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Development and Characterization of an IL-4-Secreting Human Ovarian Carcinoma Cell Line¹

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Human ovarian carcinoma cell lines were genetically engineered to secrete the cytokine interleukin-4 (IL-4) by retroviral-mediated gene transduction. These cells were transduced with the LXS_N retroviral vector containing the human IL-4 gene and the neomycin resistance selection marker. Numerous IL-4-secreting clones were isolated from different papillary serous carcinoma cell lines, including SKOV-3, UCI-101, and UCI-107, and one clone derived from UCI-107 extensively characterized. This clone, termed UCI 107E IL-4 GS, was shown to constitutively express high levels of IL-4 (i.e., 900 to 1300 pg/ml/10⁵ cells/48 hr) for over 35 passages and 6 months of study. Like the parental cell line (UCI-107), UCI 107E IL-4 GS cells expressed MHC class I and Her-2/*neu* surface antigens but did not express detectable MHC class II, ICAM 1, CA 125, or IL-4 receptors. No increase in expression of surface proteins was noted between parental and UCI 107E IL-4 GS. The morphology of this clone did not differ from that of the parental or LXS_N vector control cells; however, parental cells had a faster growth rates than transductants. UCI 107E IL-4 GS was sensitive to γ irradiation since as little as 2500 rad killed most of the cells within 10 days of irradiation. However, after irradiation, IL-4 secretion continued until about Day 8. The potential use of these IL-4-secreting ovarian carcinoma cells as vaccines for woman with advanced ovarian cancer will be discussed. © 1995 Academic Press, Inc.

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 the disease,

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65 to 75% of patients initially present with tumor disseminated throughout the peritoneal cavity. Although many patients with such disseminated tumor respond completely to the standard combination of surgical and cytotoxic therapy, nearly 90% develop recurrence and inevitably succumb to their disease [1]. Thus, we are compelled to offer patients with advanced ovarian carcinoma trials with experimental modalities.

The development and progression of solid malignancies such as ovarian carcinoma may be a result of the failure of the host immune system to eliminate spontaneously arising tumors. The mechanisms by which tumors escape the immune response may involve downregulation in the expression of tumor-associated cell surface antigens, major histocompatibility (MHC-I and MHC-II) molecules, costimulatory and adhesion molecules such as ICAM [2], and the production of blocking factors [3].

Recent studies with murine tumors, genetically altered to secrete immune system-enhancing cytokines (genetically altered tumor cells, GATC),³ offer new approaches for the development of vaccines for cancer therapy and prevention [4-16]. Several general phenomena have been observed in these studies: (1) Naive animals inoculated with parental, unaltered tumor cells universally develop lethal tumors; (2) naive animals "vaccinated" with GATC develop resistance to rechallenge with parental tumor cells; (3) when coinjected at the same subcutaneous site, GATC can induce immunity to the unaltered parental cells or to a bystander unrelated tumor; (4) in some cases, inoculation with GATC can induce regression of established tumors; and (5) immunization with

³ Abbreviations used: GATC, genetically altered tumor cells; IL-4, interleukin-4; PPC, percentage of positive cells; MFI, mean channel fluorescence intensity.

GATC can induce a host response against tumors that were previously considered to be nonimmunogenic.

The ability of GATC to stimulate a host antitumor immune response appears to be a general phenomenon because it has been observed with many different types of cytokine-tumor combinations [4–16]. In these models, the strength of host antitumor immunity appears to depend on the level of cytokine released. The choice to transduce human epithelial ovarian cancer cells with the IL-4 gene was based on the powerful immune reactions known to be stimulated by GATC-secreting IL-4 in several murine tumor models [7, 8, 16]. Tepper *et al.* have shown that mice challenged with IL-4-transduced plasmocytoma or mammary adenocarcinoma cells are able to reject the experimental tumors as well as a variety of unrelated tumors when such cells are mixed with the IL-4-secreting cell line [8]. Golumbek *et al.* have shown that IL-4-secreting cells can also develop specific antitumor immunity against an apparently not immunogenic spontaneously arising murine renal tumor [7]. Finally, Pericle *et al.* demonstrated that IL-4-transduced tumor cells could induce high levels of specific immunity even against a spontaneous poorly immunogenic murine mammary adenocarcinoma (TSA) [16]. IL-4 is a pluripotent cytokine endowed of a broad range of stimulating activities on both B and T lymphocytes as well as on hematopoietic cells [17]. One of its main effects may be to recruit and activate CD4⁺ antigen-presenting T cells [18]. In addition, IL-4 has the ability to induce infiltration and activation of eosinophils, mast cells, and macrophages at the local tumor site [7, 8, 16]. Finally, IL-4 has a role in the induction, differentiation and generation of human cytolytic T lymphocytes [18–21]. Taken together, these pleiotropic properties of IL-4 make it an attractive candidate for the development of GATC for human vaccine studies.

