Cancer Immunol Immunother (1991) 33: 183–188
034070049100050Y

Cancer Immunology Immunotherapy

Inhibition of protein synthesis enhances the lytic effects of tumor necrosis factor α and interferon γ in cell lines derived from gynecological malignancies

L. Stewart Massad, David G. Mutch*, Ming-Shian Kao, C. Bethan Powell, and John Leslie Collins

Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO 63 110, USA

Received 5 November 1990/Accepted 24 January 1991

Summary. Few clinical responses have occurred in preliminary studies using the cytokines tumor necrosis factor α (TNFα) or interferon γ (IFNγ) in cancer patients. This may be related to the observation that many malignant cell lines are resistant to lysis by these cytokines *in vitro*. Resistance to lysis by TNFα or IFNγ in many cells is controlled by a protein-synthesis-dependent mechanism, such that when protein synthesis is inhibited cells become sensitive to lysis by these cytokines. Because there is some evidence that TNFα and IFNγ act through different lytic mechanisms and are opposed by different resistance mechanisms, we treated a panel of eight cell lines, five derived from human cervical carcinomas (ME-180, MS751, SiHa, HT-3, and C-33A) and three derived from ovarian carcinomas (Caov-3, SK-OV-3, and NIH: OVCAR-3) with both TNFα and IFNγ to determine whether such combination treatment might maximize *in vitro* cell lysis. Our results showed that pretreatment with IFNγ followed by exposure to TNFα in the presence of protein synthesis inhibitors increased lysis of seven of the eight cell lines above that seen with either TNFα or IFNγ and inhibitors of protein synthesis. Only the cell line C-33A was resistant to lysis by TNFα and IFNγ, when exposed to these agents both alone and in combination with protein synthesis inhibitors. Clinically, combining the cytokines TNFα and IFNγ with protein synthesis inhibitors may maximize the *in vivo* lytic effects of these cytokines.

Key words: TNFα – IFNγ – Gynecological cancer

Introduction

The cytokines tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ) have been shown to have *in vitro* antiproliferative activity for wide variety of malignant cell lines [8, 15, 20, 23]. In spite of this *in vitro* activity, clinical responses have been rare in early trials of TNFα and IFNγ [5–7, 11–13]. This discrepancy between laboratory and clinical results may be explained in part by our observation that TNFα, IFNγ and their combination are cytostatic for a variety of cell lines derived from human gynecological malignancies *in vitro* [17] but are not cytolytic when used at pharamcological concentrations for up to 24 h [14, 18].

Since the elimination of cancer cells rather than the inhibition of their growth is the goal of cancer therapy, we have attempted to identify agents that might increase the lytic potential of these cytokines. We and others have shown that chemotherapeutic drugs that inhibit protein synthesis are such agents. When protein synthesis is inhibited in cells that are normally resistant to the cytotoxic effects of TNFα or IFNγ, they become sensitive to these cytokines [9, 10, 14, 18].

The inability of TNFα and IFNγ to lyse many cancer cell lines in the absence of protein synthesis inhibition indicates that these cells normally express resistance mechanisms to TNFα and IFNγ. Although the inhibition of protein synthesis increases lysis by both TNFα and IFNγ, the lytic mechanisms activated by the two cytokines are different, in that the TNFα lytic mechanism exists constitutively while that of IFNγ requires induction [14, 18]. The existence of independent lytic mechanisms activated by TNFα and IFNγ, both of which are opposed by protein-synthesis-dependent resistance mechanisms, suggest that treating cells with TNFα and IFNγ while inhibiting protein synthesis may result in still further increases in the lysis of cancer cells.

Materials and methods

Cell lines. ME-180, SiHa, HT-3, MS751, and C-33A are cell lines derived independently from human cervical carcinomas. SK-OV-3,

* Supported by American Cancer Society Career Development Award 90-221

Offprint requests to: J. L. Collins, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University School of Medicine, 4911 Barnes Hospital Plaza, St. Louis, Missouri 63 110, USA

