF-α in A549 cells was not effected by anti-IFN-β antiserum, it should be noted that in these cells, TNF-α exerted no antiviral activity when used as a single agent (Wong and Goeddel, 1896).

The induction of $(2'–5'A)_n$ synthetase in HEp-2 cells appears to occur through an indirect mechanism since the levels of synthetase specific mRNA is reduced when TNF treatment occurs in the presence of cycloheximide (Mestan et al., 1988) similar to the observed synthetase induction by IFN-γ. In contrast, synthetase induction by IFN-β appears to be a primary response, not inhibited in the presence of protein synthesis inhibitors (Faltyneck et al., 1985).

In terms of combination treatments, treatment of A549 cells with TNF-α plus IFN-γ or treatment of A549, T24 or 7860 cells with TNF-β plus IFN-γ led to enhanced levels of $(2'–5'A)_n$ synthetase along with enhanced inhibition of virus replication (Wong and Goeddel, 1986). The situation is different in HEp-2 cells, where enhanced antiviral effects were observed with combinations of TNF-α plus IFN-γ or IFN-β while enhanced $(2'–5'A)_n$ synthetase levels were observed only with the combination of TNF-α plus IFN-β (Mestan et al., 1988).

Effect of TNF on retrovirus replication in vitro

Human immunodeficiency virus (HIV), the virus identified as the etiological agent of AIDS, is a member of the lentivirus family of retroviruses. HIV binds to specific surface receptors (CD4) on receptive cells and after internalization and uncoating, the viral RNA is transcribed into DNA by a viral reverse transcriptase. Viral DNA accumulates in the cell cytoplasm and/or is integrated into the genomic DNA of the host cell. The virus can enter into a latent state which may persist for long periods of time. Upon activation, transcription and translation of the viral information leads to production of virus particles which then bud from the cell surface (reviewed in Rosenberg and Fauci, 1989).

In studying the effects of TNF-α on HIV infection in vitro, investigators have examined: (i) infection of various cell types including T cells and monocyte/macrophages; (ii) acutely and chronically-infected cells; and (iii) treatment of cells with cytokine prior to and post-virus infection. Inhibition of HIV replication has been observed in cells treated with TNF-α (Wong et al., 1988; Banerjee et al., 1992) and additionally, Kornbluth et al. (1989) demonstrated the ability of LPS to protect cultured macrophages from infection with a macrophage-tropic strain of HIV-1. Wong et al. (1988) examined acute HIV infection of HuT78 (CD+human T leukemic) cells, RPMI 1788 (B lymphoblastoid) cells or normal CD4+ T cells (from HIV-seronegative

human peripheral blood leukocytes). They treated cells with TNF-α or IFN-γ 24 h prior to infection with HIV and for 7–8 days after infection. After 7 days in the presence of cytokines, decreased production of infectious HIV and HIV mRNA, was observed. IFN-γ alone was effective, TNF-α alone had minimal effect and the combination of the two showed enhanced inhibition. They also found that enhanced cytotoxicity of infected cells was observed in response to treatment with the two cytokines in combination.

Two other groups (Ito et al., 1989; Matsuyama et al., 1989b) examined the effect of pretreating T cells with TNF-α prior to acute infection with HIV-1. In these reports, treatment of human T cell lines (Molt-4, Jurkat, TLOm-1, H9) with TNF-α enhanced HIV-1 replication as measured by reverse transcriptase (RT) activity and viral antigen production and enhanced cytotoxicity for infected cells. Ito et al. (1989) also showed that not all CD4+ cells are sensitive to the stimulatory effects since HIV-1 replication was not enhanced in H9 or HuT78 cells treated with TNF-α. These latter results are consistent with those of Wong et al. (1988) who found minimal change in HIV p24 antigen expressions in HuT78 cells treated with TNF-α.

