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Development and *in vitro* characterization of a GM-CSF secreting human ovarian carcinoma tumor vaccine

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Abstract. Santin AD, Ioli GR, Hiserodt JC, Rose GS, Graf MR, Lander JK, DiSaia PJ, Pecorelli S, Granger GA. Development and *in vitro* characterization of a GM-CSF secreting human ovarian carcinoma tumor vaccine. *Int J Gynecol Cancer* 1995; **5**: 401–410.

A human ovarian carcinoma cell line (UCI-107) was genetically engineered to secrete the cytokine granulocyte–macrophage colony stimulating factor (GM-CSF), by retroviral medicated gene transduction. This line was transduced with the LXSN retroviral vector containing the human GM-CSF gene and the neomycin resistance selection marker. Numerous GM-CSF secreting clones were randomly isolated and one clone, termed UCI-107M GM-CSF-MPS, extensively characterized. This clone was shown to constitutively secrete high levels of GM-CSF (ie 420–585 pg ml^{-1} 10^5 $cells^{-1}$ 48 h^{-1}) for over 35 passages and 6 months of study. Like the parental cell line UCI-107, UCI-107M GM-CSF-MPS cells expressed MHC class I and Her2/Neu surface antigens but did not express detectable MHC class II, ICAM-1 or CA-125. No change in the expression of these surface proteins was noted between the parental cells and this GM-CSF secreting clone. The morphology of UCI-107M GM-CSF-MPS did not differ from that of the parental or LXSN vector control cells; however, parental cells had a slightly faster growth rate than the transductants. UCI-107M GM-CSF-MPS was sensitive to gamma irradiation, since as little as 2500 rads killed the cells within 10 days of irradiation. However, even after higher doses of irradiation (ie 10 000 rads), GM-CSF secretion continued *in vitro* until about day 8. Interestingly, irradiation induced up-regulation of the surface antigens previously expressed, and they remained up-regulated for as long as the cells remained viable. The potential use of these GM-CSF secreting ovarian carcinoma cells as vaccines for women with advanced ovarian cancer will be discussed.

KEYWORDS: cytokine, GM-CSF, immunotherapy, ovarian cancer, tumor vaccine.

Ovarian carcinoma is the fourth most frequent cause of cancer death in women in the USA. Approximately 1 in 70 will develop ovarian cancer in their lifetime and only about 20% will be alive 5 years after the diagnosis of advanced ovarian cancer[1]. Thus, despite the introduction of new drug-based chemotherapy, the outcome for ovarian cancer patients is still poor and therefore we need to offer trials with experimental modalities to patients with this disease.

Although immunotherapy using autologous cancer

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cells as vaccines has been explored as a novel treatment, only in a few patients in a restricted group of malignancies has this resulted in a radical change in prognosis. However, recent studies employing tumor cells genetically altered to secrete cytokines (GATC), have provided a new enthusiasm for strategies to improve the efficacy of such vaccinations[2–25]. Several general phenomena have been observed in these studies: (i) naive animals inoculated with parental, unaltered tumor cells universally develop lethal tumors; (ii) when injected cutaneously, GATC are rejected by naive animals; (iii) naive animals 'vaccinated' with GATC develop resistance to re-challenge with parental, unmodified tumor cells; (iv) in some cases, inoculation with GATC can induce the regression of small established tumors and metastases; (v) when co-injected at the same subcutaneous site, GATC can induce immunity to unaltered parental cells or to a bystander unrelated tumor. In these models, the strength of host anti-tumor immunity appears to depend on the level of cytokine released by the GATC. The possibility of inducing specific immunity, even against untransduced parental cells or with an unrelated tumor when such cells are admixed with the GATC, has clearly shown that the expression of the cytokine by the tumor itself is unnecessary, and that what is required is the presence of the tumor antigen(s) at the local site of cytokine production.

The induction of a specific immune response (even against spontaneous and poorly immunogenic murine tumors when genetically altered to secrete cytokines) has revealed that even these tumors can express antigens that are potential targets for immune recognition[2–25]. Hence, it is possible that the immune system fails to eliminate tumors that arise spontaneously, not because of the absence of specific tumor antigens, but because the response to these antigens is inadequate in normal conditions.

The choice of transducing an ovarian carcinoma cell line with the GM-CSF gene has been made in light of several recent reports which showed the important role of GM-CSF in the maturation and function of specialized antigen-presenting cells[26]. The possibility that high local levels of GM-CSF production by GATC could specifically enhance tumor-antigen presentation by host antigen presenting cells has merit, since the majority of epithelial tumors do not appear to express detectable levels of MHC class II molecules[27], and are therefore unlikely to be able to prime antigen-specific $CD4^+$ cells. In addition, recent reports comparing the relative potency of irradiated GATC engineered to secrete different cytokines, identified GM-CSF as one of the most potent stimulators of systemic anti-tumor immunity[20,24,25].

