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Interferon-gamma and cytotoxic agents studied in combination using a soft agarose human tumor clonogenic assay

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Summary. The in vitro antiproliferative activity of human recombinant interferon-gamma (IFN-gamma) was tested against human tumor cells in vitro in combination with doxorubicin, cisplatin, or vinblastine. Using a human tumor clonogenic assay (HTCA), IFN-gamma alone showed dose-dependent inhibition of colony growth in six or seven human tumor cell lines as well as in each of nine fresh ovarian tumor specimens. The combination of IFN-gamma and either doxorubicin or cisplatin showed additive antiproliferative effects against all the cell lines with the exception of an IFN-gamma-resistant endometrial cancer cell line (HEC-1A). In combination with vinblastine, IFN-gamma rarely had an additive effect.

Inclusion of macrophages from malignant effusions in the HTCA potentiated the antiproliferative effect of IFN-gamma alone as well as the combination of IFN-gamma and doxorubicin; however, the efficacy of the two agents was never more than additive.

The results show that combinations of IFN-gamma with doxorubicin or cisplatin are additive and warrant further investigation. The antitumor effect of IFN-gamma alone or in combination with cytotoxic drugs may be significantly enhanced by tumor-associated macrophages.

Introduction

Recent advances in DNA technology have enabled the detailed investigation of the range of biological effects of interferons (IFNs). It is now known that all three types of IFN (alpha, beta, and gamma) have antitumor acivity in addition to their antiviral effects [5, 10].

The efficacy of IFNs as anticancer agents has been investigated clinically, but these early phase I trials have not demonstrated them to be of sufficient potency to cause complete clinical response in solid tumors when used as single agents [24, 37]. According to the results of in vitro studies, immune IFN (IFN-gamma) has somewhat more potent antitumor activity than the class I interferons (alpha and beta) [5, 10]. This may reflect impurtities present in the early preparations or may be a result of differences in sensitivity of the target cells to the different compounds. However, results of phase I clinical trials with IFN-gamma in cancer patients indicate marginal antitumor activity at tolerable doses [11, 12, 19, 28, 33]. Objective assessment of the action of IFN-gamma against cancer will require phase II trials empoying multiple-treatment regimens.

An alternative therapeutic application of IFNs would be in combination with conventional cytotoxic drugs. Several reports have indicated usefulness of combinations of antineoplastic agents with alpha or beta IFNs using animal models [3, 21] and in vitro tests [1, 9, 37]. Combinations of cytotoxic agents with IFN-gamma have not been investigated as extensively as with the other IFNs.

Because IFN-gamma has been shown to act as a macrophage-activating factor [22, 25, 27], indirect antitumor effects mediated through macrophages should also be considered. An indirect antiproliferative effect of IFN-gamma, mediated by macrophages from human malignant effusions, has been demonstrated using a soft agarose clonogenic assay [26].

The purpose of this study was to determine the effectivness of combinations of IFN-gamma and cytotoxic drugs on tumor cells using a human tumor clonogenic assay (HTCA). In order to investigate the effect on different types of tumors, various human cell lines were used as targets in this experimental model. Furthermore, inclusion of adherent cells from malignant effusions in the assay allowed the assessment of indirect effects of IFN-gamma.

Materials and methods

Cell lines. Seven different human cancer cell lines were used in this study. They were derived from a human ovarian carcinoma (BG-1) [37], an endometrial carcinoma [18], a melanoma (SK-MEL 28) [7], two cervical carcinomas (ME-180 [31] and CaSki [23]), a breast carcinoma (MCF-7) [30], and a myeloma line (RPMI-8226) [20].

Cells were maintained by serial passage in culture flasks and plated in soft agarose culture 72 h after prior reseeding in liquid culture to achieve logarithmic phase growth. This results in consistent cloning efficiencies over the course of serial passages as described perviously [26].

Fresh human tumor cells. Human tumor specimens were obtained from nine ovarian cancer patients having either surgery or draining of malignant effusions. Single-cell suspensions of tumor cells were prepared from either effusions or solid tumors using mechanical and enzymatic techniques [37].