In the earliest report of TNF-α effects on retrovirus replication in chronically-infected cells, Yagi et al. (1987) utilized a cell line which was established by transfection of mouse NIH 3T3 cells with cloned proviral DNA from the leukemogenic SL-3 virus. After 24 h of treatment of these cells with rHuTNF-α alone (1–40 ng) the levels of extracellular virus were increased by 7.7- to 10.3-fold (measured by purification of radiolabeled virus). Similar enhancement was observed after an additional 24 h incubation with this cytokine. In contrast, treatment of cells with natural MuIFN-α/β, or rMuIFN-γ or rHuIL-2 reduced virus production by 30–85%. These studies also showed that incubation of cells with rHuTNF-α for as short an interval as 30 min was sufficient to lead to a 2-fold enhancement of virus production. When cells were treated with rHuTNF-α in combination with MuIFN-α/β or rMuIFN-γ, the enhancing effect of the rHuTNF-α was reduced; however, the antiviral effect of the MuIFN-α/β or MuIFN-γ was abrogated and the resultant effect was still 2- to 6-fold enhancement compared to control, untreated cultures. Similar enhancement of HIV-1 replication by TNF (rHuTNF-α or natural TNF-β) treatment was also observed in chronically-infected T cells [Molt-4/$HIV_{HTLV-IIIB}$ (Ito et al., 1989; Matsuyama et al., 1988a; 1988b; 1989a; 1989b); CCRF-CEM, Jurkat, H9 (Matsuyama et al., 1989b); ACH-2 (Rosenberg and Fauci, 1989; Clouse et al., 1989; Folks et al., 1987; 1989)]; monocyte/macrophage cultures U937 (Matsuyama et al., 1989a) and U1 (Rosenberg and Fauci, 1989); and human peripheral blood mononuclear cells from HIV-infected individuals (Michihiko et al., 1989). Matsuyama et al., (1989a) observed enhancement of HIV replication from as early as 1 h to as late as 6 days after exposure to TNF-α.

A critical step in the immunopathogenesis of HIV infection is the conversion of a latent or low level infection to active virus replication with the subsequent destruction of T cells. While the mechanisms by which this activation occurs

have not been fully established, in vitro activation of HIV infection in both T cells as well as monocytes has been demonstrated. The level of virus replication appears to correlate directly with the state of T lymphocyte activation and mitogens (McDougal et al., 1985; Folks et al., 1986; Zagury et al., 1986) or antigen (Margolick et al., 1987) can act as signals to bring about this activation. A regulatory factor which has been shown to play a key role in T cell activation is NF-κB. NF-κB was first identified as a DNA-binding protein which recognized and bound to a 10 base pair sequence in the κ light chain enhancer (κB) and was implicated in κ enhancer function regulating transcription (Sen and Baltimore, 1986a). NF-κB was later shown to be inducible by phorbol myristate acetate (PMA) and lipopolysaccharide in a variety of cell types (Sen and Baltimore, 1986b). In the cell cytoplasm, NF-κB appears to be complexed with an inhibitory protein, (IκB) and activation by inducers involves modification of IκB and translocation of NF-κB to the nucleus where it binds to DNA recognition sites (Lenardo and Baltimore, 1989).

There is thought to be only one NF-κB or a family of closely related proteins, acting as regulators of transcription of many genes in a variety of cell types. Viruses such as SV-40 (Mitchell et al., 1987; Chiu et al., 1987), CMV (Boshart et al., 1985) and HIV-1 (Muesing et al., 1987; Nabel and Baltimore, 1987) have NF-κB-binding sites in their enhancers and viral transactivators of CMV and hepatitis B virus induce NF-κB activity (Sen and Baltimore, 1986a; Cherrington and Mocarskii, 1989). Additionally, in activated T lymphocytes and phorbol ester treated HeLa cells, NF-κB-binding has been shown to modulate transcriptional inducibility of the HIV long terminal repeat (LTR) and to stimulate HIV production (Nabel and Baltimore, 1987). Similarly, HIV gene expression in monocyte/macrophages has been shown to be regulated by NF-κB although the control of NF-κB in monocytes and T cells may differ (Griffin et al., 1989).

In studies of the molecular mechanisms by which TNF-α activates HIV expression in ACH2 cells (a chronically-infected T-cell line), increases in steady-state HIV RNA and HIV transcription were observed and the activation appeared to be mediated by the induction of cellular transcriptional factors binding to the NF-αB sequences in the viral LTR (Duh et al., 1989). Similar results were observed in Jurkat (T cells), pre-B (70Z) and promonocytic (U937) leukemia cell lines and HT2 cells (Osborn et al., 1989) as well as in primary human macrophages (Mellors et al., 1991).