The possibility that: GATC induces specific host anti-tumor immunity, even against spontaneous tumors or tumors which have been previously shown to be non-immunogenic; the occurrence of this phenomenon among a wide variety of solid tumors in animals; the lack of a suitable preclinical model for ovarian carcinoma; and the dismal prognosis for patients with advanced ovarian carcinoma treated with conventional therapy, provide the rationale for vaccine trials in patients with this disease.

Materials and methods

Cell lines and culture reagents

The human ovarian cell line UCI-107 was established from a previously untreated patient with a primary stage III serous papillary adenocarcinoma of the ovary. UCI-107 has been previously characterized[28] and was kindly provided by Dr A. Manetta (University of California, Irvine Medical Center). UCI-107 cells were maintained at 37 °C, 5% CO_2 in complete media (CM) containing RPMI 1640 (Gibco Life Technologies, Grand Island, NY), 10% fetal bovine serum (FBS, Gemini Bioproducts, Calabassas, CA), and 1% penicillin/streptomycin sulfate (Irvine Scientific, Santa Ana, CA).

Construction of retroviral vectors

The pLXSN plasmid was kindly provided by Dr A. D. Miller (Fred Hutchinson Cancer Center, Seattle, WA). This plasmid, derived from a Maloney Murine Leukemia Virus (MMLV), contains the neophosphotransferase gene whose constitutive expression is driven by the SV40 enhancer/promoter and the 5′ retroviral LTR of the integrated vector which drives the expression of an inserted gene[29]. The human GM-CSF cDNA was obtained from ATCC[30] in the Okaiama and Berg pCD cloning vector and was excised using the *Bam*HI restriction enzyme. The cDNA was then cloned into the *Bam*HI restriction site in the multiple cloning region of pLXSN[29,31]. Proper orientation of the cDNA was determined by diagnostic restriction endonuclease digests. Once constructed, the retroviral plasmid DNA was then purified by CsCl gradient density centrifugation.

Retroviral transfection and selection of clones

Purified retroviral plasmid DNA (LXSN/GM-CSF) was used to transfect the murine ecotropic packaging

cell line GP-E86[32] by the calcium phosphate method[31]. Forty-eight hour supernatant from these cells was then used to infect the murine amphotropic packaging cell line, PA317[33]. The PA317 packaging cell line was obtained from ATCC and maintained in CM. Transduced PA317 cells were selected by resistance to G418 (Geneticin, Gibco Life Technologies). Isolated clones were expanded, aliquoted and frozen under liquid nitrogen in a master cell bank. The supernatant from a transduced PA317 clone, containing infectious, replication-incompetent retrovirus, was used to infect the UCI-107 cell line. Briefly, UCI-107 cells were seeded into 100 mm tissue culture dishes at densities of 1×10^6 cells in 10 ml CM and incubated for 4 h at 37 °C, 5% CO_2 to allow adherence. After incubation, the media was aspirated and replaced with 5 ml of 2% polybrene in phosphate buffered saline, pH 7.3 (PBS, Aldrich Chemical Company Inc., Milwaukee, WI). After 30 min at 37 °C, 5% CO_2, 10 ml of retroviral supernatant was added, and retroviral mediated gene transfer was accomplished by overnight incubation. Supernatants were then aspirated and replaced with CM. After an additional 48 h incubation in CM at 37 °C, 5% CO_2, selection of transduced clones was accomplished by culture in CM containing 0.075% G418. Clones were isolated after 14 days using sterile 8×8 mm cloning cylinders (Belco Glass Inc., Vineland, NJ) and expanded for 3 weeks in CM containing G418. Parental cell lines were used as positive controls for G418 resistance. After clonal selection in G418, transduced cell lines were returned to CM for expansion and study.

In vitro growth characteristics

Cells were established in CM at a density of 0.5×10^6 cells/10 ml in 100 mm tissue culture dishes. Cell counts were conducted every 12, 24, 48, 72 and 96 h, and the number of viable cells was determined using trypan blue exclusion. Experiments were conducted to compare the growth of non-transduced (parental) and transduced tumor cell lines (UCI-107M GM-CSF-MPS and LXSN vector controll cells) and to evaluate the level of cytokine production over time. Supernatants were collected and frozen at −20 °C (for subsequent ELISA evaluation of cytokine levels) and culture dishes trypsinized to determine cell count and viability.