Caov-3, and NIH:OVCAR-3 are independent cell lines originally derived from human ovarian carcinomas. All cell lines were obtained from the American Type Culture Collection (Rockville, Md) and were maintained as exponentially growing cultures. All cell lines except HT-3 and NIH:OVCAR-3 were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 30 mg/ml L-glutamine (Sigma Chemical Co., St. Louis, Mo.), 450 U/ml penicillin (Sigma), 40 μg/ml streptomycin (Sigma), and 10% fetal bovine serum (HyClone Lab., Logan, Utah). HT-3 cells were grown in McCoy's medium containing the same supplements as DMEM. NIH:OVCAR-3 cells were grown in RPMI-1640 medium containing the same supplements as DMEM with an additional 10 μg/ml recombinant human insulin (Eli Lilly and Co., Indianapolis, Ind.). NIH:OVCAR-3 cells were grown at 37° C in a humidified atmosphere of 5% CO_2 and 95% air. All other cells were grown at 37° C in a humidified atmosphere of 10% CO_2 and 90% air.

Cytokines. Recombinant human IFNγ (2.5×10^7 units/mg) was obtained from Genzyme (Boston, Mass.) and was reconstituted with distilled water (10^5 units/ml) and stored at –70° C. Recombinant human TNFα (3.2×10^7 units/mg) was obtained from Bachem (Torrance, Calif.) and was reconstituted with distilled water (3×10^4 units/ml) and stored at –70° C. Both cytokines were diluted in the appropriate medium used for the routine growth of cells prior to their addition to the cytolytic assay.

Protein synthesis inhibitors. Stock solutions of emetine (EM, Sigma), an inhibitor that blocks protein synthesis at the level of translation, and actinomycin-D (Act-D, Sigma), an inhibitor that blocks protein synthesis at the level of transcription, were prepared by reconstituting with sterile water or saline, as recommended by the manufacturer. Further dilutions were prepared with media used for the routine growth of cells.

The cytolytic assay. In IFNγ pretreatment experiments, $(1.0-2.0) \times 10^6$ cells were plated in 100-mm plastic tissue-culture dishes (Corning Glassworks, Corning, N. Y.) containing 10 ml medium and allowed to adhere for 6–18 h before 0.1 ml IFNγ was added at a concentration of 10000 units/ml (final concentration 100 units/ml). Following 24 h of incubation, control (no IFNγ) and IFNγ-containing media were removed and replaced with RPMI-1640 medium containing 3% bovine serum albumin (Sigma) and 50 μCi/ml ^{51}Cr as sodium chromate (NEN/DuPont, Boston, Mass.). Cells were labelled with ^{51}Cr for 1 h in a humidified atmosphere of 5% CO_2 and 95% air at 37° C and then washed with DMEM. Cells were removed from the plates with 0.04% EDTA in phosphate-buffered saline, pH 7.2, and washed with DMEM. Cells were suspended in medium used for the routine growth of cells, counted, and transferred to 96-well microtiter plates (Corning) at a concentration of 10^4 cells/well in a final volume of 0.15 ml. TNFα, EM, and Act-D were added to effect the final concentration indicated. Released of ^{51}Cr was determined after 24 h of incubation at 37° C in a humidified atmosphere of 10% CO_2 and 90% air for all cell lines except NIH:OVCAR-3, which was incubated in a humidified atmosphere of 5% CO_2 and 95% air. Total incorporation of ^{51}Cr was determined by counting the radioactivity of 1×10^4 cells. The spontaneous release of ^{51}Cr was determined for cells incubated in medium for 24 h. Three replicate wells were assayed for the determination of the percentage specific lysis. The percentage specific lysis (^{51}Cr release, cpm) was calculated by the following formula:

Specific lysis (%) =

$$100 \times \frac{[^{51}\text{Cr (experimental)}]-[^{51}\text{Cr (spontaneous)}]}{[^{51}\text{Cr (total)}]-[^{51}\text{Cr (sponanteous)}]}$$

Overall, the percentage spontaneous release [^{51}Cr (spontaneous)/^{51}Cr (total)] ranged from 30% to 52%. In those experiments that involved comparison of separate IFNγ-pretreated and control (i.e. not pretreated) cells, the percentage spontaneous release did not differ by more than 10% and was not related to the presence or absence of IFNγ.