Early gene transcription of many viruses, including herpesviruses, is dependent on enhancer elements. Since, as mentioned above, NF-κB has been shown to recognize similar sequences in several primate virus enhancers (Mitchell et al., 1987; Chiu et al., 1987; Boshart et al., 1985) it is possible that similar to the mechanisms by which TNF stimulates HIV replication, TNF-α may also stimulate the replication of other viruses (retroviruses and DNA-containing viruses) which proceed through a DNA synthesis phase in their replicative cycle and which possess NF-κB-binding sequences in enhancer elements (see below).

As mentioned above, NF-κB-binding can also be stimulated by PMA (Sen and Baltimore, 1986a; Nabel and Baltimore, 1987), protein synthesis inhibitors such as cycloheximide or anisomycin (Sen and Baltimore, 1986a) or tax_1, the transactivator of human T cell lymphotropic virus type 1 (HTLV-1) (Leung and Nabel, 1988). The activation of NF-κB-binding by TNF-α appears to be similar to activation by cycloheximide, at least in Jurkat T-lymphoma cells (Osborn et al., 1989). However, the finding that NF-κB-binding is enhanced in TNF-α-treated HT2 cells which show no increase in response to PMA, suggests that this effect can occur through multiple mechanisms.

The number of reports identifying genes whose transcription is regulated by NF-κB is rapidly increasing (reviewed in Lenardo and Baltimore, 1989). In addition to genes encoding the immunoglobulin κ chain and viruses such as SV-40, CMV and HIV-1, NF-κB sites are found in genes encoding the Interleukin-2α receptor (IL-2Rα) (Leung and Nabel, 1988; Bohnlein et al., 1988; Ruben et al., 1988), IL-2 (Shibuya et al., 1989), major histocompatability complex (MHC) class I (Baldwin and Sharp, 1987; 1988), β_2-microglobulin (Israel et al., 1987) and IFN-β (Lenardo et al., 1989). TNF-α treatment of cells alters transcription of many genes, decreasing transcription of genes such as those encoding lipoprotein lipase and α1 collagen, while inducing transcription of genes such as those encoding IFN-β, IL-6, *c-fos* and *c-myc*. While NF-κB appears to play a role in TNF-induced expression of IL-6 (Ray et al., 1988), IL-2Rα (Lowenthal et al., 1989a; 1989b and HIV-1 (Duh et al., 1989; Osborn et al., 1989) its role in expression of other TNF-induced genes remains to be elucidated. Lenardo et al. (1989) showed that PRDIII, the critical virus-inducible element of the human IFN-β gene, is interchangeable with the NF-κB-binding site of the immunoglobulin κ chain enhancer (κB), suggesting that NF-κB is involved in the induction of IFN-β by viruses. Similarly, the induction of IFN-β by TNF-α may also involve NF-κB activation. The demonstration that NF-κB binds to PRDIII (Lenardo et al., 1989) also suggests that regulation of TNF-induced gene expression may involve binding of NF-κB not only to κB sites but to sites functionally related to the already identified κB sites, as well.

Effect of TNF on virus infection in vivo

Virus infection of mice

EMCV is a member of the Picornavirus fairly which includes the enteroviruses (Poliovirus, Coxsackievirus and Echovirus) as well as Rhinovirus. These viruses are the cause of a wide spectrum of human disease including nonspecific febrile illness, aseptic meningitis, pericarditis, myocarditis and encephalitis. Inoculation of mice with this virus results in an infection which closely resembles human disease caused by enteroviruses. In the infected mice, viremia is followed by infection of target organs and death occurs primarily from central nervous system involvement (Stringfellow et al., 1974).

The pathogenesis of disease in this model has been well characterized. Animals with detectable viremia die from infection and encephalitis is generally the primary cause of death.