The lack of a suitable preclinical immunocompetent animal model for ovarian carcinoma and the dismal prognosis for patients with advanced ovarian carcinoma treated with conventional therapy, together provide the rationale for vaccine trials in patients with this disease. Therefore, we have generated clones of human ovarian carcinoma cells genetically engineered to secrete high levels of IL-4 by retroviral-mediated gene transduction. This is the first report of such GATC and their characterization.

MATERIALS AND METHODS

Cell Lines and Culture

All ovarian carcinoma cell lines were derived from human serous papillary adenocarcinomas of ovarian epithelial origin. SKOV-3 was purchased from the American Type Culture Collection (ATCC). Human ovarian carcinoma cell lines UCI-101 and UCI-107 have been previously characterized [22, 23] and were kindly provided by Dr. Alberto Manetta,

University of California, Irvine (UCI). All human ovarian cell lines were maintained at 37°C, 5% CO₂ in complete medium (CM) containing RPMI 1640 (Gibco Life Technologies), 10% fetal bovine serum (FBS, Gemini Bioproducts, Calabasas, CA), and 1% penicillin/streptomycin sulfate (Irvine Scientific, Santa Ana, CA).

Construction of Retroviral Vectors

The pLXSN plasmid was kindly provided by Dr. A. Dusty 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, the 5' retroviral LTR of the integrated vector drives the expression of an inserted gene [24]. The human IL-4 cDNA was obtained from ATCC [25] in the Okaiama and Berg pCD cloning vector and was excised using *Bam*HI restriction enzyme. The cDNA was then cloned into the *Bam*HI restriction site in the multiple cloning region of pLXSN [24, 26]. Proper orientation of the cDNA was determined by diagnostic restriction endonuclease digests. Once constructed, retroviral plasmid DNA was then purified by CsCl gradient density centrifugation.

Retroviral Transfection and Selection of Clones

Purified retroviral plasmid DNA (LXSN/IL-4) was used to transfect the murine esotropic packaging cell line GP-E86 [27] by the calcium phosphate method [26]. Forty-eight-hour supernatant from these cells was then used to infect the murine amphotropic-packaging cell line, PA317 [28]. The PA317-packaging cell line was obtained from the ATCC and maintained in CM. Transduced PA317 cells were selected by resistance to G418. 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 human carcinoma cell lines. Briefly, human ovarian carcinoma cell lines were seeded in 100-mm tissue culture dishes at densities of 1×10^6 cells in 10 ml CM and incubated for 4 hr at 37°C, 5% CO₂ to allow adherence. After incubation, the medium was aspirated and replaced with 5 ml of 2% polybrene in phosphate-buffered saline (PBS), (Aldrich Chemical Co. Inc., Milwaukee, WI). After 30 min at 37°C, 5% CO₂, 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-hr incubation in CM at 37°C, 5% CO₂, selection of transfectant clones was accomplished by culture in CM containing 0.075% G418 (geneticin, Gibco Life Technologies). 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 negative controls for G418 resistance. After clonal selection in G418, transfected cell lines were returned to CM for maintenance and expansion.

In Vitro Growth Characteristics

Cells were established in complete medium 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, and 72 hr, and the number of live cells was determined using trypan blue exclusion. Experiments were conducted to compare the growth of nontransduced (parental) and transduced tumor cell lines (107E IL-4 GS and LXSXN vector control) 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 were trypsinized to determine cell count and viability.

Analysis of Human IL-4 Secretion

Parental, IL-4 transductants, and vector control cells were seeded in 100-mm tissue culture dishes (Corning) at a density of 1×10^6 cells/ml in 10 ml CM. After 48-hr incubation at 37°C , 5% CO_2 , the supernatant was aspirated, rendered cell-free by centrifugation at 1500 rpm for 10 min, and then stored at -20°C . IL-4 concentration was then determined by ELISA, employing a commercially available kit (Research & Diagnostic Systems, Minneapolis, MN). All samples were assayed in duplicate along with known standards (standard regression lines, generated by plotting \log_{10} concentration vs \log_{10} optical density, projected correlation coefficient greater than 0.98 in all the cases). The maximal allowed sample duplicate error was 10%. The biologic activity of IL-4 in cell-free supernatants was determined by Dr. Monica Tsang (Research and Diagnostic Systems) with a proliferative assay using a human IL-4-dependent cell line, TF-1 [29]. The level of biologic activity was found to have a direct correlation with the level of IL-4 detected by ELISA.

Southern Blot Analysis

DNA was extracted by a modification of the method of Strauss [30]. 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 $\mu\text{g}/\text{ml}$ proteinase K overnight at 37°C , and then extracted with phenol and chloroform. The DNA solution was precipitated in 100% ethanol, spooled out, and resuspended in 10 mM Tris, 0.1 mM EDTA (pH 8). Ten micrograms of high-molecular-weight DNA was digested with *Sst*I (GIBCO/BRL, Grand Island, NY), separated by electrophoresis on a 0.8% agarose gel and transferred to Gene Screen Plus (DuPont NEN, Boston, MA). Transfer, hybridization, and washing were performed according to manufacturer's specifications. Random primer *neo* probe was pre-

pared by using the 0.7-kb *Nco*I fragment of pLXSXN [24] by the method of Tabor and Struhl [31].

