Human Monocytes in the Presence of Interferons Alpha2a and Gamma Are Potent Killers of Serous Ovarian Cancer Cell Lines in Combination with Paclitaxel and Carboplatin

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Interferons (IFNs) play an important role in immune surveillance of tumors; however, their efficacy in the treatment of malignancies has been limited. Monocytes are mononuclear phagocytes that are critical to the generation of an innate immune response to tumors. The authors and others have shown that treatment of tumor cell lines *in vitro* and *in vivo* with human monocytes primed with type I and type II IFNs results in killing. We now expand on this work, in an extended panel of ovarian cancer cell lines. In this study, we hypothesized that there would be variable sensitivity amongst cell lines to the killing properties of monocytes and IFNs. To this end, we explored the interactions of IFN primed monocytes in conjunction with the standard of therapy for ovarian cancer, taxane, and platinum-based chemotherapeutics. Using 6 ovarian cancer cell lines, we demonstrated that there is variation from cell line to cell line in the ability of IFN- α 2a and IFN- γ primed monocytes to synergistically kill target tumor cells, and further, there is an additive killing effect when target cells are treated with both IFN primed monocytes and chemotherapy.

Introduction

BOTH THE INNATE AND THE ADAPTIVE immune system are critical in the immune surveillance and control of malignancies (Mittal and others 2014). In mice, M1 inflammatory macrophages and interferon gamma (IFN- γ) are critical in immunoediting of tumors (O'Sullivan and others 2012). Consistent with these observations, the presence of M1 macrophages in human tumor biopsies are indicative of a proinflammatory state in ovarian cancer, and correlate with increased survival (Zhang and others 2014). Manipulation of the adaptive immune system is currently under clinical development for the treatment of ovarian cancer, with T cell therapy or vaccine-based clinical trials. However, there has been limited implementation of the innate immune system in conjunction with IFNs as cell-based therapy for the treatment of ovarian cancer.

Type I and type II IFNs have limited indications in the treatment of malignant cancers in humans (Bekisz and others 2013). IFNs comprise a family of proteins that protect the host from viral and bacterial infections, and inhibit cell growth. The most significant problem associated with IFN therapy is lack of target specificity. All nucleated cells have the IFN alpha receptors, thus making intravenous IFN in-

jection systemically active instead of tumor specific. To address this problem, we and others have shown that a combination of IFN-alpha2a (IFN- α 2a) and IFN- γ exerts more direct antiproliferative and tumoricidal effects in the presence of monocytes (Griffith and others 1999; Baron and others 2011; Nakashima and others 2012). Synergy between the antiproliferative effects of the IFNs on the tumor cells in combination with the proinflammatory and tumoricidal effects of these IFNs on monocytes results in enhanced killing of tumor cells.

The current treatment for patients with stage 3 or higher metastatic ovarian cancer of serous origin is surgical debulking of metastases in the peritoneal cavity, followed by intraperitoneal or intravenous administration of the chemotherapeutic agents paclitaxel and carboplatin (Coleman and others 2013). There is a high mortality in patients with ovarian cancer due to the inability to remove all tumors during surgery and subsequent chemotherapy-resistant tumor growth. However, there is a strong correlation between increased survival times and the presence of a proinflammatory gene signature (Verhaak and others 2013).

Recent genetic studies have shown that ovarian cancers comprise a heterogeneous cell population (Cancer Genome Atlas Research Network 2011; Creighton and others 2012).

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This variability from patient to patient necessitates the study of multiple cell lines to test the efficacy of IFNs and monocytes in killing ovarian cancer cells. Previous studies focused primarily on the cell line OVCAR-3 (Griffith and others 1999; Baron and others 2011). Whereas these cells provide a good model for study, they may not be representative of ovarian cancer lines in general. Furthermore, no studies have addressed the effects of type I and type II IFNs and monocytes on the standard chemotherapeutic treatment of ovarian cancers. In this study, we show that there is wide variability in ovarian cell lines' sensitivities to IFN- α 2a, IFN- γ , and monocyte killing. Additionally, there is an additive killing effect when IFNs and monocytes are combined with clinically relevant adjuvant therapy, paclitaxel and carboplatin.