Utilizing this model, we studied the effects of exogenously administered recombinant murine TNF-α (rMuTNF-α) alone and in combination with recombinant murine interferon-γ (rMuIFN-γ) (Czarniecki and Chiu, 1986). As shown in Fig. 1, rMuIFN-γ administered intraperitoneally (i.p.) 24 and 4 h prior to infection effectively protected mice from lethal virus challenge (100 PFU of EMCV, administered i.p.) in a dose-dependent manner. Only one mouse out of 20 died in the group that received rMuIFN-γ at a concentration of 10 μg/mouse. The number of survivors in each group was subjected to statistical analysis (Cox-Mantel Test). By this analysis, the number of surviving mice was significantly greater ($P<0.005$) in the groups receiving rMuIFN-γ doses of either 1 or 10 μg/mouse compared to those receiving PBS. These results confirm those reported earlier by Shalaby et al. (1985).

The treatment of mice with rMuTNF-α (administered i.p. 24 and 4 h prior to infection) in doses ranging from 0.05 to 3 μg/mouse had minimal beneficial effect in this model. While the survival curves for the groups receiving 0.05 and 0.5 μg/mouse were not significantly different from each other ($P=0.7$), it is interesting to note that the number of survivors was significantly ($P=0.04$) greater in the group receiving rMuTNF-α concentrations of 0.05 μg/mouse than in the PBS control group.

The results of these studies clearly demonstrate that although TNF-α may exhibit inhibitory activity against EMCV replication in certain in vitro cell systems, it is only marginally effective in protecting mice from in vivo challenge with this virus. In contrast, the in vitro antiviral activities of MuIFN-γ, reported in the scientific literature, correlate well with the in vivo protective effects shown in Fig. 1.

We were next interested in examining the effects of combined cytokine treatment on the course of EMCV infection in this murine model. Cytokines were administered i.p. and for combination treatments, separate sites were inoculated with rMuIFN-γ administered immediately prior to rMuTNF-α. As shown in Fig. 1, treating mice with combinations of rMuTNF-α and rMuIFN-γ resulted in enhanced protection against EMCV infection. Addition of 0.05 μg/mouse of rMuTNF-α to a suboptimal concentration of rMuIFN-γ (1 μg/mouse) resulted in a survival curve similar to that obtained with 10 μg/mouse of rMuIFN-γ, administered as a single agent. The number of mice surviving in the group receiving the combination dose of rMuTNF-α (0.05 μg/mouse) and rMuIFN-γ (1 μg/mouse) was significantly greater than in the groups receiving either rMuIFN-γ alone (1 μg/mouse) ($P=0.03$) or rMuTNF-α alone (0.05 μg/mouse) ($P=0.002$). While there was no significant difference between the combination doses comprised of 1 μg/mouse rMuIFN-γ plus either 0.05 or 0.5 μg/mouse rMuTNF-α ($P=0.22$), similar to results of single agent treatments, the lower rMuTNF-α dose appeared to be more beneficial than the 0.5 μg/mouse dose.

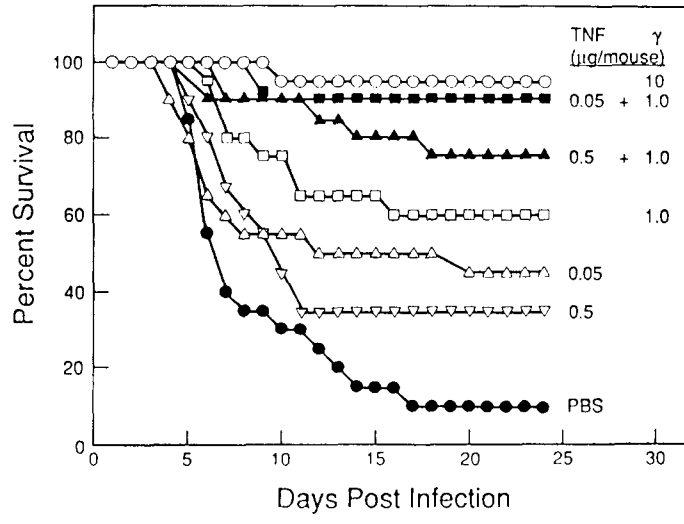

Fig. 1. Effect of rMuTNF-α and rMuIFN-γ on EMCV infection of mice. CD1 mice (20/group) were treated i.p. with PBS (●); rMuIFN-γ in concentrations of 1.0 (□) or 10 (○) μg/mouse; rMuTNF-α in concentrations of 0.05 (△) or 0.5 (▽) μg/mouse; rMuTNF-α (0.05 μg/mouse) plus rMuIFN-γ (1.0 μg/mouse) (■); or rMuTNF-α (0.5 μg/mouse) plus rMuIFN-γ (1.0 μg/mouse) (▲). All treatments were administered 24 h and 4 h prior to infection with EMCV.