Cell Surface Antigen Analysis

Monolayers of parental, vector controls, and IL-4 transductant cells were harvested with 0.1% trypsin, 0.2% EDTA. Harvested cells were labeled with 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 anti-IL-4 receptor (Genzyme Diagnostic, Cambridge, MA) and analyzed for antigens expression using a FACS Analyzer (Becton-Dickinson) [32].

Irradiation of Cell Lines

Lymphokine-producing UCI 107E IL-4 GS cells were irradiated in a 15-ml conical tube in CM at room temperature with γ ray (cesium-137) at a dose rate of 200 rad/min. 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, 2500, 5000, and 10,000 rad were applied. Irradiated cells were cultured at 37°C in a 5% CO_2 atmosphere and the medium was completely changed every 4 days in all the dishes. Every 48 hr, culture supernatant was collected from the dish to assess levels of cytokine production and the number of viable cells was assessed by light microscopy using trypan blue exclusion. In addition, irradiated cells cultured in separate T75 tissue culture flasks (Corning, Corning, NY) were harvested at Days 2 and 8 for determination of surface antigen expression using FACS scan analysis.

Statistical Analysis

Significance analysis was performed using a paired Student's *t* test. Only *P* values <0.05 were considered significant.

RESULTS

Transduction and selection of a clone of human ovarian cancer cells secreting high levels of IL-4. Three different human ovarian carcinoma cell lines were transduced with LXSXN and LXSXN-IL-4, and then clones were identified and expanded after selection in neomycin containing medium. Standardized cultures of each clone were established and after 48 hr, the medium was tested for the presence of IL-4 by ELISA as described under Materials and Methods. The results shown in Table 1 reveal that the majority of the clones randomly selected from transfected cells produced IL-4. However, clones derived from the cell lines SKOV3

TABLE 1

Interleukin-4 Production of Multiple Clones Derived from Three Different Human Serous Papillary Ovarian Carcinoma Cell Lines^a

SKOV-3 IL-4-secreting clones		UCI-101 IL-4-secreting clones		UCI-107 IL-4-secreting clones	
Clone	IL-4 pg/ml	Clone	IL-4 pg/ml	Clone	IL-4 pg/ml
A	32	A	140	A	32
C	16	B	ND	B	83
D	20	C	49	C	90
F	63	D	40	D	35
G	34	E	87	E	1300
H	121	G	93	F	30
I	103	H	38	G	80
L	11	I	93	H	513
X	97	L	42	L	170
	Average, 55.2	M	32	M	297
		N	ND	N	† 265
		O	ND	P	330
			Average, 51.1	Q	615
				X	79
				Y	68
					Average, 265.8

^a All ovarian carcinoma cell lines were derived from human serous papillary adenocarcinomas of ovarian epithelial origin. All clones were randomly selected and evaluated for cytokine secretion by seeding 1×10^6 cells/ml in 10 ml CM in 100-mm tissue culture dishes. After 48-hr incubation, supernatant was aspirated, rendered cell-free by centrifugation at 1500 rpm for 10 min, and then stored at -20°C . IL-4 concentration was then determined by ELISA, employing a commercially available kit (Research & Diagnostic Systems, Minneapolis, MN). All samples were assayed in duplicate along with known standards as described under Materials and Methods. ND, not detectable.

and UCI 101 produced less IL-4 than the clones derived from UCI 107. As expected, each parental line and cells transduced with vector alone did not produce detectable levels of IL-4. The best IL-4-producing clone, termed UCI 107E IL-4 GS, was expanded and employed to form a master cell bank for further testing and extensive characterization.

Morphology and growth characteristics of UCI 107E IL-4 GS cells *in vitro*. The parental cell line UCI 107 has the characteristic morphology of ovarian epithelial cells grown *in vitro* as previously described [23]. The morphology of UCI 107 cells transduced with the LXS vector alone or LXS containing the IL-4 gene was indistinguishable from that of parental 107 cells (Fig. 1). The doubling time of parental, vector control, and UCI 107E IL-4 GS cells was determined to be 15.3, 15.7, and 18.6 hr, respectively (Fig. 2A). No changes in the growth rate of these cells have been observed *in vitro* over 35 passages and 6 months of culture.

Kinetics and longevity of IL-4 production by UCI 107E IL-4GS cells *in vitro*. Supernatants were collected from UCI 107E IL-4GS cell cultures at various intervals and levels of IL-4 were measured by ELISA. The results in Fig. 2B show IL-4 production by 0.5×10^6 cells/10 ml over a period of 96 hr. In addition, the level of IL-4 production by UCI 107E IL-4GS was studied over a period of 6 months and a total of 35 passages. We found that levels of IL-4 production were consistently in the range of 900 to 1300 pg/ml/ 10^5 cells/48 hr.