Materials and Methods

Cell lines, IFNs, and monocytes

The human ovarian serous carcinoma OVCAR-3 cell line was obtained from ATCC (Manassas, VA). CaOV3, OVCAR-4, OVCAR-5, OVCAR-8, and SKOV-3, all human ovarian serous carcinomas, were obtained from Dr. Annunziata at the National Cancer Institute, National Institutes of Health (NIH). All lines were verified through short tandem repeat analysis (Hsu and others 2012). Cell lines were maintained in RPMI-1640 (Life Technologies Cooperation, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin–streptomycin.

Human IFN- α 2a, was a gift of Hoffmann LaRoche (Nutley, NJ), and IFN- γ purchased from Intermune Pharmaceutical, Inc. (Brisbane, CA). Human elutriated monocytes (>85% purity) were obtained from the NIH Clinical Research Center Department of Transfusion using the Gambro Elutra Method. Monocytes were cryopreserved at -80°C in FBS supplemented with 10% DMSO until use. Carboplatin, paclitaxel, and crystal violet solution were obtained from Sigma-Aldrich (St. Louis, MO).

Assays

Carboplatin and paclitaxel EC50s. Cell lines were seeded at 10^4 cells/well in a 96-well plate in 100 μL of media and incubated until adherence (4 h, 37°C , 5% CO_2). Serial dilutions of carboplatin (dissolved in water) and paclitaxel (dissolved in 100% methanol) were added and incubated for 3 days. Media was then removed and cell viability was determined by crystal violet dye uptake in fixed, live cells. Dye absorbance was read at 570 nm using a spectrophotometer. EC50 is defined as the half maximal effective concentration.

IFN, carboplatin, paclitaxel, and monocyte treatments. Cell lines were seeded at 10^4 cells/well in a 96-well plate in 100 μL of media and incubated until adherence (4 h, 37°C , 5% CO_2). Human elutriated monocytes, 10^5 cells/well, were added to adhered ovarian serous carcinomas in 100 μL of media. IFNs, carboplatin, and paclitaxel were added and the plate was incubated for 3 days. Media was then removed and cell viability was determined by crystal violet dye uptake in fixed, live cells. Dye absorbance was read at 570 nm using a spectrophotometer. For the 144 h experiments cells were seeded at time 0, the assay was terminated by the addition of crystal violet to the monolayer at 144 h.

Statistical analysis

Drug treatments were analyzed for significance through Student's *t*-test and 2-way analysis of variance with Bonferroni post test analysis. In all graphs data are mean $\pm$ standard error of the mean with *P* values ≤ 0.05 (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001). All data were obtained from at least 3 different experiments and at least 3 different monocyte donors.

Synergism, additive effects, and antagonism for multiple drug treatments were quantified by combination index (CI) values obtained from the CompuSyn software (Chou 2006).

Results

Sensitivity of ovarian cancer cell lines to IFNs with or without monocytes

It has been reported that 85% of ovarian adenocarcinomas are of serous origin. To study the role of IFN primed monocyte killing of serous cancer cell lines, 6 adherent lines were treated with IFN- α 2a, IFN- γ , or IFN- α 2a and IFN- γ in combination (Fig. 1). Cell lines cultured with monocytes were also tested with IFN- α 2a, IFN- γ , or IFN- α 2a and IFN- γ at a fixed effector to target ratio of 10:1. A 4 log₁₀ range of IFN concentrations (0.2–200 ng/mL) was chosen to examine dose-related effects (Fig. 1).

The antiproliferative and cytotoxic effects of IFN- α 2a with and without monocytes on serous ovarian tumors were examined (Fig. 1A). All cell lines showed some cytotoxicity when treated with IFN- α 2a or IFN- α 2a and monocytes. However, there was variable sensitivity among cell lines. CaOV3 and OVCAR-3 were highly sensitive to both IFN- α 2a alone and in the presence of monocytes. OVCAR-5 and SKOV-3 showed moderate sensitivity, having $\sim 50\%$ viability at the highest concentration of IFN- α 2a (200 ng/mL). The addition of monocytes significantly enhanced the killing efficiency of CaOV3, OVCAR-3, OVCAR-5, and SKOV-3 at multiple concentrations. Conversely, OVCAR-4 and OVCAR-8 were least sensitive to IFN- α 2a at the highest concentration with or without monocytes.