Analysis of human GM-CSF secretion

Parental (GM-CSF transductants and vector control cells) were seeded in 100 mm tissue culture dishes (Corning, Corning, NY) at a density of 1×10^6 cells ml^{-1} in 10 ml CM. After 48-h incubation at 37 °C, 5% CO_2, supernatant was aspirated, rendered cell-free by centrifugation at 1500 r.p.m. for 10 min, then stored at −20 °C. GM-CSF concentration was then determined by ELISA, employing a commercially available kit (Research & Diagnostic Systems, Minneapolis, MN) utilizing GM-CSF standards ranging from 7.8 to 500 pg ml^{-1}. All samples were assayed in duplicate along with known standards prepared in CM (standard regression lines were generated by plotting $\log_{10}$ concentration vs $\log_{10}$ optical density, giving correlation coefficients greater than 0.98 in all cases). The maximum allowed sample duplicate error was 10%. Duplicates falling outside this error were re-analyzed. The biologic activity of GM-CSF was measured in a cell proliferation assay using a GM-CSF factor-dependent human cell line, TF-1[34] provided by Dr M. Tsang (Research and Diagnostic Systems). The level of biologic activity was found to have a very good correlation with the level of GM-CSF detected by ELISA.

Southern blot analysis

DNA was extracted by parental UCI-107 and GM-CSF transduced cell lines with a modification of the method of Strauss[35]. Briefly, concentrated suspensions of tissue culture cells were lysed in TNE buffer (10 mM Tris, 100 mM NaCL, 1 mM EDTA, pH 7.5) containing 0.5% SDS, treated with 50 μg ml^{-1} proteinase K overnight at 37 °C, then extracted with phenol and chloroform. The DNA solution was precipitated in 100% ethanol, spooled-out and re-suspended in 10 mM Tris, 0.1 mM EDTA (pH 8). Ten micrograms of high molecular weight DNA was digested with *Bam*H1 (GIBCO/BRL, Grand Island, NY), separated on a 1.0% agarose gel and transferred to Gene Screen Plus (Dupont NEN, Boston, MA). Transfer, hybridization and washing were performed according to the manufacturer's specifications. Random primer GM-CSF probe was prepared by using the 0.9 kb *Bam*H1 fragment of pLGM-CSFSN[29] by the method of Tabor & Struhl[36].

Cell surface antigen analysis

Monolayers of parental, vector controls and GM-CSF transductant cells were harvested with 0.1% Trypsin in 0.2% EDTA. Harvested cells were labeled with an isotype control (mAb IgG_2a; Becton Dickinson, Mountain View, CA) and with monoclonal antibodies anti-HLA class I (mAb W6/32, Accurate Chemical and Scientific Corp., Westbury, NY); anti-HLA class II (mAb

CR3-43, Accurate Chemical and Scientific Corp.); anti-ICAM-1 (mAb LB-2, Becton-Dickinson); anti-CA125 (mAb OC125, Signet Laboratories, Dedham, MA); anti-HER-2/neu p185 (mAb TA-1, Oncogene Science, Uniondale, NY); and analyzed for antigen expression using a fluorescent activated cell sorter (FACS) analyzer with log amplifier (Becton-Dickinson)[37].

Irradiation of cell lines

UCI-107M GM-CSF-MPS cells were irradiated in a 15 ml conical tube in CM at room temperature with gamma rays (Cesium-137) at a dose rate of 200 rads min^{-1}. Immediately after irradiation, cells were seeded in a Petri dish culture plate at a density of 1×10^6 cells in 10 ml of CM. Test doses of 1000 to 10000 rads were applied. Irradiated cells were cultured at 37 °C in a 5% CO_2 atmosphere and the media was completely changed every 4 days in all the dishes. Every 48 h, culture supernatants were collected from the dish for cytokine production and the number of viable cells was assesssed by light microscopy using trypan blue exclusion. Irradiated cells cultured in separate T75 tissue culture flasks (Corning, Corning, NY) were harvested at days 2 and 8 for the determination of surface antigen expression using FACS analysis.

Table 1. GM-CSF production by transduced clones derived from UCI-107 parental cell line*

Clone ID	GM-CSF (pg ml^{-1})	Clone	IDGM-CSF (pg ml^{-1})
A	55	L	126
A1	15	L1	149
B	25	M	420
B1	98	M1	67
C	9	N	31
C1	7	N1	63
D	83	O	79
D1	73	O1	115
E	7	P	31
E1	61	Q	49
F	5	R	35
F1	21	S	ND†
G	47	T	74
G1	39	U	34
H	13	V	ND
H1	134	X	8
I	86	Y	146
I1	12	Z	22

*All clones were randomly selected and evaluated for cytokine secretion by seeding 1×10^6 cells ml^{-1} in 10 ml CM in 100 mm tissue culture dishes. After 48 h incubation, supernatant was aspirated, rendered cell-free by centrifugation at 1500 r.p.m. for 10 min, then stored at −20 °C. GM-CSF concentration was then determined by ELISA, employing a commercially available kit (Research & Diagnostic Systems). All samples were assayed in duplicate along with known standards as described in the methods.

