Title

Differential Effects of High-Dose Gamma Irradiation on the Production of Transforming Growth Factor-Beta in Fresh and Established Human Ovarian Cancer

Journal

Gynecologic Oncology, 61(3)

ISSN

0090-8258

Authors

Santin, Alessandro D
Hiserodt, John C
DiSaia, Philip J
et al.

Publication Date

1996-06-01

DOI

10.1006/gyno.1996.0164

GYNECOLOGIC ONCOLOGY **61,** 403–408 (1996)
ARTICLE NO. 0164

Differential Effects of High-Dose Gamma Irradiation on the Production of Transforming Growth Factor-Beta in Fresh and Established Human Ovarian Cancer[1]

ALESSANDRO D. SANTIN,*,†,§,[2] JOHN C. HISERODT,*,‡ PHILIP J. DISAIA,†
SERGIO PECORELLI,†,§ AND GALE A. GRANGER*,¶

**Department of Molecular Biology and Biochemistry, University of California, Irvine, Irvine, California 92717; Divisions of †Gynecologic Oncology and ‡Pathology, University of California Irvine Medical Center, Orange, California 92668; §Division of Gynecologic Oncology, University of Brescia, Brescia, Italy; and ¶Memorial Cancer Institute, Long Beach, California 90801*

Received October 20, 1995

Tumor cells from five freshly isolated ovarian tumors and four established human ovarian carcinoma cell lines were analyzed for the production of the immunoinhibitory cytokine transforming growth factor-beta (TGF-β) before and after exposure to gamma irradiation and/or the cytokines TNF-α plus IFN-γ. All fresh tumors secreted high levels of TGF-β when compared to the levels produced by the established ovarian carcinoma cell lines. TGF-β produced by fresh tumors was significantly reduced after high doses of gamma irradiation (10,000 cGy). In contrast with the established cell lines, irradiation significantly increased TGF-β secretion. Exposure of fresh tumor cells to cytokines followed by irradiation caused significant reduction of TGF-β released when compared to the amount released after exposure to cytokines only. However, in the established cell lines, cytokines followed by irradiation again significantly increased TGF-β production. These data indicate that high doses of irradiation in fresh ovarian tumors, unlike established ovarian carcinoma cell lines, can significantly reduce the local production of this potent immunoinhibitory cytokine. This effect could work to further amplify weak immunological responses within the tumor. In addition, these findings indicate major differences between fresh tumor samples and established cell lines and warn against the sole use of continuous cell lines as models for tumors growing *in vivo*.

INTRODUCTION

Ovarian carcinoma remains the fourth most frequent cause of cancer death in women in the United States and Europe. Because of the insidious onset and progression of this disease, about 75% of patients initially present with tumor disseminated throughout the peritoneal cavity. Although many patients with such disseminated tumor respond initially to standard combinations of surgical and cytotoxic therapy, nearly 90% will develop recurrence and inevitably succumb to their disease [1].

The poor prognosis of patients with advanced ovarian cancer may reflect an increased capacity of this tumor to evade detection and containment by host immune responses. Examples of mechanisms by which tumor cells can escape the immune response include (1) expression of tumor-associated cell-surface antigens of weak immunogenicity; (2) lack of expression or down-regulation of major histocompatibility molecules, costimulatory molecules, or adhesion molecules; (3) masking or shedding of tumor antigens; and (4) secretion of blocking factors that directly suppress inflammatory responses and specific immune cell functions [2, 3].

Because ovarian cancer remains confined to the peritoneal cavity even in advanced stages of the disease it has been suggested that the growth of this malignancy could be related to a local phenomenon of immunosuppression [4, 5]. Indeed, several studies have documented the local deficiency of antitumor immune effector mechanisms in the peritoneal cavity of patients with advanced stages of ovarian cancer [4, 5]. While some of these immunosuppressive factors have been identified as cytokine receptors shed into the ascitic fluid [3, 6], others have been identified as inhibitory cytokines [7, 8].

Among the many cytokines that have been shown to be produced by ovarian cancer cells [9] TGF-β[3] appears as the best characterized inhibitory cytokine. TGF-β is a multifunc-

[1] This work was supported in part by grants from Memorial Health Services of Long Beach Memorial Hospital (Long Beach, CA), the Schmidt Foundation of Irvine, California, and the Camillo Golgi Foundation, Brescia, Italy.