Statistical analysis. The means of triplicate experiments were compared using Student's *t*-test. To ensure reproducibility of the results obtained, multiple such assays were performed. The results presented in Tables 1 and 2 are from representative assays. Each assay was performed using multiple concentrations of each protein synthesis inhibitor; for presentation in the tables, concentrations of EM or Act-D were selected that produced some toxicity but did not cause such high levels of cell lysis as to obscure additional lysis by cytokines. The standard errors of the means of all data sets used to calculate percentage specific lysis were in all cases less than 9%. When comparing the results of lytic experiments using cells pretreated and not pretreated with IFNγ, the δ method was employed [4]. Statistical significance was assumed when $P < 0.05$. Synergistic interactions were determined to be present when the combination of one or more inactive agents with a lytic agent or combination [3] resulted in a further increase in lytic activity. All other interactions resulting in increased lysis were presumed to be additive.

Results

The cervical carcinoma cell lines ME-180, MS751, HT-3, SiHa, and C-33A and the ovarian carcinoma cell lines Caov-3, SK-OV-3, and NIH:OVCAR-3 are resistant to *in vitro* lysis by TNFα and IFNγ under conditions that approximate those achievable *in vivo*. However, inhibiting protein synthesis in cells incubated with TNFα or preincubated with IFNγ increases their lysis to levels above those attributable to either of these cytokines alone or to protein synthesis inhibitors [14, 18]. As shown in Tables 1 and 2, the cell lines ME-180, MS751, HT-3, SiHa, Caov-3, SK-OV-3, and NIH:OVCAR-3 are sensitive to lysis by TNFα when protein synthesis is inhibited, and the cell lines ME-180, MS751, Caov-3, and SK-OV-3 are sensitive to lysis by IFNγ when exposed to IFNγ prior to protein synthesis inhibition. Because there is some evidence that the mechanism of lysis mediated by TNFα is distinct from that mediated by IFNγ [14, 18], we set out to determine whether the combination of TNFα and IFNγ in the presence of inhibitors of protein synthesis might further increase the lysis of these cells. The results of analysis of the specific lysis of cell lines derived from cervical carcinomas are shown in Table 1, while Table 2 shows the specific lysis of cell lines derived from ovarian carcinomas.

As shown in Table 1, ME-180 cells are relatively resistant to lysis by TNFα alone, reaching only 10% specific lysis at 500 units/ml (first line). Pretreatment with 100 units/ml IFNγ for 24 h did not cause any lysis of ME-180 cells (second line, 0 units/ml TNFα), nor did it increase the level of lysis by TNFα (second line, $P > 0.05$).

Table 1 shows that the exposure of ME-180 cells to TNFα and inhibitors of protein synthesis causes a level of lysis significantly greater than is observed with TNFα alone or with protein synthesis inhibitors alone. At 500 units TNFα/ml, ME-180 cells are lysed 48% in the presence of Act-D and 45% when protein synthesis is inhibited by EM (third and fifth lines). This is significantly greater than the 8% and –2% lysis of ME-180 cells in the presence of Act-D or EM alone (third and fifth lines, 0 units/ml TNFα) or the 10% lysis of ME-180 cells when protein synthesis is 500 units of TNFα/ml alone (first line). A dose response to TNFα is apparent, in that lysis by the combination of TNFα and protein synthesis inhibitors increases with increasing concentrations of TNFα (third and fifth lines).

Table 1 also shows that the lysis of ME-180 cells pretreated with 100 units/ml IFNγ is increased by subsequent exposure to protein synthesis inhibitors, although this in-

Table 1. The percentage specific lysis of cells derived from human cervical malignancies in the presence of tumor necrosis factor α (TNFα) and protein synthesis inhibitors with or without pretreatment with 100 U/ml interferon γ (IFNγ)