†ND = not detectable.

Results

Transduction and selection of a clone of human ovarian cancer cells secreting high levels of GM-CSF

UCI-107 human ovarian carcinoma cells were transduced with LXSN and LXSN-GM-CSF vectors, and clones were identified and expanded after selection in G418-containing media. Standardized cultures of each clone were then established and after 48 h, the media tested for the presence of GM-CSF by ELISA and bioassay as described in the methods section. Clones were randomly selected from transduced cells. As expected, parental UCI-107 cells, and cells transduced with the LXSN vector alone did not produce detectable levels of GM-CSF. Of the 36 clones originally selected (Table 1), the highest GM-CSF producing clone, termed UCI-107M GM-CSF-MPS, was expanded and employed to form a master cell bank for further testing and extensive characterization.

Morphology and growth characteristics of UCI-107M GM-CSF-MPS cells *in vitro*

The parental cell line UCI 107 has the characteristic morphology of ovarian epithelial cells grown *in vitro*, as previously described[28]. The morphology of UCI 107 cells transduced with the LXSN vector alone or LXSN containing the GM-CSF gene was indistinguishable from that of parental 107 cells. The doubling time of the parental, vector control and UCI-107M GM-CSF-MPS cells was determined to be approximately 20–26 h (Fig. 1a); however, parental cells had a slightly faster growth rate than either of the transductants. No changes in the growth rate of these cells have been observed *in vitro* for over 35 passages and 6 months of culture.

Kinetics and longevity of GM-CSF production by UCI-107M GM-CSF-MPS cells *in vitro*

Supernatants were collected from UCI-107M GM-CSF-MPS cell cultures at various intervals and levels of GM-CSF measured by ELISA. The results (Fig. 1b) show GM-CSF production by 0.5×10^6 cells/10 ml over a period of 96 h. In addition, the level of GM-CSF production by UCI-107M GM-CSF-MPS was studied over a period of 6 months and a total of 35 passages. During this observation period, levels of GM-CSF production were consistently in the range of 420 to 585 pg ml^{-1} 10^5 $cells^{-1}$ 48 h^{-1}.

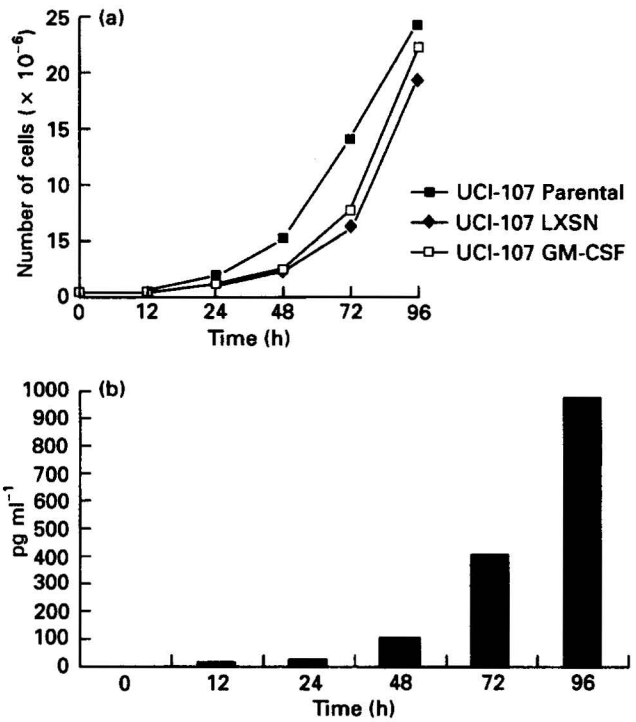

Fig 1. (a) The growth rates of UCI 107 parental, UCI-107 LXSN (vector) and UCI-107M GM-CSF-MPS. Cells were established in complete media at a density of 0.5×10^6 cells/10 ml in 100 mm tissue culture-treated dishes. Cell counts were conducted every 12, 24, 48, 72 and 96 h and the total number of viable cells was determined using trypan blue exclusion. The result shown is the average of two different experiments. The variation was not greater than 10%. (b) The kinetics of GM-CSF production by UCI-107M GM-CSF-MPS cells. These cells were established in complete media at a density of 0.5×10^6 cells/10 ml in 100 mm tissue culture-treated dishes. Supernatants were collected from cultures every 12, 24, 48, 72 and 96 h, and levels of GM-CSF measured by ELISA. The results from one experiment are shown and are representative of two separate studies.