In the next experiment, polyclonal rabbit antiserum raised against purified MuIFN-β (Lee Biomolecular) was administered to CD 1 mice simultaneously with rMuIFN-γ and rMuTNF-α prior to virus challenge. The results are presented in Fig. 2. As in the previous experiment, rMuTNF-α (0.05 μg/mouse) alone had no beneficial effect on the course of survival; administration of rMuIFN-γ protected these mice in a dose-dependent manner and administration of the two cytokines in combination resulted in enhanced antiviral activity.

Administration of anti-MuIFN-β at the same time as the combination of rMuIFN-γ (1 μg/mouse) and rMuTNF-α (0.05 μg/mouse) completely abrogated the enhancement of antiviral activity and the survival curve for this group of mice was not statistically different from that of the group treated with 1 μg/mouse rMuIFN-γ alone ($P=0.4$). Similar responses were observed with lower doses of rMuIFN-γ (0.1 μg/mouse) and rMuTNF-α (0.05 μg/mouse). The administration of anti-MuIFN-β at the same time as rMuIFN-γ (0.1 μg/mouse) plus rMuTNF-α (0.05 μg/mouse) abrogated the potentiated protective response and the survival curve of this treatment group was similar to that of the group receiving 0.1 μg/mouse rMuIFN-γ alone (data not shown).

These results are consistent with in vitro data suggesting that induction of IFN-β plays a crucial role in the antiviral effect of TNF-α as observed in in vitro studies described above. However, this induction of IFN-β was not sufficient to protect mice from EMCV infection since rMuTNF-α alone had no beneficial effect on survival of infected mice.

Utilizing another model of EMCV infection, Sriram et al. (1991) also

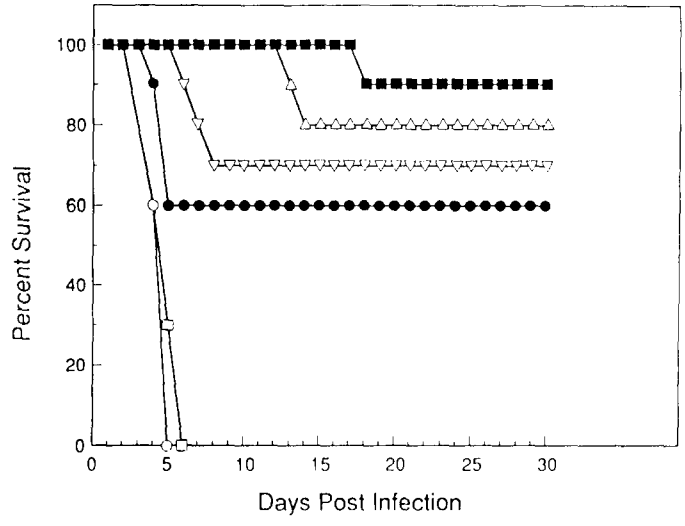

Fig. 2. Anti-MuIFN-β abrogates the potential anti-EMCV effects of combination cytokine treatment. CD1 mice (20/group) were treated i.p. with PBS (○); 1.0 μg/mouse rMuIFN-γ (▽); 10.0 mg/mouse rMuIFN-γ (△); 0.05 μg/mouse rMuTNF-α (□); 1.0 μg/mouse rMuIFN-γ plus 0.05 μg/mouse rMuTNF-α (■); 1.0 μg/mouse rMuIFN-γ plus 0.05 μg/mouse rMuTNF-α plus anti-MuIFN-β (●).

reported in vivo effects of TNF-α. In this model, infection of female BALB/c mice with the M variant of EMC virus (EMC-M) results in the development of a monophasic paralytic illness. As shown in these studies, treatment of mice with one dose of TNF-α (5 μg 24 h before infection) or with two doses (24 h prior to and 24 h following virus infection) had no effect on the course of the disease. However, administration of a lower dose of TNF-α (0.5 μg) on alternate days beginning 24 h before infection to day 11 after infection led to a delay in onset of disease, reduced pathology and delayed replication of virus in the brain.