Cell line	IFNγ[a]	Specific lysis (%) for [TNFα] =				
		0	1 U/ml	10 U/ml	100 U/ml	500 U/ml
ME-180[b]						
No PSI[c]	–	0	2	6[d]	8[d]	10[d]
No PSI	+	0	1	10[d]	14[d]	24[d]
Act D 1 μM	–	8	10	19[d]	35[d]	48[d]
Act D 1 μM	+	41[e]	45[f]	59[f, g]	69[f, g]	76[f, g]
EM 0.1 μM	–	–2	5[d]	18[d]	35[d]	45[d]
EM 0.1 μM	+	7	39[f, g]	55[f, g]	70[f, g]	73[f, g]
MS751						
No PSI	–	0	–1	–1	0	2
No PSI	+	0	1	1	5[d]	3
Act D 0.1 μM	–	20	31[d]	37[d]	46[d]	54[d]
Act D 0.1 μM	+	39[e]	53[f, g]	57[f, g]	63[f, g]	69[f, g]
EM 0.1 μM	–	13	16	15	19[d]	23[d]
EM 0.1 μM	+	22	30	27[f, g]	35[f, g2D]	46[f, g]
HT-3						
No PSI	–	0	–1	4	–5	6
No PSI	+	0	3	–4	–5	11[e]
Act D 1 μM	–	45	42	42	39[d]	54
Act D 1 μM	+	49	44	41	39	45
EM 10 μM	–	25	16	29	43[d]	55[d]
EM 10 μM	+	34	36[f]	48[f, g]	51[g]	67[f, g]
SiHa						
No PSI	–	0	–2	–1	–1	0
No PSI	+	0	–1	–2	–3	–2
Act D 10 μM	–	–3	–3	2[d]	13[d]	19[d]
Act D 10 μM	+	0	6	17[f, g]	33[f, g]	45[f, g]
EM 10 μM	–	4	2	8[d]	21[d]	30[d]
EM 10 μM	+	0	24[f, g]	32[f, g]	57[f, g]	65[f, 3g]
C-33A						
NoPSI	–	0	–6	–4	–6	6
No PSI	+	0	–1	2	8	7
Act D 1 μM	–	44	38	36	44	48
Act D 1 μM	+	43	52	42	42	46
EM 1 μM	–	45	47	43	45	41
EM 1 μM	+	50	57	37[g]	41	35[g]

[a] Results for experiments that did (+) or did not (–) include pretreatment of cells with 100 U/ml IFNγ for 24 h before incubation with TNFα and inhibitors of protein synthesis

[b] The percentage spontaneous release of ^{51}Cr of cells not pretreated (IFN–) or pretreated (IFN+) with IFNγ for each cell line was: ME-180 IFN–, 41%, IFN+, 39%; HT-3 IFN–, 44%, IFN+, 45%; SiHa IFN–, 30%, IFN+, 32%; MS751 IFN–, 42%, IFN+, 47%; C-33A IFN– 47%, IFN+, 43%. None of the differences between cells pretreated and not pretreated was statistically significant ($P > 0.05$)

[c] Results in the absence of protein synthesis inhibitors

[d] Lysis significantly greater than at the same concentration of protein synthesis inhibitor but in the absence of TNFα ($P < 0.05$)

[e] Lysis of cells preincubated with IFNγ and then treated with protein synthesis inhibitor greater than lysis by protein synthesis inhibitor alone ($P < 0.05$)

[f] Lysis of cells preincubated with IFNγ and then treated with protein synthesis inhibitor and TNFα greater than lysis by protein synthesis inhibitor and TNFα only ($P < 0.05$)

[g] Lysis of cells preincubated with IFNγ and then treated with protein synthesis inhibitor and TNFα different from lysis by IFNγ preincubation and protein synthesis inhibition ($P < 0.05$)

Table 2. The percentage specific lysis of cells derived from human ovarian malignancies in the presence of TNFα and protein synthesis inhibitors with or without pretreatment with 100 U/ml IFNγ

Cell line	IFNγ[a]	Specific lysis (%) for [TNFα] =				
		0 U/ml	1 U/ml	10 U/ml	100 U/ml	500 U/ml
Caov-3[b]						
No PSI[c]	–	0	–4	8[d]	10[d]	15[d]
No PSI	+	0	4	10[d]	10[d]	15[d]
Act D 0.1 μM	–	37	42[d]	62[d]	71[d]	74[d]
Act D 0.1 μM	+	62[e]	65[f]	74	76[g]	77[g]
EM 1 μM	–	37	51[d]	61[d]	77[d]	75[d]
EM 1 μM	+	69[e]	72[f]	77[f]	80[f, g]	84[f, g]
SK-OV-3						
No PSI	–	0	1	3	2	4
No PSI	+	0	2	3	2	3
Act D 10 mM	–	4	8[d]	10[d]	12[d]	19[d]
Act D 10 mM	+	16[e]	18[f]	16	22[f, g]	22
EM 10 mM	–	20	30[d]	40[d]	46[d]	52[d]
EM 10 mM	+	25	39[g]	52[f, g]	57[g]	67[f, g]
NIH:OVCAR-3						
No PSI	–	0	1	–1	4	5
No PSI	+	0	7	9	9	15
Act D 10 mM	–	16	16	32[d]	36[d]	60[d]
Act D 10 mM	+	12	26[g]	37[g]	45[g]	51[g]
EM 10 μM	–	12	16	31[d]	50[d]	46[d]
EM 10 μM	+	12	34[f, g]	48[f, g]	57[g]	48[g]