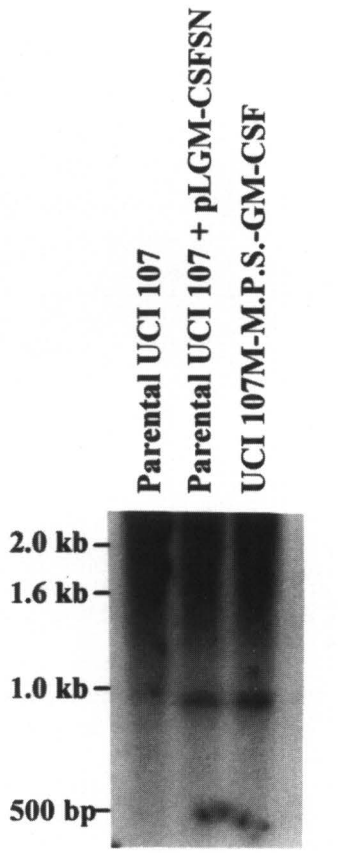

Fig 2. Southern blot analysis of UCI-107 parental, parental UCI-107+pLGM-CSFSN and UCI-107M GM-CSF-MPS. *Bam*H1-digested DNAs were hybridized with the random primer GM-CSF probe. The autoradiogram shown indicates the expected 0.9 kb band in UCI-107M GM-CSF-MPS cells and not parental UCI 107 cells. The line indicated as parental UCI-107+pLGM-CSFSN represent 10 μg of *Bam*H1-digested parental DNA mixed with 20 pg of *Bam*H1-digested pLGM-CSFSN plasmid.

The LXSN GM-CSF vector is inserted into the DNA of the UCI-107M GM-CSF-MPS cell line

Figure 2 shows a Southern blot analysis of UCI-107M GM-CSF-MPS and the parental UCI 107. GM-CSF secreting clone transduced with LXSN-GM-CSF and selected in G418 was evaluated for successful gene insertion by Southern hybridization probing for the cDNA of GM-CSF after 20 passages. In the tumor cell line, the presence of the inserted gene was confirmed.

Survival and stability of GM-CSF secretion by UCI-107M GM-CSF-MPS cells after irradiation *in vitro*

UCI-107M GM-CSF-MPS cells received different levels of gamma irradiation and were established as monolayers in complete medium, as described in the methods. Supernatants from individual subcultures were collected, evaluated for cytokine production by ELISA, and cell number and viability determined in each culture. In addition, irradiated cells were evaluated at days 2 and 8 for surface antigen expression by FACS analysis. No effects on cell growth were observed at doses of less than 1000 rads. At higher doses, approximately 90% of the cells were viable 48 h after irradiation, with 30% and 10% viability at 4 and 6 days, respectively; all the cells were dead after 3 weeks. No statistically significant differences in survival were seen among cells irradiated with 2500 (data not shown), 5000 and 10 000 rads (Fig. 3a). Secretion of GM-CSF by irradiated cells is shown in Fig. 3b. As can be seen, GM-CSF production continued until about day 8, suggesting a decrease in GM-CSF levels which correlated with a decrease in the number of viable cells, but not an inhibition in the biosynthesis and release of the cytokine.