[2] To whom correspondence should be addressed at UCI Medical Center, Building 23, Room 314, University of California, Irvine, 101 The City Drive, Orange, CA 92668-3298. Fax: 714-456-5039.

[3] Abbreviations used: TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; TGF-β, transforming growth factor-beta; MHC, major histocompatibility complex; ICAM-1, intercellular adhesion molecule-1.

0090-8258/96 $18.00

tional protein endowed with a broad range of biological activities on cells of different lineages [for review, 10]. Secretion of TGF-β has been detected from normal human ovarian epithelium as well as from a wide variety of tumors [11–14]. The predominant effect of TGF-β on most normal cells *in vitro* is reported to be inhibition of proliferation [15]. Because of this effect, it has been proposed that the unrestrained proliferation of some cancers may, in part, be due to the loss of normal growth inhibitory pathways such as that caused by TGF-β [15]. However, it has recently been shown that TGF-β also markedly inhibits immune activity including T, B, and NK cell effector functions [16, 17]. These immune inhibitory effects could allow tumors secreting TGF-β to suppress and escape the immune response, thus contributing to tumor growth.

An exciting recent approach to cancer immunotherapy has been the use of genetically altered tumor cells (GATC) engineered to secrete immune-enhancing cytokines capable of augmenting and stimulating antitumor immune responses [for review, 18]. Transduction of cytokine genes into tumor cells allows these proteins to function in their most physiologic way (i.e., paracrine) and to reach high local concentrations within the tumor microenvironment at the immunization site. Moreover, the use of such GATC has been associated with the induction of specific antitumor immunity in a variety of tumor–cytokine combinations [for review, 18].

Recent studies investigating the immunizing effects of cytokine-transduced tumor cells have suggested that the immunizing potential of a particular GATC is due to not only the specific cytokine transduced into the tumor but also the inhibitory cytokine(s) intrinsically secreted by such tumors [19–22]. Such data support the concept that the balance between the quantity of immune-enhancing and suppressive cytokines produced within the tumor milieu can affect the ability of the immune system to respond to the tumor.

We have recently developed human ovarian carcinoma tumor vaccines secreting different cytokines to be used as vaccines for women with advanced ovarian cancer [23, 24]. We have proposed that such cytokine-secreting vaccines will be mixed with irradiated autologous tumor cells obtained at the time of surgical debulking. Because of this, we have directed our interest in evaluating the production of certain immune inhibitory cytokines, particularly TGF-β, in several fresh tumors and four established ovarian cell lines. The purposes of our study were (1) to quantify the production of TGF-β by these various ovarian tumor cells and (2) to evaluate the effects of high doses of gamma irradiation, TNF-α + IFN-γ exposure, and the combination of the two procedures on the secretion of TGF-β.

MATERIALS AND METHODS

Fresh tumors and tumor cell lines. Single-cell suspensions from five adenocarcinomas of the ovary of primary or metastatic origin obtained at the time of surgery and four human epithelial ovarian carcinoma cell lines (UCI-101, UCI-107, SKOV-3, and T-222) were used for this study. UCI-101 and UCI-107 were kindly provided by Dr. Alberto Manetta, University of California, Irvine (UCI). SKOV-3 was purchased from American Type Culture Collection (ATCC), while T-222 was kindly provided by Dr. Benjamin Bonavida, University of California, Los Angeles. All the established tumor cell lines were maintained at 37°C, 5% CO_2 in complete media (CM) containing RPMI 1640 (Gibco Life Technologies, Grand Island, NY), supplemented with 10% fetal bovine serum (FBS, Gemini Bioproducts, Calabasas, CA). All of the fresh tumors (OVA-2, OVA-4, OVA-5, OVA-7, and OVA-13) were serous papillary adenocarcinomas and were maintained at 37°C, 5% CO_2 in CM containing RPMI 1640 supplemented with 5% FBS. Briefly, single-cell suspensions were obtained by processing solid tumor samples under sterile conditions at room temperature. Viable tumor tissue was mechanically minced in RPMI 1640 to portions no larger than 1–3 mm^3 and washed twice with RPMI 1640. The portions of minced tumor were then placed into 250-ml trypsinizing flasks containing 30 ml of enzyme solution [0.14% Collagenase Type I (Sigma) and 0.01% DNAse (Sigma, 2000 kU/mg)] in RPMI 1640 and incubated on a magnetic stirring apparatus overnight at 4°C. Enzymatically dissociated tumor was then filtered through 150-μm nylon mesh to generate a single-cell suspension. The resultant cell suspension was then washed twice in RPMI 1640. The percentage of tumor cells was determined by cytokeratin expression using immunohistochemical techniques. In this regard, all the fresh tumor samples evaluated contained $>$99% tumor cells.