The LXS IL-4 vector is inserted into the DNA of the UCI 107 E IL-4 GS cell line. Figure 3 shows Southern blot analysis of UCI 107E IL-4 GS and the parental UCI 107. IL-4-secreting clone transduced with LXS IL-4 and selected in G418 was evaluated for successful gene insertion by Southern hybridization probing for the Neo^R gene after 20 passages. In the IL-4-transduced tumor cell line, the presence of the vector was confirmed.

Survival and stability of IL-4 secretion by UCI 107E IL-4 GS cells after irradiation *in vitro*. UCI 107E IL-4 GS cells received different levels of γ irradiation and then were established as monolayers in complete medium as described under Materials and Methods. Supernatants from individual subcultures were collected and evaluated for cytokine production by ELISA, and cell numbers and viability were 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 rad. At higher doses, 90% of the cells were viable 48 hr after irradiation; however, only 30 to 10% were still viable 4 and 6 days later and all of 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 rad on Days 2 ($P = 0.72$), 4 ($P = 0.14$), 6 ($P = 0.10$), and 8 ($P = 0.3$) (Fig. 4A). Secretion of IL-4 by irradiated cells is shown in Figs. 4B and 4C. As can be seen, IL-4 production was detectable

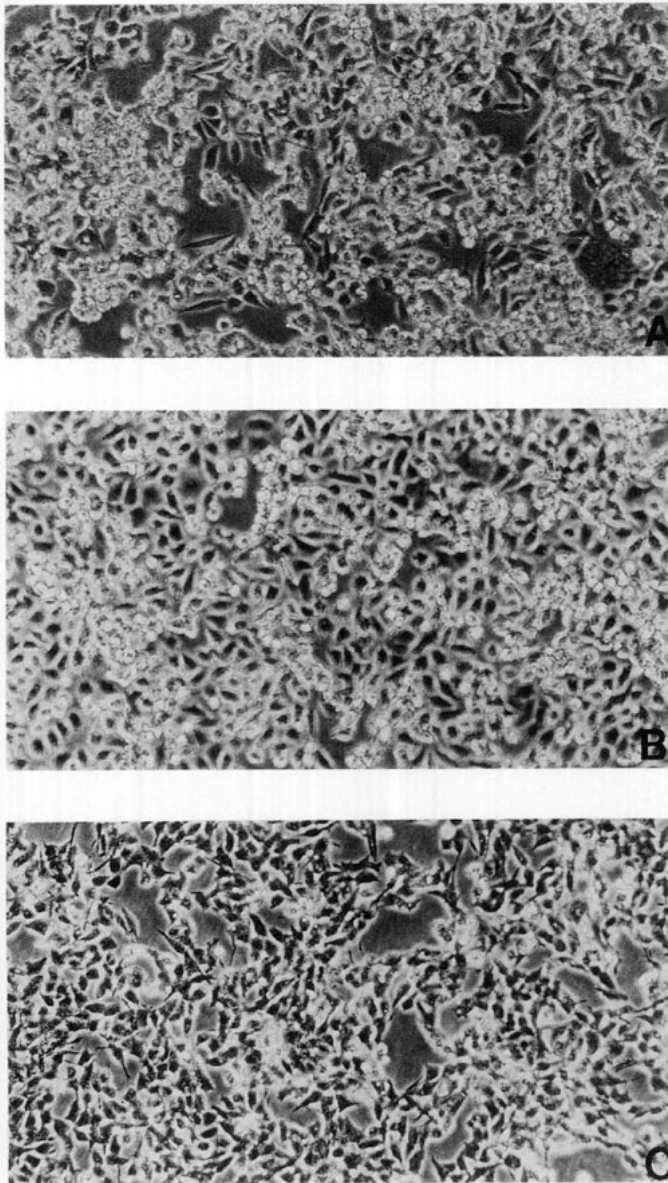


FIG. 1. Morphologic features of UCI 107 parental (A), UCI 107 LXS (vector) (B), and UCI 107 E IL-4 GS (C). Photograph 9 \times magnification.

until Day 8, suggesting a decrease in IL-4 levels which correlated with a decrease in the number of viable cells, but not an inhibition in the biosynthesis and release of the cytokine. Indeed, when IL-4 production after irradiation is standardized to pg/ml/ 10^5 viable cells/48 hr (Fig. 4C), IL-4 secretion was clearly maintained in irradiated viable cells and no statistically significant differences were noted in cytokine production in cells irradiated with 5000 and 10,000 rad on Days 2 ($P = 0.99$), 4 ($P = 0.27$), 6 ($P = 0.15$), and 8 ($P = 0.8$).