Macrophages from malignant effusions. Macrophages from human malignant effusions were used in experiments test-

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ing for indirect effects of IFN-gamma, as previously described in detail [26]. Briefly, ascitic fluid was obtained from patients with histologically proven ovarian cancer. Mononuclear cells were separated by gradient centrifugation on Ficoll-Paque. The cells were resuspended in RPMI-1640 medium with 20% heat-inactivated fetal calf serum at a concentration of 10^6 cells/ml, and allowed to adhere to plastic for 1 h at 37 °C in a humidified atmosphere of 7.5% CO_2 in air. After thorough washing off of nonadherent cells, the adherent cells were removed from the flasks with a rubber policeman and included in the agarose underlayer at a density of 5×10^4 cells per dish. Cytochemical identification of macrophages was made on slides prepared using cytobucket centrifugation followed by fixation and staining for nonspecific esterase [16]. Only the adherent cells comprised of more than 80% cytochemically positive macrophages were used in the study.

Soft agarose clonogenic assay. Tumor cells were cloned in a two-layer agarose matrix as described by Hamburger and Salmon [15] with slight modification [26]. Plating densities of the cell lines were between 5×10^4 and 1×10^5 cells per dish, selected in order to achieve at least 200 colonies per control dish. Primary human tumors were plated at 5×10^5 monoclear cells per dish. Cytotoxic drugs or IFN-gamma were applied as continuous liquid overlay onto the upper layer. To test the effect of single agents, 0.22 ml of 10 times the final concentration of drug was applied onto the upper layer and remained present throughout the incubation of the culture. For in vitro combination chemotherapy, 0.11 ml of 20 times the final concentration of each agent was applied simultaneously. Control cultures were incubated with 0.22 ml of PBS alone on the upper layer. Each experiment was set up in triplicate.

In experiments investigating the indirect as well as the direct effect of IFN-gamma, 5×10^4 ascites macrophages were included in the underlayers. Upper layers and application of drugs were as previously described.

Agarose cultures of human tumor cell lines and fresh human tumors were incubated at 37 °C in a humidified incubator with 7.5% CO_2 for 7–14 days. Colonies were counted with a computerized feature analysis system (Omnicon FAS II, Baush & Lomb, Rochester, NY, USA) [17]. Colonies were defined as cell aggregates greater than 50 μm in diameter arising from a single-cell suspension. Only those experiments with a minimum of 200 colonies in control plates were evaluated for IFN-gamma activity. Results were expressed as the percent colony growth in the treated cultures compared with the untreated controls.

Cytotoxic drugs. Doxorubicin, cisplatin, and vinblastine were used in combination with IFN-gamma. Concentrations of cytotoxic drugs producing 50% inhibition of colony formation (IC_{50}) were used for combination experiments. The IC_{50} of each drug was determined from dose-response curves of colony growth of each cell line treated with increasing doses of the single agent. Table 1 presents the IC_{50} of each drug for each cell line used for the described investigation. Drug solutions were prepared fresh just before each experiment from aliquots in liquid nitrogen.

Interferon-gamma. Human recombinant IFN-gamma (2.8×10^7 IU/mg protein) prepared by recombinant DNA technology was provided by Biogen Research Corp. (Cambridge, Mass, USA). Concentrations of IFN-gamma used in this study were between 1 IU/ml (0.036 ng/ml) and 10000 IU/ml (360 ng/ml).

Table 1. IC_{50} of cytotoxic drugs in the HTCA

Cell line	Doxorubicin (ng/ml)	Cisplatin (ng/ml)	Vinblastine (pg/ml)
BG-1	1.4	22	0.003
SK-MEL 28	1.2	11	10.0
ME-180	5.0	101	11.0
MCF-7	1.0	29	0.3
HEC-1A	0.6	63	0.1
RPMI-8226	0.1	98	300.0
CaSki	1.6	30	1.3

The IC_{50} of each drug was determined from dose-response curves of colony growth of each cell line treated with increasing doses of the single agent

Table 2. Definitions of drug combination effects

Negative interaction	FCG(AB) > = FCG(B) when FCG(A) > = FCG(B)
Positive interaction	FCG(AB) > FCG(B) when FCG(A) > = FCG(B)
Subadditive	FCG(AB) > FCG(A) × FCG(B)
Additive	FCG(AB) = FCG(A) × FCG(B)
Synergistic	FCG(AB) < FCG(A) × FCG(B)

FCG, fractional clonal growth; (A), (B), drugs used as single agents; (AB), drugs used in combination

Determination of drug combination effects. The activity of drug combinations was interpreted according to criteria developed by Valeriote and Lin [34] with some modification (Table 2). The mean fractional colony growth (FCG) of cultures treated with two-agent regimens was compared to that of single-agent treatments. When two-drug treatments resulted in less colony inhibition (greater FCG) than the more effective of the two single agent's effects, the drug interaction was termed "negative". In cases where FCG of cultures treated by drug combinations was less than that of the better of the two single agents' FCG, the drug combination was described as "positive". Statistical significance ($P<0.05$) was determined using Student's *t*-test (two-tailed).