[a] Results for experiments that did (+) or did not (–) include pretreatment of cells with 100 U/ml IFNγ for 24 h before incubation with TNFα and inhibitors of protein synthesis

[b] The percentage spontaneous release of ^{51}Cr of cells not pretreated (IFN–) or pretreated (IFN+) with IFNγ for each cell line was: SK-OV-3 IFN–, 38%, IFN+, 37%; Caov-3 IFN–, 43%, IFN+, 47%; NIH: OVCAR-3 IFN–, 42%, IFN+, 52%. None of the differences between cells pretreated and not pretreated was statistically significant ($P > 0.05$)

[c] Results in the absence of protein synthesis inhibitors

[d] Lysis by TNFα and protein synthesis inhibitor significantly greater than lysis by protein synthesis inhibitor alone ($P < 0.05$)

[e] Lysis of cells preincubated with IFNγ and then treated with protein synthesis inhibitor greater than lysis by protein synthesis inhibitor alone ($P < 0.05$)

[f] Lysis of cells preincubated with IFNγ and then treated with protein synthesis inhibitor and TNFα greater than lysis by protein synthesis inhibitor and TNFα only ($P < 0.05$)

[g] Lysis of cells preincubated with IFNγ and then treated with protein synthesis inhibitor and TNFα greater than lysis by IFNγ preincubation and protein synthesis inhibition ($P < 0.05$)

crease only attains significance when protein synthesis is inhibited by Act-D. ME-180 cells are lysed 41% when protein synthesis was inhibited by Act-D after IFNγ pretreatment (fourth line; 0 units TNFα/ml). This is compared to 0% lysis by IFNγ alone, and 8% lysis by Act-D alone (second and third lines; 0 units TNFα/ml). Incubation of IFNγ-pretreated ME-180 cells with EM at a concentration of 0.1 μM caused 7% lysis (line 6). This was not significantly more lysis than the –2% caused by EM alone (line 5), but incubation of pretreated cells with higher concentrations of EM did significantly increase lysis above levels observed with either EM or IFNγ alone (data not shown).

When IFNγ-pretreated cells were exposed to TNFα (500 units/ml), 76% lysis of ME-180 cells occurred when protein synthesis is inhibited by Act-D and 73% lysis when

protein synthesis is inhibited by EM (fourth and sixth lines). This is significantly more lysis than was obtained when cells that had not been pretreated with IFNγ were exposed to TNFα and either Act-D (48% lysis) or EM (45% lysis) and also significantly more lysis than was obtained when cells pretreated with IFNγ were exposed to either Act-D (41% lysis) or EM (7% lysis). Thus the combination of TNFα and IFNγ in the presence of inhibitors of protein synthesis is more efficacious in causing lysis of ME-180 cells than either cytokine alone in the presence of protein synthesis inhibitors.

Although all the cell lines in Tables 1 and 2 show individual differences in sensitivity to particular agents, they can be broadly divided into three groups based on their sensitivity to TNFα and IFNγ. The first group is composed of the cervical carcinoma cell lines ME-180 and MS751 (Table 1) and the ovarian carcinoma cell lines Caov-3 and SK-OV-3 (Table 2). All of these cell lines are relatively resistant to lysis by TNFα and by pretreatment with IFNγ. Inhibition of protein synthesis renders each of these cell lines sensitive to lysis by TNFα. Pretreatment with IFNγ similarly increases the lysis of each of these four cell lines when protein synthesis is subsequently inhibited, although this increase in lysis attained statistical significance for ME-180, MS751 and SK-OV-3 cells only when protein synthesis was inhibited by Act-D. Finally, for all of the cell lines in this group, combining IFNγ pretreatment with exposure to TNFα and protein synthesis inhibition resulted in greater lysis than was achieved by either TNFα or IFNγ in combination with protein synthesis inhibitors.