Analysis of human TGF-β secretion. Tumor cell were seeded at a density of 1×10^5 cells/ml in CM in tissue culture flasks (Corning) and after a 48-hr incubation at 37°C, supernatants were aspirated, rendered cell-free by centrifugation at 1500 rpm for 10 min, and stored at −20°C. TGF-β concentration was determined by ELISA, employing a commercially available kit (Research & Diagnostic Systems, Minneapolis, MN) utilizing TGF-β standards ranging from 31.2 to 2000 pg/ml. All samples were assayed in duplicate. Standard regression lines were generated by plotting $\log_{10}$ concentration vs $\log_{10}$ optical density, creating correlation coefficients greater than 0.98 in all cases. The maximal allowed sample duplicate error was 10%. Duplicates falling outside this error were reanalyzed.

IFN-γ and TNF-α preincubation. Cells from fresh tumors and established cell lines were cultured in CM containing a combination of IFN-γ and TNF-α (500 units/ml each) for 3 days, washed, and reseeded at a density of 1×10^5 cells/ml in CM for subsequent studies. Recombinant human IFN-γ (sp act 2.5×10^7 U/mg) was purchased from

TABLE 1

TGF-β Production in Fresh Ovarian Tumor Cells and Established Ovarian Carcinoma Cell Lines before and after High Doses of Gamma Irradiation

Tumor cells	TGF-β pg/ml untreated	TGF-β pg/ml 10,000 cGy
OVA-2	841	802
OVA-4	1793	981
OVA-5	741	37
OVA-7	636	144
OVA-13	1777	1497
UCI-101	419	865
UCI-107	416	515
SKOV-3	424	452
T-222	480	998

Note. TGF-β levels were determined by ELISA after culturing unirradiated (untreated) or irradiated (i.e., 10,000 cGy) tumor cells for 48 hr in tissue culture flasks. All samples were assayed in duplicate along with known standards as described under Materials and Methods. *P* value for unirradiated vs irradiated fresh tumor cells was <0.03. *P* value for unirradiated vs irradiated established tumor cells was <0.05. Data from one experiment are shown and are representative of two different experiments with similar results.

Genzyme Corp. (Cambridge, MA) and recombinant human TNF-α (sp act 1.0×10^7 U/mg) was obtained from Genentech, Inc. (San Francisco, CA).

IFN-γ and TNF-α preincubation followed by high-dose gamma irradiation. Tumor cells were irradiated with a total dose of 10,000 cGy using a Cs^{137} source at a rate of 200 cGy/min with or without prior preincubation with cytokines and then seeded at a density of 1×10^5 cells/ml in CM without cytokines. An aliquot of the cytokine-treated cells was also seeded in CM without additional cytokines to follow the natural course of TGF-β secretion but without the influence of irradiation. Media collected from flasks containing irradiated cells previously preincubated with cytokines and media collected from flasks containing irradiated control cells were collected after 48 hr for TGF-β evaluation.

Statistical analysis. Significance analysis was performed using a paired Student *t* test. Only *P* values <0.05 were considered significant.

RESULTS

Secretion of TGF-β by fresh tumors and established ovarian cell lines. Cell-free supernatants from freshly isolated tumors and established ovarian cell lines were collected and analyzed for the levels of TGF-β by ELISA. The results in Table 1 show that all freshly isolated tumors as well as the established cell lines secreted TGF-β with a range of secretion between 636 and 1793 pg/ml/10^5 cells/48 hr (mean 1158) and 415 to 480 pg/ml/10^5 cells/48 hr (mean 435), respectively. This difference in the levels of TGF-β production was shown to be significant ($P < 0.02$).