In another report, Rossel-Voth et al. (1991) showed that administration of recombinant human TNF-α (rHuTNF-α) to DBA/2, BALB/c and C57BL/6 mice 4 h and 8 h after HSV-1 infection resulted in increased survival.

SVV Infection of non-human primates

The herpesvirus, simian varicella virus (SVV), is antigenically and biochemically similar to human varicella-zoster virus and in the African green monkey (*Cercopithecus aethiops*), it causes a severe, potentially lethal disease similar to varicella-zoster virus infection in immunocompromised hosts. This model has been utilized effectively for predicting the activity of therapeutic agents against varicella-zoster virus infection in humans and we utilized this model to evaluate the in vivo antiviral effects of rHuTNF-α (Soike et al., 1989).

Monkeys, free of antibodies to SVV, were infected by combined intratracheal and subcutaneous inoculation of SVV. Treatment with

TABLE 5

Effect of rHuTNF-α on SVV infection in rhesus monkeys

rHuTNF-α[a] μg/kg/day	Severity of rash on post-infection day[b]							Viremia on post-infection day[c]				
	7	8	9	10	11	12	14	3	5	7	9	11
Control[d]	1+	3+	Dead					4	217	>1000	Dead	
	1+	2+	3+	4+	4+	4+	3+	14	134	647	562	1
	3+	4+	4+	4+	4+	4+	3+	6	157	>800	7	3
1.0	1+	1+	2+	2+	1+	1+	±	4	139	497	2	2
	–	Dead						26	>1000	>1000	Dead	
	–	2+	Dead					5	167	>1000	Dead	
3.0	–	Dead						4	331	>1000	Dead	
	±	1+	Dead					7	277	>1000	Dead	
	±	Dead						2	306	>1000	Dead	
10.0	2+	Dead						3	>1000	>1000	Dead	
	1+	Dead						35	>1000	>1000	Dead	
	Dead							12	>500	Dead		

[a]Treatment was begun 21 h after virus inoculation and was administered by intravenous injection in divided doses twice daily. [b]Rash was scored by severity on a scale of 1+ to 4+. [c]Mean plaque forming units obtained from lymphocytes separated over a Ficoll-Hypaque gradient and cocultured with Vero cells (number of plaques obtained from 1.0 ml of heparinized blood). [d]Control Monkeys received placebo (PBS). (Modified with permission from Soike et al., 1989).

rHuTNF-α (1, 3 or 10 μg/kg per day) was initiated 24 h after infection. This is a time frame at which virus replication is not yet detectable and which has been used to demonstrate therapeutic efficacy of rHuIFN-α (Soike et al., 1983), rHuIFN-β (Soike et al., 1987) and rHuIFN-γ (Soike and Czarniecki, unpublished results) administered as single agents. The rHuTNF-α was administered in divided doses twice daily to groups of three monkeys by i.v. bolus injection into the saphenous vein. The doses and route of administration were chosen as those shown to be well tolerated and efficacious in previous studies of uninfected monkeys and in Phase 1 studies with cancer patients (Blick et al., 1987). Virus infection was monitored by collecting blood samples on days 3, 5, 7, 9 and 11 after virus inoculation for determination of viremia (by plaque assay) and hematological and clinical chemistry tests. Additionally, all monkeys that died underwent a complete necropsy with histopathological examination of tissues.

As shown in Table 5, all of the rHuTNF-α treated monkeys developed marked varicella infection. While only one of 3 infected, non-rHuTNF-α monkeys died of SVV infection, all monkeys receiving 10 or 3 μg/kg/day and 2 of 3 monkeys receiving 1 μg/kg/day died with severe viral disease. Treatment of uninfected monkeys with similar doses of rHuTNF-α resulted in no mortality or detectable toxicity. It is important to note that deaths in rHuTNF-α treated, virus-infected monkeys occurred within 6 and 8 days after initiation of