The cervical carcinoma cell lines HT-3 and SiHa (Table 1) and the ovarian carcinoma cell line NIH:OVCAR-3 (Table 2) comprise the second group. Like the cells in group 1, all these cell lines were resistant to lysis by TNFα or by pretreatment with IFNγ, and inhibition of protein synthesis rendered all three cell lines sensitive to lysis by TNFα. However, unlike the cell lines in group 1, none of the cell lines in this second group was lysed by pretreatment with IFNγ followed by protein synthesis inhibitors. Nevertheless, pretreatment with IFNγ before exposure to TNFα and protein synthesis inhibitors increased lysis to levels above those seen when cells were exposed only to TNFα and protein synthesis inhibitors.

Group 3 contains only the cervical carcinoma cell line C-33A. Although similar to the cells in groups 1 and 2 in their resistance to lysis by TNFα and IFNγ in the absence of protein synthesis inhibitors, C-33A cells were unique in that they did not become sensitive to lysis by either TNFα or IFNγ when protein synthesis was inhibited. Combining the two cytokines also did not increase lysis when protein synthesis was inhibited.

The increased lysis resulting from the combination of TNFα and IFNγ with protein synthesis inhibitors shown by all cell lines except C-33A was analyzed for the presence of synergy [3]. For the cells that showed an increase in lysis when protein synthesis was inhibited after pretreatment with IFNγ (group 1), the increase in lysis resulting from the combination of IFNγ pretreatment, TNFα, and inhibition of protein synthesis was an additive effect when compared to lysis by either cytokine with protein synthesis inhibitors. For the cells that were resistant to IFNγ even when protein synthesis was inhibited (group 2), the increase in lysis resulting from the combination IFNγ pretreatment, TNFα, and protein synthesis inhibitors was synergistic relative to lysis by either TNFα or IFNγ and protein synthesis inhibitors.

Discussion

In summary, Tables 1 and 2 show that all the human cervical and ovarian carcinoma cell lines tested were resistant to lysis by clinically achievable concentrations of TNFα and IFNγ during 24 h of incubation. Pretreating cells with IFNγ and then exposing them to TNFα while inhibiting protein synthesis resulted in three types of response. ME-180, MS751, SK-OV-3, and Caov-3 cells (group 1) showed additive increases in lysis when the two cytokines were combined and protein synthesis was inhibited. HT-3, SiHa, and NIH:OVCAR-3 cells (group 2) showed synergistic increases in lysis under the same conditions. C-33A cells (group 3) remained resistant to the combination of TNFα and IFNγ even when protein synthesis was inhibited.

Because the lytic and resistance mechanisms of TNFα and IFNγ are all dependent on protein synthesis, differences in the ability of Act-D and EM to increase the lysis mediated by these cytokines may reflect individual differences among cell lines in the relative induction, synthesis, or degradation of the TNFα and IFNγ lytic and resistance mechanisms. In Tables 1 and 2, there are several instances in which protein synthesis inhibition by EM but not by Act-D renders cells sensitive to TNFα. Act-D is an inhibitor of DNA-dependent mRNA transcription, while EM is an inhibitor of the translation of mRNA into protein. Sensitivity to lysis by TNFα revealed by incubation with EM but not Act-D may reflect the existence of an intracellular pool of mRNA encoding the protein-synthesis-dependent resistance to TNFα that is too large to be depleted during a 24-h incubation with Act-D. One example of a cell line in which this may occur is HT-3 (Table 1). There are also instances of protein synthesis inhibition by Act-D but not EM rendering cells sensitive to pretreatment with IFNγ. This may be because an intracellular pool of mRNA encoding the lytic mechanism induced in these cells by exposure to IFNγ continues to be translated when protein synthesis is inhibited by Act-D but not by EM. ME-180, MS751, and SK-OV-3 are all examples of cell lines that demonstrate this.

Protein synthesis inhibitors block resistance to lysis by TNFα and by preincubation with IFNγ in the cell lines in group 1. The presence of lytic mechanisms in these cell lines indicates that they possess receptors for TNFα and IFNγ. The lytic mechanisms activated by TNFα and IFNγ in these cell lines must be different, since the TNFα lytic mechanism exists constitutively while that of IFNγ requires induction [14, 18]. The lack of synergy between TNFα and IFNγ in group 1 suggests that the TNFα and IFNγ lytic mechanisms do not interact. In contrast to group 1, the cell lines in group 2 express only the TNFα lytic mechanism. Although these cells do not express the IFNγ lytic mechanism, pretreatment of these cell lines with IFNγ