Positive drug interactions were further categorized according to the observed FCG of drug combination cultures relative to an "expected" FCG value which was calculated as the product of the FCG observed from each of the single-agent cultures [4, 34]. These interactions were termed "subadditive", "additive", and "synergistic" when the observed FCG was greater, equal to, or less than the calculated FCG respectively. The data were tested for statistical significance ($P<0.05$) using a recently described analytical model [37].

Results

Direct antiproliferative effects of IFN-gamma on different human tumor cell lines and fresh ovarian tumor cells

Seven cell lines were tested for the direct antiproliferative activity of IFN-gamma using continuous exposure

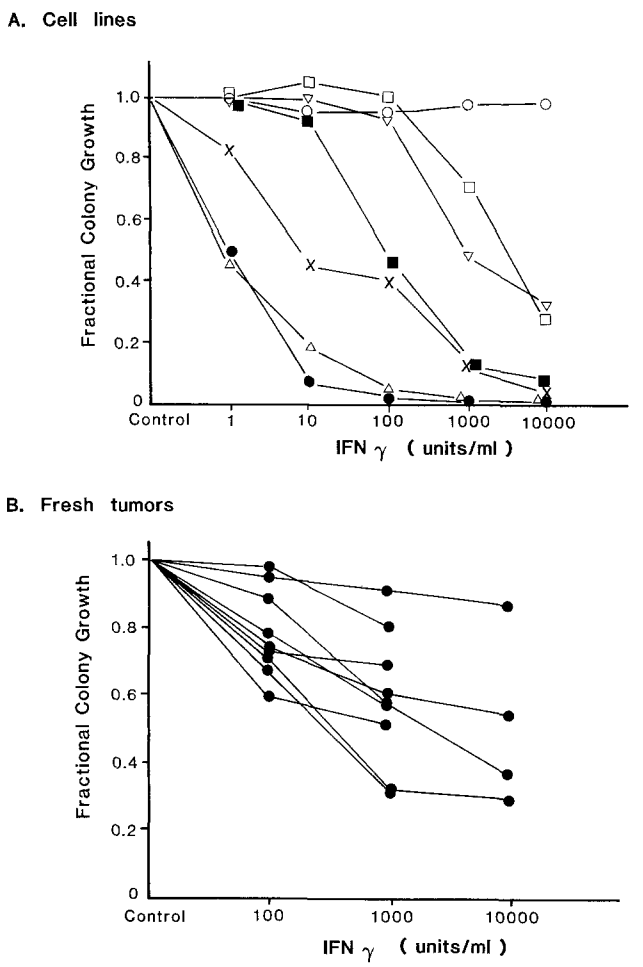

Fig. 1 A, B. Direct antiproliferative effect of IFN-gamma on seven human tumor cell lines **(A)** and fresh human ovarian cancer cells from nine patients **(B)**. Cell lines used in the clonogenic assay were BG-1 (□), HEC-1A (○), MCF-7 (▽), SK-MEL 28 (■), RPMI-8226 (X), Caski (△), and ME-180 (●)

(Fig. 1A). The endometrial cell line, HEC-1A, showed resistance at all concentrations tested. The other six cell lines showed sensitivity to IFN-gamma in a dose-responsive fashion. Interestingly, the two cervical cancer cell lines showed high sensitivity, with 70% colony reduction occurring between 1 and 10 IU/ml.

In a similar fashion, dose-dependent antiproliferative activity was observed on the fresh ovarian cancer cells (Fig. 1B). Reduction of colony growth exceeded 50% for two of the nine specimens at a concentration of 1000 IU/ml, which can be clinically achieved in serum [19].

Interaction of IFN-gamma and cytotoxic drugs

Doxorubicin. Seven cell lines were tested for the efficacy of IFN-gamma in combination with doxorubicin (Table 3). Additive effects were observed at all the concentrations of IFN-gamma for the melanoma (SK-MEL 28) and myeloma (RPMI-8226) cell lines. For the endometrial cell line, HEC-1A, the combined effect was not different from doxorubicin alone at all concentrations. The other four cell lines demonstrated additive effects at two concentrations of IFN-gamma.