TABLE 6

Aspartate aminotransferase (SGOT) values in sera from African green monkeys infected with SVV and treated with rHuTNF-α

rHuTNF-α[a] μg/kg/day	SGOT values on post-infection day:				
	0	3	7	9	11
Control[b]	50	133	228	Dead	
	49	95	133	1435	500
	32	67	180	1245	156
1.0	39	82	226	400	155
	33	83	10240	Dead	
	49	237	2080	Dead	
3.0	39	121	10380	Dead	
	33	113	4220	Dead	
	36	72	2320	Dead	
10.0	45	94	6690	Dead	
	33	92	9160	Dead	
	49	90	Dead	Dead	

[a]rHuTNF-α treatment was begun 24 h after inoculation of virus. [b]Control monkeys received placebo (PBS). (Modified with permission from Soike et al., 1989).

treatment. These results are in contrast with the TNF-α induced mortality observed by Kettlehut et al. (1987) who showed that rats injected i.v. with high doses of TNF-α died within several hours after injection.

All of the data collected in these studies suggest that treatment with rHuTNF-α enhanced SVV infection in these monkeys and the effect was dose-related since the monkeys treated with 10 μg/kg/day of rHuTNF-α had more severe disease than those receiving 1 μg/kg/day. Viremia was more severe in the rHuTNF-α treated monkeys and appeared earlier. Hepatitis, which is a characteristic of this disease in African green monkeys, was monitored by measuring serum aspartate aminotransferase (SGOT) levels and as shown in Table 6, SGOT levels of rHuTNF-α treated infected monkeys rose earlier and to higher titers. Necropsies performed on monkeys that died in these studies showed severe systemic SVV infection and the severity of gross and histopathological lesions was greater in the cytokine-treated monkeys compared to control monkeys receiving no cytokine treatment. The more severe hepatic and pulmonary necrosis and hemorrhage present histologically in the rHuTNF-α treated, infected monkeys might be attributable to enhancement of virus infection induced by cytokine treatment. Additionally, TNF-α has been shown to exert many direct actions on endothelium associated with endothelial cell activation, playing a role in endothelial injury (Pober, 1987; Movat, 1987). Since the vasculature, with particular involvement of endothelial cells, has been shown to be a major target of SVV infection (Pober, 1987), it is possible that TNF-α induced endothelial cell activation may lead to

enhanced damage to the vasculature during SVV infection.

From the results of in vivo studies described above, it is evident that the effects of exogenously administered recombinant TNF-α on the course of virus infection are highly dependent upon the specific model, including host and viral pathogen. Administration of rMuTNF-α to mice had no beneficial effects against lethal infection with EMCV. However, extremely low doses of rMuTNF-α did enhance the protective effects of rMuIFN-γ in this infectious model, and these enhanced protective effects were abrogated with simultaneous administration of antibodies which neutralize MuIFN-β. In contrast, TNF-α did show benefit in other murine models, preventing viral encephalitis following infection with EMC-M virus (Sriram et al., 1991) and increasing survival of mice infected with HSV-1 (Rossel-Voth et al., 1991).

Very different results were observed when rHuTNF-α was administered to monkeys infected with SVV. In this virus infection model, rHuTNF-α enhanced severity of virus infection and subsequent mortality. While several reports have described TNF-induced enhancement of HIV replication in vitro (see above) this (Soike et al., 1989) is the first report of TNF-α mediated enhancement of in vivo virus infection. Possible mechanisms by which virus infection can be modulated and enhanced include: (i) induction of virus receptors on cells which can then provide new reservoirs for progeny virus and/or (ii) activation and enhancement of transcription and translation of viral gene products within the host cell. While further studies are necessary to determine the mechanisms by which rHuTNF-α enhanced the replication of SVV in our in vivo studies, it is tempting to speculate that TNF may modulate virus infection at either or both levels.

Viruses utilize specific surface proteins as portals of entry into the host cell. These surface proteins, most likely, serve other functions besides their role as receptors for specific viruses and their expression might be modulated by immunoregulatory molecules. In the case of SVV infection, if endogenous TNF-α is induced during virus infection and plays a role in pathogenesis of disease by inducing virus receptors on specific cell types (e.g., endothelial cells), then administration of exogenous rHuTNF-α to infected monkeys would exacerbate that disease. The identification of the cell surface receptor for Rhinovirus as the previously described integral membrane protein, intercellular adhesions molecule-1 (ICAM-1) (Greve et al., 1989; Staunton et al., 1989) and the observations that expression of ICAM-1, as well as that of other adhesion molecules (such as ELAM-1 on endothelial cells) is modulated by TNF-α (Cotran and Pober, 1989) is supportive of the above hypothesis.