Expression of surface antigens on parental, vector-containing, and IL-4-secreting UCI 107 cells in vitro. Proteins of the major histocompatibility complex, adhesion mole-

cules, and tumor-associated antigens (TAA) are important for both recognition and destruction of tumor cells by the immune system. The expression of these cell membrane proteins as well as the expression of IL-4 receptors was examined by FACS analysis on parental, LXS-containing, and 107E IL-4 GS cells. The expression of MHC class I and Her-2/*neu* antigens are shown in Fig. 5. Parentals, vectors (data not shown), and 107E IL-4 GS cells constitutively express MHC class I antigens [PPC, percentage positive cells is 87.5%; MFI, mean channel fluorescence intensity is 33.6, and PPC = 96.7%, MFI = 46.7, respectively], Her-2/*neu* [PPC = 95.8%, MFI = 20.8 and PPC = 62.1%, MFI = 16.5, respectively], but did not express MHC class II determinants, CA 125, ICAM-1, or IL-4 receptors (data not shown). The production of IL-4 or the expression of the

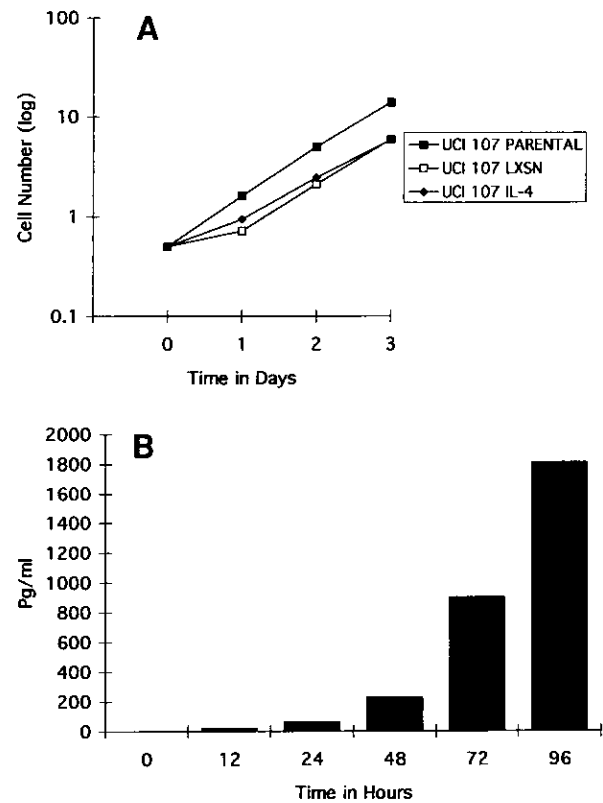


FIG. 2. (A) The growth rates of UCI 107 parental, UCI 107 LXS (vector), and UCI 107 E IL-4 GS cells. Data are shown in a semilog format. Cells were established in complete medium at a density of 0.5×10^6 cells/10 ml in 100-mm tissue culture treated dishes. Cell counts were conducted every 12, 24, 48, and 72 hr and the total number of viable cells was determined using trypan blue exclusion. The results shown are the average values from two different experiments. The variation was not greater than $\pm 10\%$. (B) Kinetics of IL-4 production by UCI 107 E IL-4 GS cells. Cells were established in complete medium 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 hr, and levels of IL-4 were measured by ELISA. The results of one experiment are shown and are representative of two separate studies.

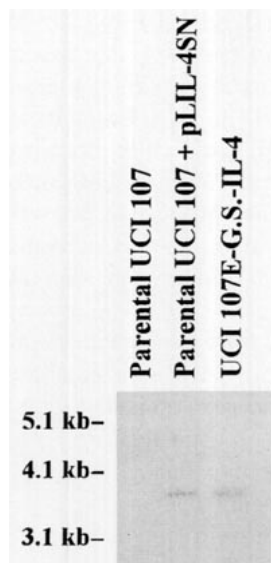


FIG. 3. Southern blot analysis of UCI 107 parental, parental UCI 107 + pLIL-4SN, and UCI 107 E IL-4 GS. *Sst*I-digested DNAs were hybridized with the random primer *neo* probe. The autoradiogram shown indicates the expected 3.5-kb band in UCI 107 E IL-4 GS cells and not parental UCI 107 cells. The line indicated as parental UCI 107 + pLIL-4SN represent 10 μ g of *Sst*I-digested parental DNA mixed with 10 pg of *Sst*I-digested pLIL-4SN plasmid.

vector had no detectable effect in up- or downregulating the antigens positively expressed in the parental line.

Expression of MHC proteins, ICAM-1, and TAA on UCI 107E IL-4 GS cells after irradiation. Membrane expression of MHC proteins and TAA in irradiated cells was examined by FACS at Days 2 and 8 *in vitro*. A significant increase in expression of MHC class I and Her-2/*neu* antigens was observed in irradiated cells when compared to the nonirradiated controls (Fig. 6 and Table 2). While this was noted for cells treated with all doses of irradiation, there was a trend toward higher expression associated with the higher doses. However, γ irradiation did not induce *neo* expression of HLA class II, ICAM-1, or the TAA CA125.