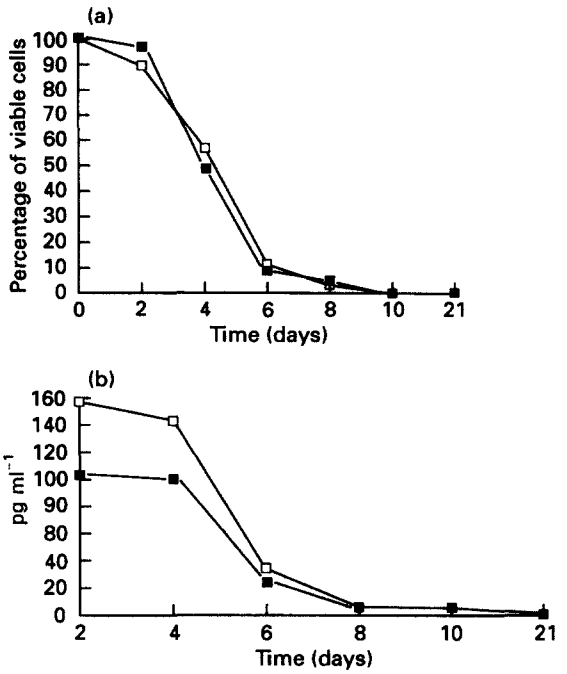

Fig 3. (a) The effects of gamma irradiation on the viability of UCI-107M GM-CSF-MPS cells *in vitro*. UCI-107M GM-CSF-MPS cells in suspension were irradiated and then established as monolayers as described in the methods. While not shown, no effects on cell growth were observed at doses of less than 1000 rads. At 5000 and 10 000 rads, 90% of the cells were viable 48 h after irradiation: however, only 10–30% were still viable 4 and 6 days later and all the cells were not viable after 3 weeks. No statistically significant differences in survival were seen among cells irradiated with 2500 (data not shown), 5000 and 10 000 rads. The results of one experiment are shown and is representative of three different studies. (b) GM-CSF production by UCI-107M GM-CSF-MPS cells after irradiation at 5000 and 10 000 rads. The result from a single experiment reveal cytokine production was detectable for 8 days after irradiation. The results are representative of three separate experiments. □ = UCI 107M GM-CSF-MPS cells after irradiation at 5000 rads. ■ = UCI 107M GM-CSF-MPS cells after irradiation at 10 000 rads.

Expression of surface antigens on parental, vector-containing and GM-CSF secreting UCI 107 cells *in vitro*

Proteins of the major histocompatibility complex, adhesion molecules and tumor-associated antigens are important for both the recognition and destruction of tumor cells by the immune system. The expression of these cell membrane proteins was examined by FACS analysis on parental, LXSN-containing and UCI-107M GM-CSF-MPS cells. The results of these studies showed that parental, vector controls (data not shown) and UCI-107M GM-CSF-MPS cells constitutively express MHC class I antigens, (87.5%, mean fluorescent channel intensity (MFI) = 33.6; and 92.9%, MFI = 53.9) respectively, Her-2/*neu*, (95.8%, MFI = 20.8; and 93.3%, MF = 22.1), respectively, but did not express MHC class II determinants, CA 125 and ICAM-1 (data not shown). The production of GM-CSF or the expression of the LXSN vector had no detectable effect in significantly up or down-regulating the antigens positively expressed in the parental line.

Expression of MHC proteins, ICAM-1 and TAA on UCI-107M GM-CSF-MPS cells after irradiation

Membrane expression of MHC proteins and TAA in irradiated cells was examined by FACS analysis at days 2 and 8 *in vitro*. Increased expression of MHC class I and Her-2/*neu* surface antigens was observed in irradiated cells when compared to the non-irradiated controls. While this was noted for cells treated with all doses of irradiation, there was a trend toward higher expression associated with the higher doses (Fig. 4 and Table 2). However, gamma irradiation did not induce the neo-expression of antigens not originally expressed on the parental cells, including HLA class II, ICAM-I or the TAA CA125.

The cell line UCI-107M GM-CSF-MPS is free of micro-organisms

The UCI-107M GM-CSF-MPS cell line was extensively tested for the presence of various micro-organisms by our own and outside laboratories. Results revealed that the UCI-107M GM-CSF-MPS cell line is free of mycoplasma, bacteria, the DNA viruses Epstein–Barr, human hepatitis B, human cytomegalovirus and replication competent retroviruses.

Discussion

Immunotherapy using systemic administration of immune enhancing cytokines, with or without activated lymphoid cells, has resulted in dramatic clinical responses in some patients with advanced cancer[38–40]. In the majority of patients however, the high levels of toxicity resulting from the systemic administration of high doses of cytokines have limited their therapeutic benefit[31,41–43]. Gene therapy using retroviral-mediated transduction to introduce genes into human cells before their infusion into patients has already been shown to be a feasible and safe procedure[43]. Local, continuous delivery of cytokines by tumor cells genetically engineered to produce these molecules provides an alternative approach for tumor immunotherapy which should have few, if any, side-effects. Furthermore, this delivery system would also bypass the short half-life of cytokines and their dilution when administered systemically.