Treatment of the cell lines with IFN- γ resulted in a similar profile as the IFN- α 2a treatments (Fig. 1B). Both CaOV3 and OVCAR-3 were not as sensitive to the cytotoxic properties of IFN- γ when compared with IFN- α 2a treatment. However, the addition of monocytes increased cytotoxicity in both cell lines, even at low concentrations (0.2 and 2 ng/mL). OVCAR-5 cells treated with IFN- γ showed a dose-dependent cytotoxicity, with increased cytotoxicity in the presence of monocytes. Although SKOV-3 showed a dose-dependent increase in cytotoxicity to IFN- γ , there was no enhancement of cell death with the addition of monocytes to the cultures. OVCAR-4 had low cytotoxicity to both IFN- γ and monocytes and did not drop below 50% viability at the highest concentrations tested (200 ng/mL). The OVCAR-8 cell line was generally refractory to all conditions tested.

Our previous work has shown that there is increased killing of tumor cells when monocytes are stimulated with both IFN- α 2a and IFN- γ . CaOV3 and OVCAR-3 cell lines were extremely sensitive to the cytotoxic effects of combination IFN treatment, and there was 99% cytotoxicity at the lowest concentration tested (0.2 ng/mL) (Fig. 1C). Treatment of OVCAR-5 cells with both IFNs increased cytotoxicity when compared with single IFN treatments. There

FIG. 1. Ovarian cell line sensitivity to IFNs and monocytes. Cell lines were treated with IFNs alone (black bar) and with IFNs and monocytes (white bar) for 72 h as in the Materials and Methods section. Tumoricidal properties of IFNs with and without monocytes were measured as percent viability of control. Concentration of IFNs is labeled on the X-axis in ng/mL. Cell lines were treated with and without monocytes at a fixed ratio of 10 monocytes to 1 ovarian cancer cell in combination with (A) IFN- α 2a, (B) IFN- γ , (C) IFN- α 2a and IFN- γ . X-axis concentrations indicate the amount of each IFN used alone and when combined. Data were collected from at least 3 separate experiments, each with different donor monocytes (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). IFN, interferon.

was increased cytotoxicity with the dual treatment of IFNs and monocytes resulting in increased killing at all concentrations. Although the SKOV-3 cells were more sensitive to the cytotoxicity of dual IFN treatment, there was not a significant increase in killing upon the addition of monocytes at any of the concentrations. Similar to SKOV-3, OVCAR-4 showed higher cytotoxicity with the IFNs combined, but only increased killing with monocytes at the lowest IFN concentration tested (0.2 ng/mL). Despite being mostly refractory to single IFN treatments, OVCAR-8 cells showed increased sensitivity with the dual IFN treatment. Additionally, there was increased cytotoxicity of IFNs with monocytes at 2, 20, and 200 ng/mL. Further, the combination of monocytes with IFNs at the highest concentration (200 ng/mL) resulted in greater than 50% cell death.

The treatment of a cell line with more than 1 component can result in synergism, addition, or antagonism (Chou 2006). To determine which type of effect we were observ-

ing, we calculated the CI for coculture of ovarian cancer cells with increasing concentrations of IFNs with and without monocytes (Table 1). The calculation of the CI showed that there was synergism between the IFNs, and in most cases the addition of monocytes slightly increased the synergism. OVCAR-3 cell lines were not included in the analysis due to complete killing by IFNs and monocytes at all concentrations.