Secretion of TGF-β after high-dose gamma irradiation. TGF-β secretion was evaluated 48 hr after irradiation (10,000 cGy) and expressed as a normalized value as pg/ml/10^5 viable cells/48 hr and the results were compared to the level of cytokine secreted by the unirradiated control cells. In all fresh tumors, irradiation caused a significant decrease in the secretion of the TGF-β (Table 1) ($P < 0.03$). In contrast with the established cell lines, irradiation induced a significant ($P < 0.05$) increase in TGF-β secretion. Viability studies (trypan blue exclusion) showed that fresh tumor cells remained >95% viable 48 hr after irradiation while established cell lines showed a lower viability (range between 40 and 70%). Despite the differences in viability between established and fresh tumor samples 48 hr after irradiation, in all cases 10,000 cGy was able to kill all tumor cells in 14–21 days.

Effects of irradiation on cytokine preincubated tumor cells. TGF-β secretion was evaluated 48 hr after the combination of cytokine exposure and irradiation and normalized to pg/ml/10^5 viable cells/48 hr. Again, with the fresh tumors, a significant decrease in TGF-β production was noted in all the specimens when compared to the production of TGF-β after cytokine exposure only ($P < 0.04$). In contrast, for the established cell lines, cytokine exposure combined with irradiation consistently and significantly increased TGF-β production ($P < 0.02$) (Table 2).

DISCUSSION

The production of inhibitory cytokines by a wide variety of tumors has been suggested as one of the major mechanisms by which these cells can evade destruction by the immune system [9–12]. The fact that ovarian cancer remains confined to the peritoneal cavity, even at advanced stages of the disease, suggests that the growth of this malignancy could be related to a local phenomenon of immunosuppression [4, 5]. The local deficiency of antitumor immune effector mechanisms in the peritoneal cavity of patients with advanced stages of ovarian cancer [4, 5] as well as the detection of high levels of inhibitory cytokines [5, 6] in the ascites fluid of these patients supports this hypothesis.

TGF-β is a multifunctional cytokine with predominantly suppressive effects on immune system function [for review, 10]. Tumors that produce TGF-β secrete active TGF-β within the local environment [16] and it is therefore likely that this cytokine could inhibit the effector functions of T, B, and NK cells at the tumor site [17]. In this regard, it has been shown that TGF-β is effective in blocking the differentiation and/or the proliferation of naive CTLs and markedly inhibits the cytotoxic activity of NK effector cells [17].

TABLE 2

TGF-β Production in Fresh Ovarian Tumor Cells and Established Ovarian Carcinoma Cell Lines after Exposure to TNF-α + IFN-γ and the Combination of 10,000 cGy Plus TNF-α + IFN-γ

Tumor cells	TGF-β pg/ml (TNF-α + IFN-γ)	TGF-β pg/ml (TNF-α + IFN-γ) + 10,000 cGy
OVA-2	1312	1189
OVA-4	1123	834
OVA-5	147	25
OVA-7	220	216
OVA-13	1989	1883
UCI-101	665	2067
UCI-107	578	874
SKOV-3	687	1028
T-222	630	1714

Note. TGF-β levels were determined by ELISA from supernatants collected from tumor cells treated with TNF-α + IFN-γ or with TNF-α + IFN-γ followed by irradiation (i.e., 10,000 cGy). All samples were assayed in duplicate along with known standards as described under Materials and Methods. *P* value for fresh tumor cells treated with TNF-α + IFN-γ vs those treated with TNF-α + IFN-γ and irradiation was <0.04. *P* value for established tumor cells treated with TNF-α + IFN-γ vs those treated with TNF-α + IFN-γ plus irradiation was <0.02. Data from one experiment are shown and are representative of two different studies with similar results.