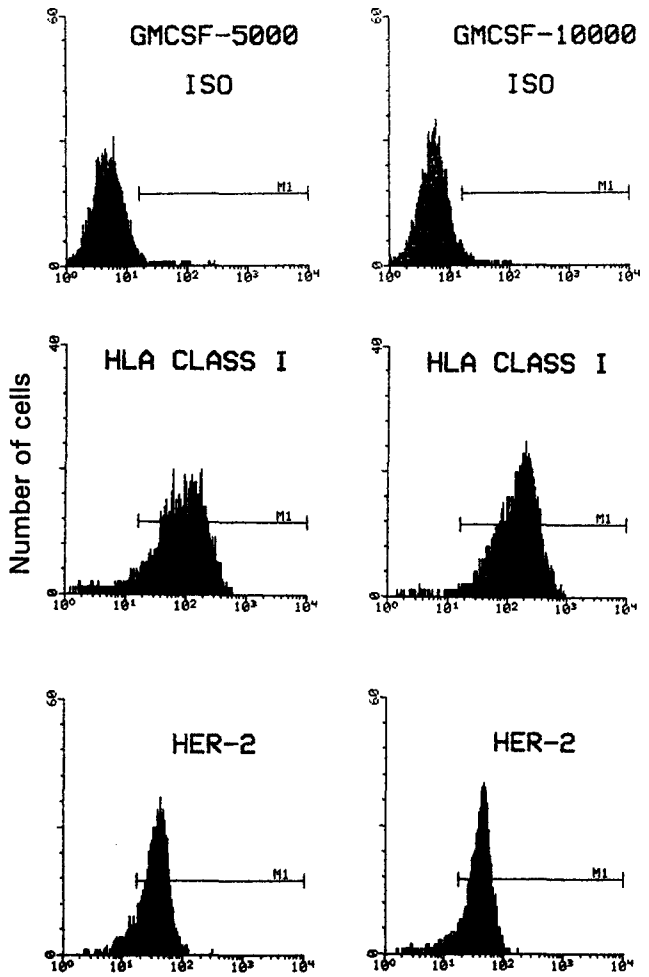

Fig 4. Expression of cell surface proteins in irradiated UCI 107M GM-CSF-MPS cells after 2 days. Membrane protein expression in irradiated cells was measured by FACS as described in the methods at day 2 *in vitro*. Increased expression of MHC class I and Her-2 (Her2/*neu*) was observed in irradiated cells as compared to the non-irradiated controls. While the data are not shown, gamma irradiation did not induce neo-expression of HLA class II, ICAM-I or the TAA CA125. The results are representative of two separate experiments.

Therefore, we initiated studies to develop GATC from human ovarian carcinoma cell lines with the intent of using these lines as vaccines for the treatment of advanced ovarian carcinoma. Our previous experience using other ovarian carcinoma cell lines indicated that clones derived from the UCI-107 cell line were generally much better cytokine producers than clones from other human ovarian carcinoma cell lines [Santin *et al.*, unpublished data]. Among the 36 randomly selected G418 resistent clones from the GM-CSF transduced UCI-107 cell line, a clone which produces high levels of GM-CSF, termed UCI-107M GM-CSF-MPS, was selected and extensively characterized. UCI-

Table 2. FACS scan analysis of UCI-107M GM-CSF-MPS cell line after radiation*

Cell line	HLA class I	Her-2-neu
UCI-107 GM-CSF	92.9%†	93.3%
(unirriadiated control)	53.9‡	22.1
UCI-107 GM-CSF	94.8%	86.9%
(irradiated 5000 rads after 2 days)	139.5	39.1
UCI-107 GM-CSF	97.3%	89.2%
(irradiated 10 000 rads after 2 days)	229.5	51.5
UCI-107 GM-CSF	81.7%	72.8%
(irradiated 5000 rads after 8 days)	119	50
UCI-107 GM-CSF	86.0%	78.3%
(irradiated 10 000 rads after 8 days)	202	47

*Membrane expression of MHC proteins and TAA in irradiated cells was examined by FACS at days 2 and 8 *in vitro*. Increased expression of MHC class I and Her-2/*neu* was observed in irradiated cells as compared to the non-irradiated controls. Gamma irradiation did not induce neo-expression of HLA class II, ICAM-I or the TAA CA 125 (data not shown). All data are shown as mean (CV for per cent positivity ranged from 0.3 to 8.9%, and for MFI ranged from 6.9 to 16.7%).

†Per cent of positive cells.

‡MFI = mean channel fluorescence intensity.

107M GM-CSF-MPS cells have the LXSN-GM-CSF vector stably inserted into their DNA, as determined by Southern blot analysis, and GM-CSF production was a stable phenotype.