Effect of chemotherapeutic agents paclitaxel and carboplatin with IFNs primed monocytes on serous ovarian cancer cell lines

Standard of care for patients with stage 3 or higher ovarian cancer includes the intraperitoneal administration of the chemotherapeutics paclitaxel and carboplatin. Based upon published reports, we tested the cell lines for 50% cytotoxicity within a physiologic relevant range (Fig. 1 and

TABLE 1. COMBINATION INDEX VALUES FOR INTERFERON COMBINATION TREATMENT

	<i>IFN-α2a + IFN-γ</i>		<i>IFN-α2a + IFN-γ</i>		<i>IFN-α2a + IFN-γ</i>		<i>IFN-α2a + IFN-γ</i>	
	0.2 ng/mL		2 ng/mL		20 ng/mL		200 ng/mL	
<i>Monocytes</i>	–	+	–	+	–	+	–	+
<i>Cell lines</i>	<i>CI values</i>							
CaOV3	0.31 [S]	0.06 [S]	0.06 [S]	0.29 [S]	0.01 [S]	0.10 [S]	1.1E-3 [S]	1.03 [Ad]
OVCAR-5	0.42 [S]	0.29 [S]	0.06 [S]	0.04 [S]	0.01 [S]	0.01 [S]	5.79E-05 [S]	8.00E-05 [S]
SKOV-3	0.14 [S]	0.30 [S]	0.08 [S]	0.08 [S]	0.47 [S]	0.13 [S]	2.10 [A]	0.39 [S]
OVCAR-4	0.24 [S]	0.12 [S]	0.02 [S]	0.01 [S]	0.01 [S]	0.05 [S]	0.03 [S]	0.41 [S]
OVCAR-8	N/A	0.69 [S]	N/A	0.02 [S]	N/A	0.08 [S]	0.37 [S]	0.48 [S]

IFN- α 2a and IFN- γ combination treatments of ovarian carcinomas were analyzed for synergism [S], additive effects [Ad], and antagonism [A]. The combination index (CI) values CI < 1, CI = 1, CI > 1 signify synergism, an additive effect, and antagonism, respectively.

IFN, interferon; N/A, not available.

Table 2). We then tested whether paclitaxel and carboplatin treatments at concentrations that resulted in greater than 50% cell death, in the absence of monocytes, were potentiated by the addition of IFNs (Fig. 2A). Paclitaxel and carboplatin treatments resulted in greater than 90% cell death in all cell lines. When the IFNs, paclitaxel, and carboplatin were cultured with cells together, we observed an additive effect resulting in nearly 100% cell death.

At a 1:5 dilution of the EC50 in the absence of monocytes, CaOV3, OVCAR-3, OVCAR-4, OVCAR-5, and OVCAR-8 cells had greater than 50% viability in all the conditions tested (Fig. 3A). SKOV-3 lines were 50% viable when treated with IFNs or IFNs and chemotherapeutics. However, this cytotoxicity was not observed in the cells treated with chemotherapeutics only, indicating a possible synergistic role between carboplatin, paclitaxel, and IFNs at the lower concentrations.

Similar to the results in Fig. 1, there was a range of sensitivity to the cytotoxic effects of monocytes and IFNs, with SKOV-3, OVCAR-4, and OVCAR-8 being the least sensitive (Fig. 2B). The combination of chemotherapeutics and IFNs at EC50 concentrations with monocytes resulted in cytotoxicity greater than 99%, similar to the treatment without monocytes (Fig. 2B and Table 2).

At concentrations of approximately the EC50, the chemotherapeutics and IFNs showed similar cytotoxic effects (Fig. 3A). The combination of the chemotherapeutics with the IFNs

resulted in significantly greater killing compared with the treatments alone. Killing was significantly enhanced when IFNs, chemotherapeutics at the EC50, and monocytes were combined (Fig. 3B). OVCAR-5, SKOV-3, OVCAR-4, and OVCAR-8 cell lines had variable sensitivity to IFNs, with or without monocytes, with the chemotherapeutics paclitaxel and carboplatin (range 60% viability to 20% viability) (Fig. 3B). However, there was a strong additive response in the triple treatment group of IFNs, chemotherapeutics, and monocytes. OVCAR-4 cells were the most refractory to treatment, but still had less than 50% viability. In the dual treatment groups of IFNs and chemotherapeutics, the OVCAR-5, SKOV-3, and OVCAR-8 cell lines were less than 10% viable (Fig. 3B).