The importance of TGF-β secretion in experimental animal models has been underscored by recent experiments of cytokine gene transduction that have found that the production of inhibitory cytokines from the transduced tumors can significantly affect the outcome of their interaction with host immune system cells [19–22]. For example, Torre-Armione *et al.* have demonstrated that a highly immunogenic murine tumor transduced to secrete TGF-β *in vitro* escapes immune recognition *in vivo* [19]. In addition, Fakharay *et al.* have shown that glioma cells transduced with TGF-β_2 antisense cDNA are capable of eliciting systemic immunity and are rejected when administered intracranially [20]. Dorigo *et al.* have also found that TGF-β can dramatically inhibit the effects of IL-2 gene therapy with TGF-β-producing tumors [21]. The failure to induce immunity against TGF-β-secreting tumors is in agreement with our unpublished data on a rat glioblastoma tumor (T9) genetically engineered to secrete IL-2 which was also shown to be a high secretor of TGF-β. In contrast, McAdam *et al.* have been able to reverse the *in vivo* inhibitory effects of TGF-β by immunizing animals with tumor cells engineered to secrete high levels of IL-2 [22]. Thus, it appears that balances in the quantity of enhancing or inhibitory cytokines within the tumor milieu may provide a possible explanation for the different outcomes reported in these studies.

Our previous studies evaluated the effects of TNF-α + IFN-γ and high-dose gamma irradiation on surface antigen expression of fresh ovarian tumors. We have shown that cytokines markedly upregulate surface antigen expression and that irradiation causes persistent expression of this upregulated state (Santin *et al.*, submitted). In this regard, several studies have indicated the importance of increased expression of surface antigens and immune recognition [25, 26]. On the basis of these observations and the potential use of GATC in phase I immunotherapy trials in advanced ovarian cancer patients we decided to evaluate the effects of these procedures on the secretion of this commonly detected and powerful immune inhibitory cytokine secreted by ovarian tumor cells.

Our data confirm that TGF-β is secreted by fresh human ovarian tumors and established cell lines. It is important to note that the level of TGF-β secreted by the fresh tumors was significantly higher than that secreted by the established cell lines ($P < 0.02$). In our study we have confirmed the purity of the tumor cells in fresh tumor specimens by differential counts of Giemsa-stained cytospin slides as well as by cytokeratin expression using immunohistochemical techniques. In this regard, our fresh tumor samples contained $>99\%$ tumor cells.

The effects of gamma irradiation on TGF-β secretion indicated that lethal doses of irradiation were able to significantly decrease the production of TGF-β in all fresh tumors tested. In contrast, however, all the established cell lines increased TGF-β secretion after lethal irradiation. One possible explanation for these differences may be due to the differential capability of the fresh tumors and established cell lines to respond with reparative processes to the lethal damages caused by 10,000 cGy. Indeed, the much slower growth rate of the fresh tumors could in part explain their higher viability 48 hr after irradiation when compared to the established cell lines. In addition, in the established cell lines, the higher cellular metabolic activity could also explain the higher TGF-β secretion following irradiation with an increase in mRNA expression in a way similar to that described for other cellular proteins after lethal irradiation [27, 28].

While the data are not shown, exposure of tumor cells to IFN-γ plus TNF-α caused an unpredictable response in the fresh tumors. Indeed, some tumors showed an increase in TGF-β secretion after cytokine exposure while others showed a decrease in its production. In contrast, in all four established cell lines, cytokine preincubation induced a significant increase in TGF-β secretion ($P < 0.006$) (data not shown).

When we evaluated the combined effects of cytokines and irradiation on TGF-β secretion, again we found an opposite effect of irradiation on the fresh tumor samples and established cell lines. Indeed, in all fresh tumors a significant decrease in TGF-β production ($P < 0.04$) after the combination of the two procedures was always noted when compared to the levels of TGF-β secretion after exposure to cytokines

only. In contrast, in the established cell lines, the combined procedures induced an increase in TGF-β secretion when compared to cytokine exposure alone ($P < 0.02$).

Taken together, these data demonstrate two important new findings. First, high-dose irradiation of fresh ovarian tumors can significantly reduce the local production of TGF-β, a powerful immunoinhibitory cytokine. The consequences of this effect, by itself, could favor weak local immune reactions within the tumor microenvironment. In addition, we have also reported that irradiation significantly and persistently increases surface expression of important immune recognition antigens, including MHC proteins and costimulatory molecules such as ICAM-1 (Santin *et al.*, submitted). These effects in combination could work to further amplify immunological responses within the tumor. Second, our data indicate that profound differences exist between freshly isolated tumor cells and established ovarian carcinoma cell lines. In particular, we found that while irradiation reduced the production of TGF-β by fresh tumor cells, it enhanced TGF-β production by the established cell lines. This important difference should be regarded as a potential warning to the sole use of continuous cell lines as models for tumors growing *in vivo*.