results in a synergistic increase in lysis when combined with TNFα and protein synthesis inhibitors. This synergy between IFNγ preincubation and TNFα indicates that these cell lines have functional IFNγ receptors even thought they are not lysed by IFNγ pretreatment followed by protein synthesis inhibition. Synergy between IFNγ and TNFα in these three cell lines also suggests that IFNγ, though not itself lytic, may amplify the TNFα lytic mechanism. IFNγ is known to increase the number of receptors for TNFα [1, 22], which might cause a synergistic increase in lysis. It is also possible that these cells express a protein-synthesis-independent resistance mechanism that prevents lysis by IFNγ and that TNFα decreases this resistance mechanism. In either case, the fact that IFNγ and TNFα are synergistic implies that the lytic mechanisms of these two cytokines are distinct. That the cell line C-33A was resistant to lysis by TNFα and IFNγ both in the presence and absence of protein synthesis inhibition suggests that C-33A cells resist both TNFα and IFNγ by mechanisms independent of protein synthesis. Absent or nonfunctional receptors or defective signalling pathways are possible specific examples of such protein-synthesis-independent mechanisms.

The possibility that TNFα and IFNγ act by increasing the sensitivity of cell lines to lysis by inhibitors of protein synthesis, rather than the converse, cannot be excluded until the molecular mechanisms of action of these agents have been fully elucidated. Data from other laboratories suggest that TNFα causes cell lysis by activating arachidonic acid metabolism and that protein synthesis inhibitors act by preventing the expression of one or more proteins that ordinarily scavenge free oxygen radicals released during arachidonic acid metabolism [24]. IFNγ also appears to exert its toxic effects through an oxygen-dependent process [2].

Although we have previously shown that exposing the eight cell lines used in this study to TNFα alone for 16 h does not cause lysis [18], the results presented in Tables 1 and 2 show that 24 h of exposure to TNFα alone causes a small but significant increase in the lysis of ME-180 and Caov-3 cells. Others have shown that there may be some lysis of certain cell lines by TNFα concentrations as high as 10000 units/ml during 72 h incubations [16, 21]. However, pharmacokinetic studies in patients with cancer have shown that the half-life of TNFα is less than 1 h, and although concentrations above 10000 units/ml can be achieved briefly, concentrations above those 500 units/ml are difficult to sustain for longer than 24 h [6, 7]. We also have shown previously that exposure to IFNγ alone for 24 h at concentrations as high as 10000 units does not cause cell lysis [14]. We and others have shown lytic effects of IFNγ on some cell lines after exposure to 100–10000 units/ml for 2–5 days [14, 16, 19]. Again, however, phase 1 trials have not achieved these high concentrations for such prolonged periods of time [5, 11, 12]. Significant numbers of clinical responses have not been achieved in early trials using TNFα and IFNγ, both alone and in combination, suggesting that these cytokines are not effective at eliminating cancer cells *in vivo* at doses that can be tolerated. The results presented here suggest that the role of TNFα and IFNγ as anticancer agents may lie not only in their combination with each other, but also in their combination with chemotherapeutic agents that inhibit protein synthesis.