Cisplatin. Five cell lines were tested for the combined effects of cisplatin at the IC_{50} and increasing dose of IFN-gamma (Table 4). An additive effect was observed for at least one concentration of IFN-gamma against four of the cell lines. For HEC-1A the combination of cisplatin and IFN-gamma demonstrated a negative interaction at all doses tested.

Vinblastine. The antiproliferative efficacy of IFN-gamma in combination with vinblastine was tested against six cell lines (Table 5). Negative interactions were observed at all concentrations tested on cell lines SK-MEL 28, ME-180, CaSki, and HEC-1A. Additive drug interactions were detected only at the highest concentration of IFN-gamma for BG-1 and MCF-7.

Result of the inclusion of ascites macrophages on the drug combination effect

The combination treatment using IFN-gamma and doxorubicin against BG-1 was performed with the inclusion of macrophages from human malignant effusions as feeder cells in the HTCA underlayers (Fig. 2). Reduction of colony growth by doxorubicin alone was not significantly altered by the presence of macrophages (Fig. 2A). However, the FCG resulting from IFN-gamma alone was significantly reduced at all concentrations of IFN-gamma by the inclusion of these feeder cells ($P < 0.01$). The combination of IFN-gamma and doxorubicin was additive in both the presence and absence of macrophages in the HTCA (Fig. 2B).

Discussion

Treatment of human cancer using combination chemotherapy is an effort to minimize development of drug resistance [13] as well as an attempt to achieve heightened therapeutic efficacy by virtue of positive drug interactions. In vitro, such interactions are usually described as synergistic or additive cytotoxic effects [4], while the in vivo manifestations of positive drug interactions may also include nonoverlapping toxicities, cell cycle synchronization of tumor cells, and indirect effects manifested as changes in the host immune status [6].

The recent description, isolation, and production of biological response modifiers may allow the introduction of combinations of cytotoxic drugs with "nontoxic" modulators which may show positive therapeutic interactions. It is in this setting that the current investigation of antiproliferative activity of combinations of IFN-gamma with antineoplastic drugs was undertaken.

It has been reported that IFNs have direct antiproliferative activity against tumor cells. A comparison of the direct antitumor effects of the three types of IFNs suggested that IFN-gamma has a more potent antiproliferative activity than either IFN-alpha or IFN-beta in mouse [10] and human [5] tumors. As more purified preparations of the compounds become available for testing and the target systems become better characterized, these differences in efficacy are becoming less significant. A clinical phase I study showed that the adverse effects of IFN-gamma were not severe, and large doses were tolerated when IFN-gamma was administered intravenously [28]. Because sustained plasma levels are tolerable and toxicity is generally not limiting, IFN-gamma may be an appropriate agent to use in

Table 3. Interaction of IFN-gamma and doxorubicin

Cell line	IFN conc. (IU/ml)	IFN-gamma alone (% control)	Combined effect of two drugs		
			Experimental (% control)	Calculated (% control)	Interaction classification
BG-1	100	86± 3.2	51± 1.9	44	negative
(51% ± 2.6%)[a]	1 000	25± 1.2	19± 1.9	12	ADDITIVE
	10 000	22± 1.6	17± 1.4	11	ADDITIVE
SK-MEL 28	100	46± 1.3	19± 1.7	27	ADDITIVE
(58% ± 4.4%)	1 000	11± 1.0	7± 1.7	6	ADDITIVE
	10 000	7± 0.1	3± 0.01	4	ADDITIVE
ME-180	1	109±13.6	34± 5.1	40	negative
(37% ± 6.3%)	10	43± 7.6	19± 2.4	16	ADDITIVE
	100	15± 8.8	6± 2.3	6	ADDITIVE
CaSki	1	45± 7.4	22± 5.0	33	ADDITIVE
(73% ± 3.9%)	10	18± 3.2	5± 1.9	13	ADDITIVE
	100	4± 0.4	3± 1.3	3	negative
MCF-7	100	79± 5.6	70±10.1	59	negative
(75% ± 2.1%)	1 000	64± 6.8	48± 1.4	48	ADDITIVE
	10 000	45± 1.7	29± 3.8	34	ADDITIVE
RPMI-8226	1	82± 2.3	31± 2.3	33	ADDITIVE
(40% ± 1.6%)	10	45± 1.8	17± 1.3	18	ADDITIVE
	100	40± 1.2	9± 0.01	16	ADDITIVE
HEC-1A	100	97± 2.7	54± 4.9	60	negative
(62% ± 1.8%)	1 000	94± 4.2	60± 5.7	58	negative
	10 000	97± 2.8	55± 2.9	60	negative