REFERENCES

1. DiSaia, P. J., and Creasman, W. T. Epithelial ovarian cancer, in *Clinical Gynecologic Oncology* (P. J. DiSaia and W. T. Creasman, Eds.), Mosby–Year Book, St. Louis, MO, pp. 333–425 (1993).
2. Moore, M. Immunity to tumors, in *Immunology* (I. M. Roitt, J. Brostoff, and D. K. Male, Eds.), Gower Medical Publishing, London, pp. 18.1–18.16 (1985).
3. Cappuccini, F., Yamamoto, R. S., DiSaia, P. J., Grosen, E. A., Gatanaga, M., Lucci, J. A., Ininns, E. K., Gatanaga, T., and Granger, G. A. Identification of tumor necrosis factor and lymphotoxin bloking factor(s) in the ascites of patients with advanced and recurrent ovarian cancer, *Lymphokine Cytokine Res.* **10,** 225–231 (1991).
4. Badger, A., Oh, S., and Moolten, F. Differential effects of an immunosuppressive fraction from ascites fluid of patients with ovarian cancer on spontaneous and antibody dependent cytotoxicity, *Cancer Res.* **41,** 1133–1139 (1981).
5. Berek, J. S., Bast, R. C., Lichtensteint, A., Hacker, N. F., Spina, C. A., Lagasse, L. D., Knapp, R. C., and Zighelboim, J. Lymphocyte cytotoxicity in the peritoneal cavity and blood of patients with ovarian cancer, *Obstet. Gynecol.* **64,** 708–714 (1984).
6. Granger, G., Gatanaga, T., Burger, R., Grosen, E., and DiSaia, P. TNF, LT, and IL-1 natural inhibitors (soluble receptors and receptor antagonists) in women with ovarian cancer, in *Ovarian Cancer 3* (F. Sharp, W. P. Mason, T. Blackett, and J. Berek, Eds.), Chapman & Hall, London, pp. 115–119 (1995).
7. Gotlieb, W. H., Abrams, J. S., Watson, J. M., Velu, T. J., Berek, J. S., and Martinez-Maza, O. Presence of IL-10 in the ascites of patients with ovarian and other intra-abdominal cancers, *Cytokine* **4,** 385–390 (1992).
8. Hirte, H., and Clark, D. A. Generation of lymphokine-activated killer cells in human ovarian carcinoma ascitic fluid: Identification of transforming growth factor β as a suppressive factor, *Cancer Immunol. Immunother.* **32,** 296–302 (1991).
9. Berek, J. S., and Martinez-Maza, O. Molecular and biologic factors in the pathogenesis of ovarian cancer, *J. Reprod. Med.* **39,** 241–248 (1994).
10. Massague, J. The transforming growth factor-β family, *Annu. Rev. Cell Biol.* **6,** 597–624 (1990).
11. Berchuck, A., Rodriguez, G., Olt, G., Whitaker, R., Boente, M. P., Arrick, B. A., Clarke-Pearson, D. L., and Bast, R. C. Regulation of growth of normal ovarian epithelial cells and ovarian cancer cell lines by transforming growth factor-β, *Am. J. Obstet. Gyn.* **166,** 676–684 (1992).
12. De Martin, R., Haendler, B., Hofer-Warbinex, Gaugitsch, H., Wrann, M., Schlusenere, H., Seifert, J. M., Bodmer, S., Fontana, A., and Hofer, E. Complementary DNA for human glioblastoma-derived T cell suppressor factor β, a novel member of the transforming growth factor-β family, *EMBO J.* **6,** 3673–3677 (1987).
13. Ikeda, T., Lioubin, M. N., and Marquardt, H. Human transforming growth factor type β_2: Production by a prostatic adenocarcinoma cell line, purification and initial characterization, *Biochemistry* **26,** 2406–2410 (1987).
14. Derynck, R., Jarret, J. A., Chen, E. Y., Eaton, D. H., Bell, J. R., Assoian, R. K., Roberts, A. B., Sporn, M. B., and Goeddel, D. V. Human transforming growth factor-β complementary DNA sequence and expression in normal and transformed cells, *Nature* **316,** 701–715 (1985).
15. Wakefield, L. M., and Sporn, M. B. Suppression of carcinogenesis: A role for TGF-β and related molecules in prevention of cancer. in *Tumor Suppressor Genes* (G. Klein, Ed.), Dekker, New York, pp. 217–243 (1990).
16. Tada, T., Ohzeki, S., Utsumi, K., Takiuchi, H., Muramatsu, M., Li, X-F., Shimizu, J., Fujiwara, H., and Hamaoka, T. Transforming growth factor-β induced inhibition of T cell function, *J. Immunol.* **146,** 1077–1082 (1991).
17. Gray, D., Hirokawa, M., and Horwitz, D. A. The role of transforming growth factor β in the generation of suppression: An interaction between $CD8^+$ T and NK cells, *J. Exp. Med.* **180,** 1937–1942 (1994).
18. Pardoll, D. M. Paracrine cytokine adjuvants in cancer immunotherapy, *Annu. Rev. Immunol.* **13,** 399–415 (1995).
19. Torre-Armione, G., Beuchamp, R. D., Koeppen, H., Park, B. H., Schreiber, H., Moses, H. L., and Rowley, D. A. A highly immunogenic tumor transfected with a murine transforming growth factor type β_1 cDNA escapes immune surveillance, *Proc. Natl. Acad. Sci. USA* **87,** 1486–1490 (1990).
20. Fakharay, H., Dorigo, O., Shawler, Lin, H., Mischel, P., Black, K. L., Roiston, I., and Sobol, R. E. Immunization with TGF-β_2 antisense gene modified tumor cells eradicates intracranial gliomas, *Proc. Am. Assoc. Cancer Res.* (1995) [abstract].
21. Dorigo, O., Shawler, D. L., Roiston, I., Sobol, R. E., and Fakhray, H. Transforming growth factor beta (TGF-β) inhibits effect of IL-2 gene therapy in TGF-β producing tumors, *Proc. Am. Assoc. Cancer Res.* (1995) [abstract].
22. McAdam, A. J., Felcher, A., Woods, M. L., Pulaski, B. A., Hutter, E. K., Frelinger, J. G., and Lord, E. M. Transfection of transforming growth factor-β producing tumor EMT6 with interleukin-2 elicits tumor rejection and tumor reactive cytotoxic T-lymphocytes, *J. Immunother.* **15,** 155–164 (1994).
23. Santin, A. D., Ioli, G. R., Hiserodt, J. C., Rose, G. S., Graf, M. R., Tocco, L. M., Lander, J. K., Eck, L. M., DiSaia, P. J., Pecorelli, S., and Granger, G. A. Development and characterization of an IL-4 secreting human ovarian carcinoma cell line, *Gynecol. Oncol.* **58,** 230–239 (1995).