References

1. Aggarwal BB, Eessalu TE (1987) Induction of receptors for tumor necrosis factor α by interferons is not a major mechanism for their synergistic cytotoxic response. J Biol Chem 262: 10000
2. Aune TM, Pogue SL (1989) Inhibition of tumor cell growth by interferon-γ is mediated by two distinct mechanisms dependent upon oxygen tension: induction of tryptophan degradation and depletion of intracellular nicotinamide adenine dinucleotide. J Clin Invest 84: 863
3. Berenbaum MC (1981) Criteria for analyzing interactions between biologically active agents. Adv Cancer Res 35: 269
4. Bishop YMM, Fienberg SE, Holland PE (1975) Discrete multivariate analysis. MIT, Boston, pp 296
5. Demetri GD, Spriggs DR, Sherman ML, Arthur KA, Imamura K, Kufe D (1989) A phase I trial of recombinant human tumor necrosis factor and interferon-gamma: effects of combination cytokine administration in vivo. J Clin Oncol 7: 1545
6. Feinberg B, Kurzrock R, Talpaz M, Blick M, Saks S, Gutterman JU (1988) A phase I trial of intravenously-administered recombinant tumor necrosis factor-alpha in cancer patients. J Clin Oncol 6: 1328
7. Foon KA, Sherwin SA, Abrams PG, Stevensom HC, Holmes P, Maluish AE, Oldham RK, Herberman RB (1985) A phase I trial of recombinant gamma interferon in patients with cancer. Cancer Immunol Immunother 20: 193
8. Fransen L, van der Heyden J, Ruysschaert R, Fiers W (1986) Recombinant tumor necrosis factor: its effect and its synergism with interferon-γ on a variety of normal and transformed human cell lines. Eur J Cancer Clin Oncol 22: 419
9. Jacobs AJ, Dawoud L, Kovacs Z, Ratliff TL (1989) Inhibition of proliferation of lines derived from human cervical carcinomas by cytotoxic drugs and by recombinant interferons. Gynecol Oncol 32: 31
10. Kim C-M, Hong W-S, Lee J-O, Kang T-W, Kim Y-W, Song J-K, Yun T-K, Kim C-Y (1989) Enhancement of cytotoxicity of cisplatin *in vitro* by recombinant human tumor necrosis factor and/or recombinant human interferon-α, -β, and -γ. Jpn J Cancer Res 80: 904
11. Kurzrock R, Rosenblum MG, Sherwin SA, Rios A, Talpaz M, Quesada JR, Gutterman JU (1985) Pharmacokinetics, single-dose tolerance, and biological activity of recombinant γ-interferon in cancer patients. Cancer Res 45: 2866
12. Kurzrock R, Feinberg B, Talpaz M, Saks S, Gutterman JU (1989) Phase I study of a combination of recombinant tumor nectosis factor-α and recombinant interferon-γ in cancer patients. J Interferon Res 9: 435
13. Lenk H, Tanneberger S, Muller U, Ebert J, Shiga T (1989) Phase II clinical trial of high-dose recombinant human tumor necrosis factor. Cancer Chemother Pharmacol 24: 391
14. Massad LS, Mutch DG, Powell CB, Kao MS, Collins JL (1990) Expression of a resistance mechanism in ovarian and cervical carcinoma cells prevents their lysis by γ-interferon. Cancer Res 50: 4923
15. Mori H, Kondoh H, Teruhiko T (1989) Synergistic effects of interferon-γ and tumor necrosis factor against proliferation of gynecologic tumor cell lines. Gynecol Oncol 35: 209
16. Morikawa K, Fidler IJ (1989) Heterogeneous response of human colon cancer cells to the cytostatic and cytotoxic effects of recombinant human cytokines: interferon-α, interferon-γ, tumor necrosis factor, and interleukin-1. J Biol Response Mod 8: 206
17. Mutch DG, Massad LS, Kao MS, Collins JL (1990) Proliferative and antiproliferative effects of interferon gamma and tumor necrosis factor alpha on cell lines derived from cervical and ovarian malignancies. Am J Obstet Gynecol 163: 1920
18. Powell CB, Mutch DG, Massad LS, Kao M-S, Collins JL (1990) Common expression of a tumor necrosis factor resistance mechanism among gynecologic malignancies. Cancer Immunol Immunother 32: 131

19. Richtsmeier WJ (1988) Interferon gamma induced oncolysis: an effect on head and neck squamous carcinoma cultures. Arch Otolaryngol Head Neck Surg 114: 432
20. Sugarman BJ, Aggarwal BB, Hass PE, Figari IS, Palladino MA, Shepard HM (1985) Recombinant human tumor necrosis factor-α: effects of proliferation of normal and transformed cells in vitro. Science 230: 943
21. Taguchi T, Abe S, Nakano K, Matsui Y, Sohmura Y (1988) Synergistic enhancement of the antitumor activity of recombinant human TNFα by recombinant human IFNγ. Cancer Detect Prev 12: 105
22. Tsujimoto M, Vilcek J (1986) Tumor necrosis factor receptors in HeLa cells and their regulation by interferon-γ. J Biol Chem 261: 5384
23. Welander CE, Homesley HD, Reich SD, Levin EA (1988) A phase II study of the efficacy of recombinant interferon gamma in relapsing ovarian adenocarcinoma. Am J Clin Oncol 11: 465
24. Wong GHW, Elwell JH, Oberley LW, Goeddel DV (1989) Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. Cell 58: 923