Target cells were cultured in HTCA with single agents or a combination of the two agents. Results were presented as % colony growth compared to control cultures without drugs (mean ± SE of triplicate cultures). Negative interaction was determined by comparing the observed combined effect with the single agents ($P < 0.05$), additive interaction by comparing the observed combined effect with the calculated combined effect ($P < 0.05$)

[a] Percent colony growth of doxorubicin alone

Table 4. Interaction of IFN-gamma and cisplatin

Cell line	IFN conc. (IU/ml)	IFN-gamma alone (% control)	Combination effect of two drugs		
			Experimental (% control)	Calculated (% control)	Interaction classification
BG-1	100	91± 5.4	66± 4.0	59	negative
(65% ± 5.2%)[a]	1 000	18± 2.0	13± 0.1	12	ADDITIVE
	10 000	12± 1.8	6± 0.1	8	ADDITIVE
SK-MEL 28	100	111± 2.5	66± 1.7	69	negative
(62% ± 0.7%)	1 000	94± 6.2	60± 2.4	58	negative
	10 000	48± 3.1	34± 3.4	30	ADDITIVE
ME-180	1	52± 5.1	32± 4.4	34	ADDITIVE
(64% ± 13.2%)	10	7± 0.3	9± 0.1	5	negative
	100	2± 0.5	3± 0.1	1	negative
MCF-7	100	95± 5.2	67± 2.8	61	negative
(64% ± 1.5%)	1 000	88±10.0	57± 6.4	56	negative
	10 000	88± 7.9	47± 4.7	56	ADDITIVE
HEC-1A	100	87±10.0	37± 1.7	38	negative
(44% ± 3.3%)	1 000	86± 6.3	48± 3.7	35	negative
	10 000	83±10.2	45± 3.2	36	negative

Results expressed as mean ± SE of triplicate cultures

[a] Percent colony growth of cisplatin alone

combination with cytotoxic drugs if it can be demonstrated that there are positive drug interactions.

The clonogenic assay described by Hamburger and Salmon [15] has been widely utilized and demonstrated to have some validity for predicting clinical activity of anticancer drugs on individual patients, particularly with respect to identification of nonresponsiveness [2, 36]. An assessment of the applicability of the HTCA for new drug screening also demonstrated its usefulness in identifying active compounds [29]. Based on these successful applica-

Table 5. Interaction of IFN-gamma and vinblastine

Cell line	IFN conc. (IU/ml)	IFN-gamma alone (% control)	Combination effect of two drugs		
			Experimental (% control)	Calculated (% control)	Interaction classification
BG-1	100	78± 0.3	76± 1.8	60	negative
(77% ± 5.0%)[a]	1000	34± 3.8	35± 0.01	27	negative
	10000	24± 1.6	12± 0.01	19	ADDITIVE
SK-MEL 28	100	74± 2.5	41± 3.3	35	negative
(47.8% ± 3.8%)	1000	40± 4.5	36± 3.5	19	negative
	10000	26± 3.4	22± 4.1	12	negative
ME-180	1	85± 5.8	58± 2.8	52	negative
(62% ± 2.8%)	10	7± 0.7	8± 0.01	4	negative
	100	2± 0.5	1± 0.01	1	negative
CaSki	1	97± 1.3	91± 1.4	64	negative
(66% ± 6.1%)	10	72± 4.5	76± 4.3	47	negative
	100	19± 1.8	20± 2.1	13	negative
MCF-7	100	82± 5.6	48± 2.6	44	negative
(54% ± 7.9%)	1000	70± 1.0	53± 2.1	37	negative
	10000	72± 2.3	35± 2.6	39	ADDITIVE
HEC-1A	100	90± 2.2	83± 2.6	70	negative
(78% ± 7.1%)	1000	76±11.0	72±11.6	59	negative
	10000	79± 9.7	70± 8.8	61	negative