The cell line UCI 107 E IL-4 GS is free of microorganisms. The UCI 107E IL-4 GS cell line was extensively tested for the presence of various microorganisms by our own and outside laboratories. The results revealed that the 107E IL-4 GS 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 [33–35]. In the majority of patients, however, systemic administration of high doses

of cytokines has been associated with severe toxicity [33, 36–38]. Local, continuous delivery of cytokines by tumor cells genetically engineered to produce these molecules provides an alternative approach for tumor immunotherapy with fewer or even no side effects. Furthermore, this delivery system would also bypass the short half-life and the dilutional effect of cytokines when administered systemically.

Three different human serous papillary ovarian carcinoma cell lines were transduced with the human gene for IL-4. We found that clones derived from the UCI 107 cell line were generally better IL-4 producers than all clones from both UCI 101 and SKOV-3, suggesting that different cell lines may have the potential to be better cytokine producers than others. A clone of UCI 107 cells which produces high

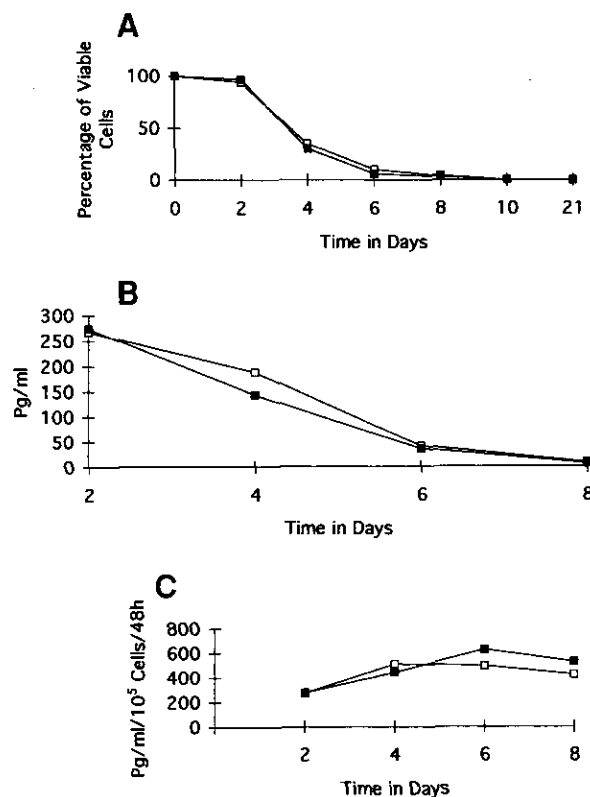


FIG. 4. (A) The effects of γ irradiation on the viability of UCI 107E IL-4 GS cells *in vitro*. No statistically significant differences in survival were seen among cells irradiated at 5000 and 10,000 rad (see Results). The results of one experiment are shown and they are representative of three different studies. (B) IL-4 production by UCI 107E IL-4GS cells after irradiation at 5000 and 10,000 rad. The results from a single experiment reveal that cytokine production was detectable for 8 days after irradiation. No significant differences in IL-4 production were detected between these two doses (see Results). The results are representative of three separate experiments. (C) IL-4 production standardized in pg/ml/ 10^5 cells/48 hr by UCI 107E IL-4GS cells after irradiation at 5000 and 10,000 rad. The results are representative of two separate experiments. No significant differences in IL-4 production were detected between these two doses of irradiation (see Results). □, UCI 107E IL-4GS cells after irradiation at 5000 rad; ■, UCI 107E IL-4GS cells after irradiation at 10,000 rad.