At a 1:5 dilution of the EC50 there was no increased killing between IFN treated cells and cotreatment of chemotherapeutics and IFN treated cells (Fig. 4A). At a 1:5 dilution of the EC50 the additive effects of the chemotherapeutics, IFNs, and monocytes was lost, similar to the results in Fig. 2C (Fig. 4B). However, monocytes and IFNs were able to kill more than 50% of the target cells in the OVCAR-5 and SKOV-3 lines, with additional killing by the chemotherapeutics in SKOV-3 and OVCAR-8 cell lines.

The OVCAR-8 cell line was mostly refractory to treatment with IFNs and monocytes. We hypothesized that this observation may be due to altered kinetics of target cell death. To address this question, we incubated OVCAR8 cells treated with IFNs with and without monocytes for 72

TABLE 2. DETERMINATION OF EC50 VALUES FOR INTERFERONS AND MONOCYTES

	<i>IFN-α2a (ng/mL)</i>		<i>IFN-γ (ng/mL)</i>		<i>IFN-α2a + IFN-γ (ng/mL)</i>		<i>Carboplatin (μM)</i>	<i>Paclitaxel (nM)</i>
	–	+	–	+	–	+	–	–
<i>Monocytes</i>	–	+	–	+	–	+	–	–
<i>Cell lines</i>	<i>EC50s</i>							
CaOV3	1.9	<0.2	0.8	<0.2	0.2	<0.2	86.1	11.2
OVCAR-3	0.3	<0.2	16	<0.2	<0.2	<0.2	179.5	5.9
OVCAR-5	35.3	9.3	3.9	1.1	1	0.4	75.7	13.5
SKOV-3	>200	19.2	1.1	<0.2	0.4	<0.2	504	12.2
OVCAR-4	>200	>200	>200	11.8	2.2	1	290.6	11.3
OVCAR-8	>200	>200	>200	>200	91.9	7.6	699.2	18.3

Ovarian cancer lines were treated with IFN- α 2a, IFN- γ , IFN- α 2a + IFN- γ , carboplatin, and paclitaxel for 72 h. The half maximal effective concentrations (EC50) were determined through a nonlinear regression curve fit, sigmoidal dose-response model. EC50s were determined by interpolation from the respective curve fit at a confidence interval of 95%. Greater or less than symbols indicate that drug concentrations were out of clinical range.

FIG. 2. IFNs and chemotherapeutics synergize to kill ovarian cancer cell lines at 5-fold EC50 drug and IFN concentrations. (A) Cell lines were treated with IFNs (black bar), carboplatin and paclitaxel (white bar), or combination of both (gray bar) for 72 h. (B) Cell lines were treated with IFNs and monocytes at a fixed ratio of 10 monocytes to 1 ovarian cancer cell (checkered bar), carboplatin, paclitaxel, and monocytes (vertical bar), or combination of both with monocytes (horizontal bar) for 72 h. (C) Concentrations of drug treatments for each cell line. IFN- α 2a and IFN- γ X-axis concentrations indicate the amount of each IFN used alone and when combined. Cell viability was measured as percent of control. Concentrations of the IFNs, carboplatin, and paclitaxel are noted in the table below the graph. Cell lines were treated using concentrations based on serial dilutions of the EC50s. Data were collected from at least 3 separate experiments (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

and 144 h (Fig. 5). The data from the 72 h time point were consistent with our previous observations (Fig. 1). However, extension of the assay to 144 h showed greater than 95% killing at 200 ng/mL and greater than 85% killing at 20 ng/mL when cells were treated with IFNs alone or in combination with monocytes.