It should be noted that in in vitro studies, we did not observe enhancement of SVV replication in monkey fibroblasts treated with rHuTNFα (Soike et al., 1989). However, these cells, prior to rHuTNF-α treatment, support SVV replication. It is possible that in vivo treatment with rHuTNF-α may exacerbate SVV infection by inducing SVV receptors on specific host cells which cannot be infected without exposure to TNF-α, and many types of target cells would have to be examined in vitro to identify the host cell that has been transformed into a

virus reservoir by TNF-α exposure.

It is also possible that rHuTNF-α enhanced SVV infection in vivo by stimulating virus replication at the level of transcription and translation of specific viral gene products. As described above, TNF-α enhances the expression of HIV in monocytes and T cells specifically by activation of a transcription factor, NF-κB. While the genome of SVV has not been cloned or sequenced, the sequence of the closely related virus, human varicella-zoster virus, has been reported (Davison and Scott, 1986). Close examination of this sequence reveals several regions showing close sequence homology (similarity in 10 of 11 base pairs) to the NF-κB-binding sites identified in the enhancer elements of HIV-1, CMV, SV-40, IL-2Rα, β_2-microglobulin and MHC class I. As discussed above, NF-κB can also recognize and bind to sequences functionally related to these κB sites, such as PRDIII sequence within the human IFN-β gene. In a similar manner, there may be additional sites functionally related to the κB sites already identified, within the varicella zoster virus genome which may also act as potential sites for NF-κB-binding.

Additional experiments are necessary to demonstrate that: (i) these sites in the human varicella-zoster virus (or similar sites in the SVV)genome are truly NF-κB-binding sites; (ii) these sites are involved in regulating transcription of the virus [as for CMV (Muesing et al., 1987) and SV-40 (Nabel and Baltimore, 1987; Cherrington and Mocarskii, 1989)]; and (iii) TNF-α enhances NF-κB-binding to these sites in target cells infected with this virus, with subsequent enhancement of virus production.

Conclusions

The establishment of a role for a particular factor in a particular disease state, is accomplished by demonstrating the presence of that factor in the host inflicted with the disease and observing an effect (beneficial or harmful) on the course of the disease by administrating the factor, exogenously, to the host or by blocking the endogenous production or activity of the factor in the host.

It is evident that viruses can act as inducers of TNF in vitro and increased levels of TNF have been observed in AIDS patients. However, it is not fully established if the TNF inducer in AIDS patients is the virus, HIV, or other microbial pathogens with which these patients are infected. In terms of effects of TNF on virus replication in vitro, TNF inhibits the replication of many RNA and DNA-containing viruses in some cell types. However, the replication of retroviruses (SL-3 and HIV-1) is enhanced in some TNF-treated cells and it is possible that by similar mechanisms the replication of other viruses may also be enhanced. The results from in vivo studies with exogenously administered TNF emphasize the need for caution in interpretation of in vitro data. While exogenously administered rMuTNF-α resulted in beneficial effects on mice infected with HSV-1 or EMC-M and in low doses potentiated the antiviral effects of rMuIFN-γ against EMCV infection of mice, rHuTNF-α treatment of

monkeys infected with SVV exacerbated virus infection and resulted in enhanced mortality.

The pleiotropic nature of TNF actions and the intricate mechanisms by which TNF interacts with other immunomodulatory factors, as well as the varied nature of the pathogenesis of disease induced by different viruses complicate the elucidation of the role of TNF in viral disease. Identification and cloning of receptors for TNF (Smith et al., 1990) and generation of TNF-receptor deficient mice (Pfeffer et al., 1993) provide us with useful tools for future studies. Only by the continuation of studies designed to understand the roles of these immunomodulatory factors in viral and other diseases can we unravel the complexities of the host immune response and thereby design rational therapeutic approaches to combat and prevent these diseases.

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