Results expressed as mean ± SE of triplicate cultures
[a] Percent colony growth of vinblastine alone

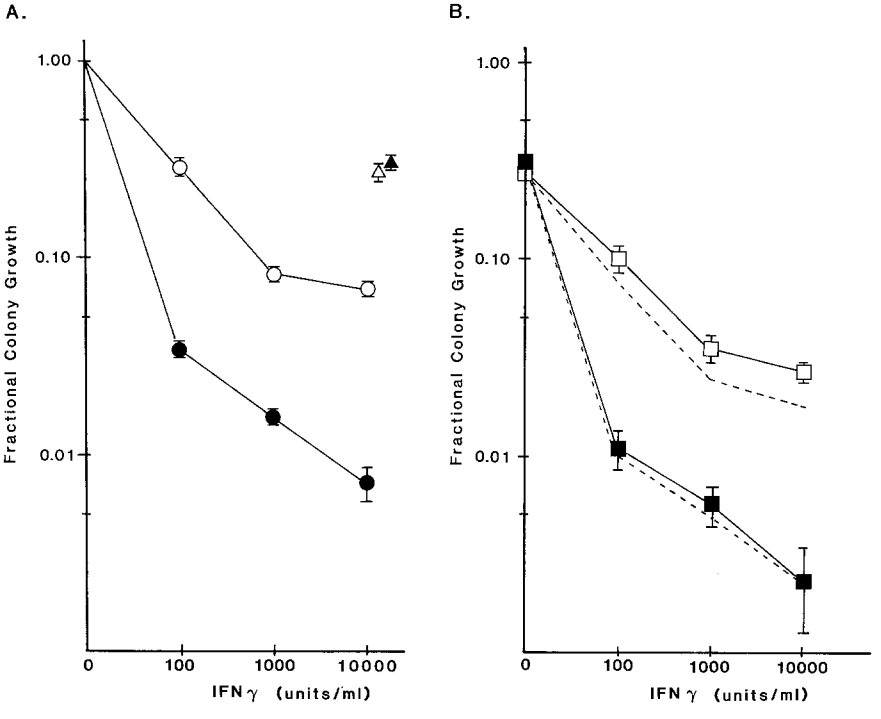

Fig. 2A. Antiproliferative effect of IFN-gamma and doxorubicin alone on ovarian cancer cell line BG-1. IFN-gamma (100 to 10000 units/ml, *circles*) and doxorubicin (1.4 ng/ml, *triangles*) were each tested as single agents with *(closed symbols)* or without *(open symbols)* ascites macrophages in the underlayer of the HTCA. Results shown are mean values from experiments performed in triplicate. **B** IFN-gamma and doxorubicin were tested in combination against BG-1 with *(closed squares)* or without *(open squares)* ascites-associated macrophages in the underlayer of the HTCA. Fractional colony growth was compared with that from the excepted, calculated growth *(dashed lines)*. This was derived from the product of the fractional colony survivals of the single agents. Colony numbers in the control dishes with and without ascites macrophages were 978±12 and 1014±11 (mean ± S. E.) respectively

tions, the screening of new drug combinations for possible beneficial interactions is reasonable in the belief that inactive combinations in the HTCA can be excluded from evaluation in clinical trials.

The results of the present investigation clearly demonstrate the differing susceptibility of seven human tumor cell lines to the direct antiproliferative activity of IFN-gamma. The endometrial cancer cell line, HEC-1A, was completely resistant to growth inhibition by IFN-gamma. Additionally, each schema of combination chemotherapy failed to demonstrate a positive interaction on this cell line. Interestingly, HEC-1A has also been shown to be resistant to both the antiviral and antiproliferative activities of IFN-alpha and IFN-beta [35].

For the other six human tumor cell lines and nine fresh ovarian tumors in this investigation, IFN-gamma exerted antiproliferative effects in a dose-responsive manner. In phase I studies with partially purified preparations, the le-

vel of IFN-gamma in serum achievable using maximum tolerated doses by continuous infusion was found to be 50–100 units/ml [14, 28]. Sustained levels of up to 50 ng/ml (approximately 1500 IU/ml) of recombinantly cloned IFN-gamma are achievable with i.m. injection [11, 19], while i.v. bolus injection with 1 mg/m^2 generates a peak plasma value of 175 ng/ml (approximately 5000 IU/ml) [11]. Doses resulting in half this value, however, required discontinuation of treatment of a significant proportion of patients [33].