Discussion

In this study, we report that treatment of multiple ovarian cancer cell lines of serous origin are killed when cocultured with IFN- α 2a, IFN- γ , and monocytes. We found that there was a wide range (4 logs₁₀) in sensitivity among the cell

FIG. 3. IFNs and chemotherapeutics synergize to kill ovarian cancer cell lines at EC50 drug and IFN concentrations. (A) Cell lines were treated with IFNs (black bar), carboplatin and paclitaxel (white bar), or combination of both (gray bar) for 72 h. (B) Cell lines were treated with IFNs and monocytes at a fixed ratio of 10 monocytes to 1 ovarian cancer cell (checkered bar), carboplatin, paclitaxel, and monocytes (vertical bar), or combination of both with monocytes (horizontal bar) for 72 h. (C) Concentrations of drug treatments for each cell line. IFN- α 2a and IFN- γ X-axis concentrations indicate the amount of each IFN used alone and when combined. Cell viability was measured as percent of control. Concentrations of the IFNs, carboplatin, and paclitaxel are noted in the table below the graph. Cell lines were treated using concentrations based on serial dilutions of EC50s. Data were collected from at least 3 separate experiments (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

FIG. 4. IFNs and chemotherapeutics synergize to kill ovarian cancer cell lines at 1/5-fold EC50 drug and IFN concentrations. (A) Cell lines were treated with IFNs (black bar), carboplatin and paclitaxel (white bar), or combination of both (gray bar) for 72 h. (B) Cell lines were treated with IFNs and monocytes at a fixed ratio of 10 monocytes to 1 ovarian cancer cell (checkered bar), carboplatin, paclitaxel, and monocytes (vertical bar), or combination of both with monocytes (horizontal bar) for 72 h. (C) Concentrations of drug treatments for each cell line. IFN- α 2a and IFN- γ X-axis concentrations indicate the amount of each IFN used alone and when combined. Cell viability was measured as percent of control. Concentrations of the IFNs, carboplatin, and paclitaxel are noted in the table below the graph. Cell lines were treated using concentrations based on serial dilutions of EC50s. Data were collected from at least 3 separate experiments (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

lines tested. Although all cell lines were sensitive to the actions of IFN- α 2a and IFN- γ individually, there was a pronounced synergistic effect when the cell lines were treated with both IFNs together. This synergy was further enhanced in the presence of monocytes. Importantly, we also showed that the IFNs and monocytes did not interfere with the cytotoxic properties of the current therapeutic treatments for ovarian cancer. In fact synergistic killing by the IFNs, chemotherapeutics, and monocytes was observed.

Previous studies of IFN primed monocyte killing of ovarian cancer cells have primarily focused on the OVCAR-3 cell line, which we found to be the most sensitive to both the IFNs alone and with monocytes (Griffith and others 1999; Baron and others 2011). The sensitivity of the OVCAR-3

cells is most likely due to the fact that the cells, when treated with IFN- α 2a, produce soluble TRAIL that acts in an auto-crine mechanism inducing apoptosis (Tsuno and others 2012). It is possible that the CaOV3 line is as sensitive as the OVCAR-3 line due to the same mechanism.

The OVCAR-5 and SKOV-3 lines showed intermediate sensitivity to the IFNs and monocytes. Both cell lines were less viable when treated with IFN- α 2a alone compared with IFN- γ alone, potentially reflecting a problem in the IFN- γ signaling pathway. The combination of the IFNs both with and without monocytes further decreased the percent viability, indicating a synergy between the IFNs and monocytes. Mathematical analysis and determination of synergistic killing showed that in almost all cell lines tested, the IFNs and

FIG. 5. Increased treatment time of OVCAR8 cells results in greater cell death. OVCAR-8 cells were treated with IFNs for 72 h (black bars) or 144 h (white bars), and cell viability was measured as percent of control. OVCAR-8 cells were also treated with IFNs and monocytes at a fixed ratio of 10 monocytes to 1 ovarian cancer cell for 72 h (dark grey bars) or 144 h (light grey bars) and cell viability was measured. IFN- α 2a and IFN- γ X-axis concentrations indicate the amount of each IFN used alone and when combined (** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$).

monocytes showed synergy rather than additive or antagonistic properties, supporting the potential of dual use of monocytes and IFNs for the treatment of patients with ovarian cancer.