24. Santin, A. D., Ioli, G. R., Hiserodt, J. C., Manetta, A., DiSaia, P. J., Pecorelli, S., and Granger, G. A. Development and characterization of an IL-2 transduced human ovarian carcinoma tumor vaccine not expressing major histocompatibility complex molecules, *Am. J. Obstet. Gynecol.* **174,** 633–640 (1996).
25. Vanky, F., Stuber, G., Rostein, S., and Klein, E. Auto-tumor recognition following in vitro induction of MHC antigen expression on solid human tumors: Stimulation of lymphocytes and generation of cytotoxicity against the original MHC-antigen negative tumor cells, *Cancer Immunol. Immunother.* **28,** 17–21 (1989).
26. Anichini, A., Mortarini, R., Alberti, S., Mantovani, A., and Parmiani, G. T-cell-receptor engagment and tumor ICAM-1 up-regulation are required to by-pass low susceptibility of melanoma cells to autologous CTL-mediated lysis, *Int. J. Cancer* **53,** 994–1001 (1993).
27. Hareyama, M., Imai, K., Kubo, K., Takahashi, H., Koshiba, H., Hinoda, Y., Shidou, M., Oouchi, A., Yachi, A., and Kazuo, M. Effect of radiation on the expression of carcinoembryonic antigen of human gastric adenocarcinoma cells, *Cancer* **67,** 2269–2274 (1991).
28. Hauser, S. H., Calorini, L., Wazer, D. E., and Gattoni-Celli, S. Radiation-enhanced expression of major histocompatibility complex class I antigen H-2D^{b} in B16 melanoma cells, *Cancer Res.* **53,** 1952–1955 (1993).