At a concentration of 100 units/ml, four of seven cell lines showed greater than 50% inhibition of colony growth. However, the data on fresh human tumors indicate that primary human ovarian tumor clonogenic cells show less sensitivity at this concentration: only 5%–40% inhibition of colony growth was achieved. At 1000 IU/ml two of nine specimens displayed an intermediate antiproliferative response below 50% survival. Results of retrospective and prospective clinical trials of the HTCA indicate that the assay has a true-negative rate of over 90% in predicting nonresponsiveness to chemotherapeutic agents [1, 29, 36]. It would be appropirate to conclude that the tumors studied in this series would be unlikely to respond to IFN-gamma as a single agent and that this compound may require combination with cytotoxic drugs to show significant antitumor effects. The role of accessory cells in mediating the antitumor effects of IFN-gamma also warrants consideration in these types of studies [26].

The assessment of drug combination effects in the HTCA was successful. Of particular interest is the observation that additive drug interactions may be detected at various activity levels of the doses used. It is evident that IFN-gamma has some direct antiproliferative effect against the cell lines tested. This compound is also capable of showing an additive interaction with some cytotoxic agents when it is used at relatively low doses. Such results suggest that regimens of moderate doses of IFN-gamma in combination with anticancer agents may be more effective and as well tolerated as cytotoxic drugs alone. This warrants phase II evaluation. Importantly, high doses of IFN-gamma were not necessary for a positive drug interaction, except in combination with vinblastine.

In the combination experiments using IFN-gamma, doxorubicin was the cytotoxic drug most effective in producing an additive antiproliferative effect. This combination showed additivity at most IFN-gamma concentrations against all the cell lines with the exception of HEC-1A. It has already been reported that doxorubicin has additive to synergistic effects in combination with IFN-alpha in an animal model [3] as well as in the HTCA [37].

Combinations of IFN-gamma and cisplatin also exhibited additive effects on the tumor cell lines, again with the exception of HEC-1A. In no instance did combinations of IFN-gamma with doxorubicin or cisplatin exceed an additive effect. However, it was clearly demonstrated that the combined antiproliferative effects were significantly higher than that of either the cytotoxic drug or IFN-gamma alone.

Combinations of IFN-gamma and vinblastine resulted in predominantly negative interactions. Additivity was evident for only two cell lines and only at the highest concentration (10000 units/ml). This is a level which cannot be achieved in serum by intravenous injection; however, intraperitoneal protocols may be one means to achieve this concentration if it were demonstrated to be tolerable and of benefit. These findings contrast with those on a combination of IFN-alpha and vinblastine, which showed synergistic activity using low doses of IFN-alpha against fresh tumors in the HTCA [1]. This discrepancy may be due to differences in the mechanisms by which IFN-alpha and IFN-gamma exert their antiproliferative effects on tumor cells.

The role of macrophages in the efficacy of cytotoxic agents is poorly understood. The work of Colotta et al. [8] indicates that the combination of cytotoxic agents and macrophages enhances the susceptibility of tumor cells to macrophage killing. The demonstration of this effect required direct contact of the macrophages with the tumor cells. Furthermore, IFN-gamma has been shown to activate macrophages for tumor cytotoxicity [22, 25, 27]. This effect can be demonstrated using the HTCA by inclusion of macrophages from malignant effusion [26].

In the present study, the inclusion of ascites-associated macrophages in the HTCA potentiated the antiproliferative effect of IFN-gamma on the ovarian cancer cell line. In contrast, the efficacy of doxorubicin was not changed by the presence of macrophages. Because of the physical separation between the macrophages and the tumor cells, no cell contact is possible. These results indicate that the role of macrophages in the enhanced efficacy of IFN-gamma against tumor cells is due to macrophage-derived antiproliferative substances which inhibit tumor growth, and not the selection of "vulnerable" tumor clones with heightened sensitivity to toxic drugs.

Two general conclusions are evident from the results of the present investigation: (1) Combinations of selected cytotoxic agents and IFN-gamma show additive direct antiproliferative efficacy against tumor cells in the HTCA. (2) Macrophages can be induced by IFN-gamma to produce antiproliferative substances which also show additive interactions with select cytotoxic agents. Specifically, doxorubicin and cisplatin show positive interactions with IFN-gamma.

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