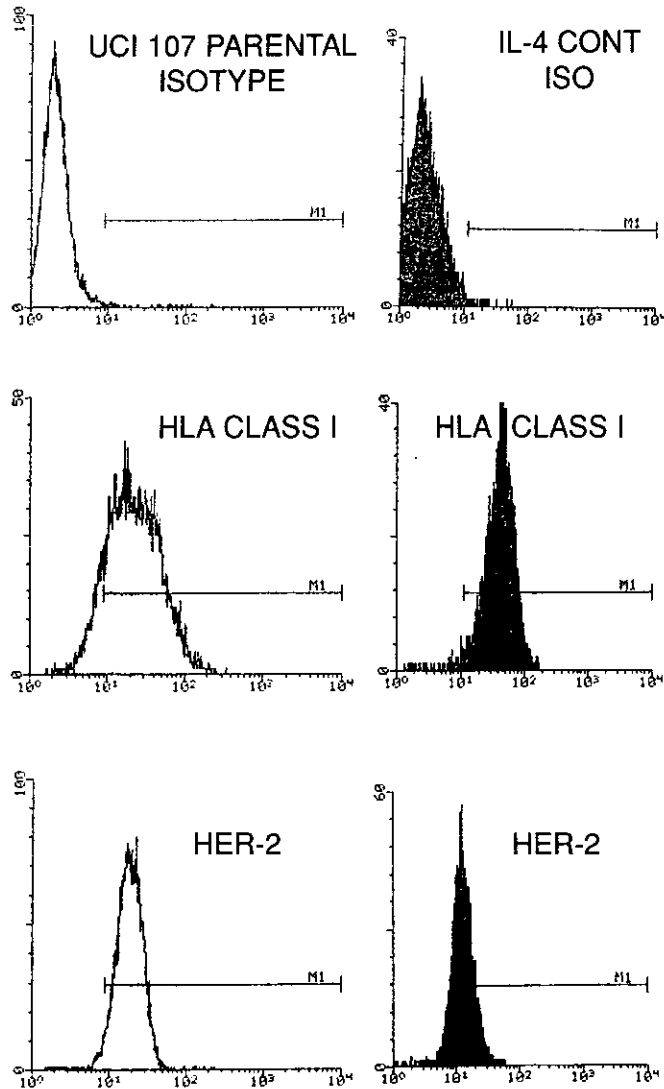


FIG. 5. Expression of cell surface proteins in parental and UCI 107E IL-4 GS cells. Parentals and UCI 107E IL-4 GS cells were subjected to FACS analysis as described under Materials and Methods. These cells constitutively express MHC class I antigens [PPC = 87.5%, MFI = 33.6 and PPC = 96.7%, MFI = 46.7, respectively], *Her-2/neu* [PPC = 95.8%, MFI = 20.8 and PPC = 62.1%, MFI = 16.5], respectively, but did not express MHC class II determinants, CA 125, ICAM-1, and IL-4 receptors (data not shown). The production of IL-4 or the expression of the vector had no detectable effect in up- or downregulating the antigens positively expressed in the parental line. IL-4 CONT, UCI 107E IL-4 GS cells; HER-2, *Her-2/neu*.

levels of IL-4, termed UCI 107E IL-4 GS, was selected and extensively characterized. UCI 107E IL-4 GS cells have the LXSIL-4 vector stably inserted into their DNA as determined by Southern blot analysis and IL-4 production appears to be a stable phenotype. These cells did not differ from parental or LXSIL-4 vector control cells in morphologic characteristics or their surface antigen expression. Like the parental UCI 107 cell line, UCI 107E IL-4 GS does not

express MHC class II, ICAM-1, or CA 125 proteins but does express MHC class I and the tumor-associated antigen *Her-2/neu*. The failure to detect changes in surface antigen expression in UCI 107E IL-4 GS cells was not surprising since UCI 107E IL-4 GS cells did not express detectable IL-4 receptors and therefore would not be able to respond to the IL-4 which they produce. Moreover, it is well known that the main action of IL-4 is activating host antitumor effector mechanisms rather than affecting tumor cells directly.