Conversely, we found that the OVCAR-4 and OVCAR-8 lines were mostly resistant to IFNs plus monocytes, and that very high concentrations of IFNs were required to see greater than 50% killing. The more refractory nature of the OVCAR-4 and OVCAR-8 lines could be due to intrinsic factors, for instance, upregulation of antiapoptotic proteins such as inhibitors of apoptosis, decreased activation of the IFN pathways, and resistance to monocyte-mediated killing. It is important to note that while OVCAR-4 and OVCAR-8 lines were more resistant to the cytotoxicity of monocytes plus IFNs, their viability was still reduced below 50% when the monocytes were primed with high concentrations of IFNs.

These 3 sensitivity profiles—high (CaOV3, OVCAR-3), intermediate (OVCAR-5, SKOV-3), low (OVCAR-4, OVCAR-8)—most likely reflect the heterogeneous nature of ovarian cancer, with some patients responding better to therapies than other patients. One of the hallmarks of ovarian cancer is the upregulation of antiapoptotic proteins, and subsequent resistance to mediators of cell death. Further, many cancer cell lines are less responsive to the antiproliferative activities of IFNs. Our observations highlight the variation in kinetics of cell death: OVCAR-8 cell lines showed greater toxicity when the incubation time of IFNs with and without monocytes were extended to 144 h. Together, these observations support a wide range in sensitivity of the cell lines to the IFNs and monocytes. Further studies are needed to elucidate pathways contributing to the variability observed.

As reported, the cell lines were highly sensitive to the combination treatment of paclitaxel and carboplatin. Paclitaxel blocks tubulin formation, which results in cell cycle arrest during cellular division and subsequent cell death by apoptosis (Jordan and Wilson 2004). Carboplatin blocks DNA repair resulting in the activation of apoptosis (Reed and others 1987). All cell lines tested were sensitive to tumoricidal properties of the compounds, as shown in previous studies. The addition of IFNs in combination with the chemotherapeutics resulted in even greater killing of target cells at the highest concentrations tested than IFNs or chemotherapeutics alone.

Further studies are needed to determine if the IFNs are also inducing cell death in the cancer cells by the extrinsic pathway through autocrine secreted TRAIL, or if IFN signaling increases cell death through the intrinsic pathway in the presence of chemotherapeutics. There are many immunomodulatory effects seen in monocytes treated with IFNs. Included in the upregulation of antiviral genes, antiproliferative genes (such as TRAIL) are upregulated (Griffith and others 1999). Furthermore, IFNs induce the maturation of monocytes to macrophages, increasing their phagocytic capabilities (Delneste 2002).

Similar results to IFNs and chemotherapeutics were obtained with the addition of monocytes to the cultures. At slightly lower (1:5 dilution of the ED₅₀) concentrations we observed synergistic killing. This synergy suggests that the monocytes and IFNs are inducing cell death as well. The chemotherapeutics worked in a very narrow range when compared with the IFNs. Interestingly, the synergy in killing was dependent on the concentration of the chemotherapeu-

tics and not the IFNs, suggesting that there is a more narrow effective concentration range with chemotherapeutics than IFN treated monocytes.

Over the last 5 years there has been rapid progress in the use of immune cell therapies in the treatment of malignancies. All of these therapies have focused on the induction of a strong antitumor adaptive immune response. The cell-based therapies have employed dendritic cells loaded with tumor antigens, and rearranged cancer specific T Cell Receptor T cells to eliminate tumor cells (Kalos and June 2013; Palucka and Banchereau 2013). A highly immunosuppressive environment characterizes ovarian cancers, with the presence of T regulatory cells and myeloid derived suppressor cells (Kipps and others 2013). Despite these suppressive conditions, monocytes could provide another treatment modality for patients with metastatic ovarian cancer.

Priming monocytes *ex vivo* with IFNs and then introducing the monocytes into the peritoneal cavity could address the limitations of IFN specificity and cell migration to the metastases (Nakashima and others 2012). Our data support this strategy as a potentially valid approach, given that IFN treated monocytes do not interfere with the tumoricidal properties of the current therapies of paclitaxel and carboplatin. Further studies are needed to elucidate the mechanism of killing and the efficacy of the proposed treatment in the mouse model.

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Author Disclosure Statement

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