These cells did not differ from parental or LXSN vector control cells in their morphologic characteristics or their surface antigen expresssion. Like the parental UCI 107 cell line, UCI-107M GM-CSF-MPS does not express MHC class II, ICAM-1 or CA 125 proteins but does express MHC class I and the tumor associated antigen Her2/*neu*. The failure to detect changes in surface antigen expression in UCI-107M GM-CSF-MPS cells was not surprising since it is well known that the main action of GM-CSF is to induce differentiation in GM precursor cells and to activate antigen presenting cells. Thus, it would not be expected that GM-CSF would have any effect on the transduced cells used in this study. Vaccines derived from allogeneic or autologous tumor cell lines require methods such as irradiation to prevent tumor formation at the immunization site. Such procedures should not reduce the capacity of these tumor cell lines to secrete high levels of the specific cytokine transduced or their immunogenecity. Because high dose gamma irradiation is one of the best methods of achieving this goal, we examined the effect of different doses of irradiation on the viability, cytokine production and antigen expression of UCI-107M GM-CSF-MPS. Cells irradiated between 2500 and 10 000 rads remained viable for about 8 days but were all dead by approximately 3 weeks. Cells irradiated with 1000 rads recuperated and

continued to grow. Cytokine production was detectable for approximately 8 days after irradiation with 5000 and 10 000 rads, and closely paralleled the number of viable cells. Interestingly, irradiation induced up-regulation of both MHC class I and Her2/*neu* surface antigens and they remained up-regulated for as long as the cells remained viable. Gamma irradiation did not induce the neo-expression of antigens previously not expressed. These data are consistent with the enhanced immunogenicity of many murine tumors following irradiation[20]. Collectively, these results indicate that UCI-107M GM-CSF-MPS cells can be irradiated effectively to stop replication, yet maintain the production of GM-CSF for up to a week. Finally, extensive tests performed on the UCI-107M GM-CSF-MPS master cell bank (MCB) revealed that this line is free of mycoplasma, bacteria and infectious viruses.

For a specific immune response to occur, T cells must recognize a target peptide in the context of an MHC molecule. In addition, secondary co-stimulatory signals are also needed[44]. In the absence of the latter signal, the T cell makes only a partial response and, more importantly, enters an unresponsive state known as clonal anergy[44]. This co-stimulatory signal results from the interaction between adhesion molecules expressed on the T cells (CD28/CTL.A4) and their natural ligand expressed only on specialized antigen presenting cells (B7). Because most tumors do not express B7 (even in the presence of tumor rejection antigens) these antigens are unlikely to induce an immunologic response strong enough to prevent tumor outgrowth.

The choice to transduce an ovarian carcinoma cell line with the GM-CSF gene has been carried out in light of its key role in the maturation and function of specialized antigen presenting cells[26], and in our belief that if a systemic immune response can ensue, the presence of activated specialized antigen presenting cells at the immunization site is essential. In addition, in recent studies using irradiated GATC which compared the relative activity of different cytokine gene products in a single tumor model[20,24,25] or examined the activity of a single gene product in multiple models[20], GM-CSF stood out as one of the most effective cytokines at inducing systemic immune responses against poorly immunogenic tumors.

There are multiple reasons for testing an allogeneic vaccine in women with advanced ovarian cancer. Unfortunately, this must be tested directly without preclinical animal studies since, at this moment, there is no relevant animal model for this disease. We have proposed a phase I clinical trial in women with advanced epithelial ovarian cancer to test the efficiacy of immunization using the presently described allogeneic GM-CSF secreting vaccine admixed with the patient's autologous tumor cells obtained at the time of surgical debulking. This cell mixture should provide three important signals for activating the host immune system in the local injection site: (i) alloantigens on the GATC; (ii) specific autologous tumor antigens; and (iii) GM-CSF production. We believe that the presence of these strong activating signals will provide the appropriate micro-environment so as to encourage the recognition of autologous tumor cell antigens by antigen presenting cells and host lymphoid cells.

Evidence to support the above treatment plan for these women includes the following: first, allogeneic cells provide a strong immunologic stimulus that can direct a host response to unrelated bystander cells. This is supported by both *in vitro* and *in vivo* studies[45,46]. Second, the presence of autologous tumor antigens at the site of the cytokine secretion overcomes the necessity of sharing the HLA class I antigens between the allogeneic vaccine and each patient for the induction of a specific immune reaction. Third, immunization with cryopreserved autologous tumor cells mixed with the GM-CSF secreting allogeneic vaccine eliminates the need to culture each autologous cell line in an attempt to obtain an autologous transduced vaccine. Numerous problems may be encountered in this regard, greatly reducing the success of such procedures. Moreover, the use of cryopreserved cells should also reduce the possibly selective loss of critical tumor specific antigens on cultured cells.

We believe that the development of a cytokine secreting allogeneic vaccine mixed with the irradiated patient autologous tumor for the treatment of advanced ovarian cancer could be an attractive alternative to currently developed autologous vaccines. Allogeneic vaccines will have the benefit of careful standardization and quality control as well as offering strong immunological signals to enhance host anti-tumor immunity. The future design and implementation of clinical trials in this regard will ultimately determine the validity of this approach.

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