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

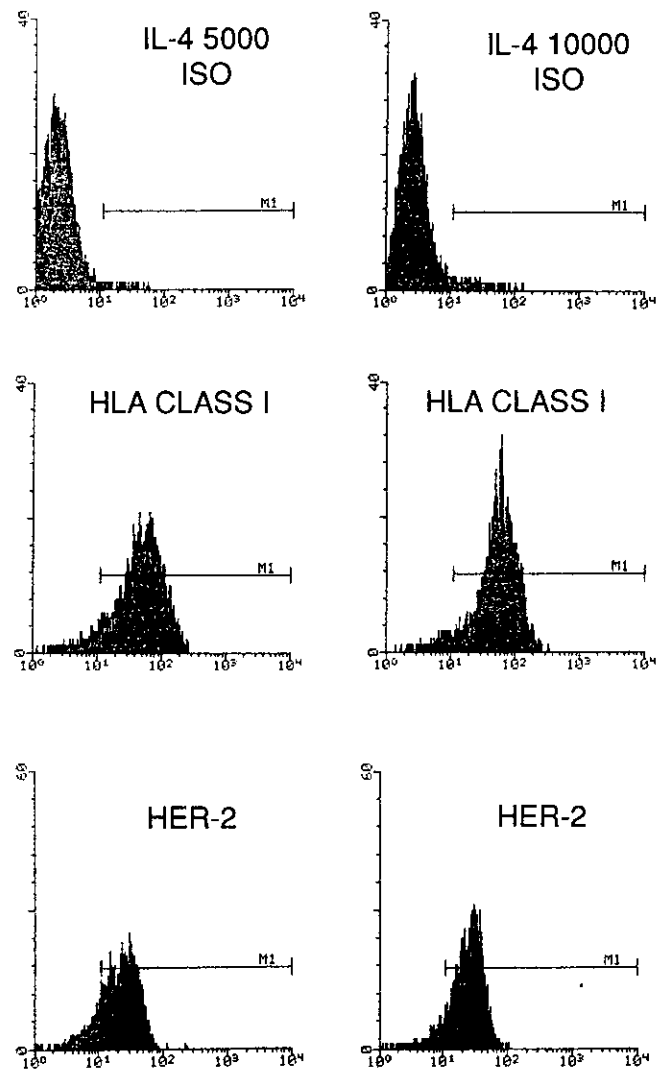


FIG. 6. Expression of cell surface proteins in irradiated UCI 107E IL-4 GS cells. Membrane protein expression in irradiated cells was measured by FACS as described in the legend to Fig. 5 at Day 2 *in vitro*. Increased expression of MHC class I and *Her-2* (*Her2/neu*) was observed in irradiated cells compared to the nonirradiated controls (see Results). While the data are not shown, γ irradiation did not induce neo expression of HLA class II, ICAM-1, or the TAA CA125.

TABLE 2
FACS Analysis of UCI-107 E IL-4 GS after Irradiation

Cell line		HLA class I		Her-2/neu	
		5000 rad	10,000 rad	5000 rad	10,000 rad
UCI-107 IL-4 GS, 2 days	PPC ^a	86.2 ± 9.8	89.0 ± 10.2	74.7 ± 22.7	79.7 ± 24.7
	MFI ^b	133.7 ± 86.9	161.5 ± 91.9	57.5 ± 24.6	58.2 ± 29.9
	<i>P</i> *	0.02	0.008	0.01	0.02
UCI-107 IL-4 GS, 8 days	PPC ^a	66 ± 19	70.5 ± 20.5	70 ± 39.5	69.5 ± 41.7
	MFI ^b	70 ± 14.1	89.5 ± 9.1	48.5 ± 16.2	88 ± 38.1
	<i>P</i> *	0.03	0.002	0.03	0.02

Note. Membrane expression of MHC proteins and TAA in irradiated cells was examined by FACS at Days 2 and 8 *in vitro*. A significant increase in expression of MHC class I and Her-2/neu was observed in irradiated cells as compared to the nonirradiated controls. γ irradiation did not induce neo expression of HLA class II, ICAM-I, or the TAA CA 125 (data not shown).

^a PPC, percentage of positive cells.

^b MFI, mean channel fluorescence intensity.

**P* values of irradiated cells vs untreated controls.

not reduce the capability of these tumor cell lines to secrete high levels of the specific cytokine transduced or their immunogenicity. Because high-dose γ irradiation is one of the best methods to achieve this goal, we examined the effect of different doses of irradiation on viability, lymphokine production, and antigen expression of the highest IL-4 producer clone (UCI 107E IL-4 GS). Cells irradiated between 2500 and 10,000 rad remained viable for about 8 days but all the cells were dead by 3 weeks. In contrast, cells irradiated with 1000 rad recuperated and continued to proliferate. Levels of cytokine production were detectable for 8 days after irradiation and closely paralleled the number of viable cells. However, the few remaining viable cells were still producing cytokines at levels comparable to unirradiated controls cells. Interestingly, irradiation induced a significant upregulation of both MHC class I and Her-2/neu surface antigens and they remained upregulated for as long as the cells remained viable. However, γ irradiation did not induce neo expression of antigens previously not expressed. These data are consistent with the enhanced immunogenicity of many murine tumors following irradiation [4]. Collectively, these results indicate that UCI 107E IL-4 GS cells can be irradiated to effectively stop replication, yet maintain production of IL-4 for up to a week. Finally, extensive tests performed on the UCI 107E IL-4 GS master cell bank (MCB) revealed that this line is free of the presence of mycoplasma, bacteria, and infectious viruses.

There are multiple reasons for testing an allogeneic vaccine in women with advanced ovarian cancer. Unfortunately, this must be tested directly without preclinical animal results for there is no relevant immunocompetent animal model for this disease. The vaccine would be employed in women with advanced epithelial ovarian cancer and the cell line will be admixed with irradiated patient's autologous tumor cells obtained at the time of surgical debulking. This cell mixture

should provide two powerful signals to activate the host immune system in the local injection site: alloantigens on the GATC and IL-4 production. We believe that these strong activating signals could provide the microenvironment that will encourage recognition of the bystander autologous tumor cells by host lymphoid cells.

There are several types of evidence that support the above treatment plan for these women. First, allogeneic cells provide a strong immunologic stimulus that can direct a host response even against tumor cells not previously recognized by autologous lymphocytes [39, 40]. Second, the presence of autologous tumor antigens at the site of the cytokine (IL-4) 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 fresh or cryopreserved autologous tumor cells mixed with the IL-4-secreting allogeneic cell line eliminates the need to culture the tumor and also reduces the possible selective loss of critical tumor-specific antigens.

We believe the development of a cytokine-secreting allogeneic vaccine for the treatment of 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 antitumor immunity. The future design and implementation of clinical trials in this regard will ultimately determine the validity of this approach.

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