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Development and characterization of an interleukin-2–transduced human ovarian carcinoma tumor vaccine not expressing major histocompatibility complex molecules

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OBJECTIVE: We initiated studies to develop cytokine-secreting human ovarian carcinoma cells for the purpose of using these cells as vaccines for the treatment of advanced epithelial ovarian cancer.
STUDY DESIGN: A human ovarian carcinoma cell line (UCI-107) was genetically engineered to secrete the cytokine interleukin-2 by retroviral-mediated gene transduction.
RESULTS: One clone, termed UCI-107A IL-2 AS, constitutively secreted high levels of interleukin-2 (i.e., 2000 to 2300 pg/ml/10^5 cells per 48 hours) for >55 passages and 8 months of study. Unlike parental- and vector-transduced cells, UCI-107A IL-2 AS cells were aneuploid and failed to express major histocompatibility complex class I and HER2/*neu* surface antigens. UCI-107A IL-2 AS cells were highly resistant to killing by gamma irradiation and continued to produce high levels of interleukin-2 even after irradiation with 10,000 cGy. Balb/C nude mice injected intraperitoneally with UCI-107A IL-2 AS cells survived significantly longer than control animals, with 25% of the animals totally rejecting their tumors. UCI-107A IL-2 AS was totally resistant to killing by fresh allogeneic peripheral blood lymphocytes in four hour chromium 51 release assays but induced high levels of killing in 72-hour long-term cytotoxic assays.
CONCLUSION: The potential use of these interleukin-2–secreting ovarian carcinoma cells as vaccines for women with advanced ovarian cancer will be discussed. (Am J Obstet Gynecol 1996;174:633-40.)

Key words: Interleukin-2, ovarian cancer, tumor vaccine

Ovarian carcinoma is the leading cause of death from gynecologic malignancy among women in the United States and Europe.[1] Even with platinum-based chemotherapy, which has led to improved response and remission rates and a prolongation of survival, only about 20% of patients with advanced disease are alive 5 years after diagnosis.[1] It is therefore obvious that other therapies must be developed to prolong survival of patients with such advanced disease.

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Spontaneously arising tumors, in contrast to chemically or virally induced tumors, are believed to undergo a process of immunoselection in which surviving tumor cells are able to escape host defenses. As a result of this selection, these tumors have been considered to lack tumor-specific antigens and therefore to be incapable of eliciting an immune response.[2] However, the oncogenic process is characterized by the accumulation of multiple mutational events within the transformed cell.[3] The large number of genetic alterations found in advanced cancers make it likely that peptides from mutated proteins may serve as tumor antigens when bound to major histocompatibility complex (MHC) class I or II molecules. The number of potential tumor antigens can include any amino acid sequence carrying a single point mutation in any cellular protein, either membrane bound or intracellular. These peptides, specific to the tumor cell, may be used as target antigens for recognition and activation of specific cytotoxic T cells.[4] Recent results indicate that even spontaneous and poorly immunogeneic murine tumors can be recognized by antigen-specific effector cells if the tumor cells are genetically altered to secrete cytokines.[5-8] Hence it is possible that the immune system fails

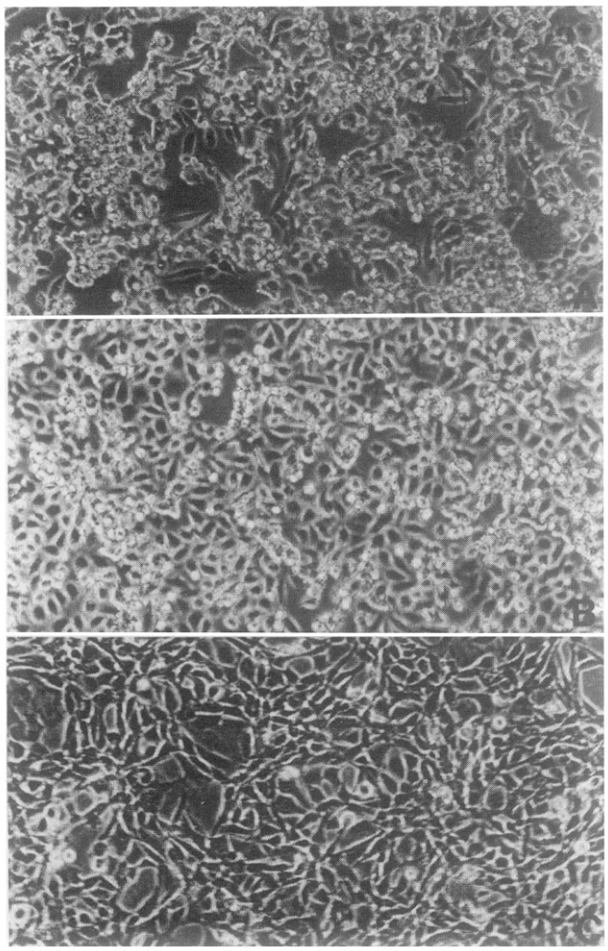

Fig. 1. Morphologic features of UCI-107 parental **(A)**, UCI-107 LXSN (vector) **(B)**, and UCI-107A IL-2 AS **(C)**. (Original magnification ×10.)

to eliminate spontaneously arising tumors not because of the absence of specific tumor antigens but because the response to these antigens under normal conditions is inadequate. The ability to induce specific immunity even against parental untransduced cells or against an unrelated tumor when such cells are combined with genetically altered tumor cells has clearly shown that the expression of the cytokine by the tumor itself is unnecessary and what is required is the presence of the tumor "antigens" at the local site of the cytokine production.

Interleukin-2 (IL-2), because of its central regulatory role in the immune response, appears to be an attractive candidate for the development of genetically altered tumor cells for human vaccine studies. IL-2 is a well-known growth factor for T-helper and T-cytotoxic lymphocytes and has since been also shown to participate in activation of B lymphocytes and natural killer cells, generate lymphokine-activated killer cell activity, and trigger nonspecific cytotoxicity in macrophages and neutrophils.[7, 9] Injection of low doses of IL-2 in vivo reverses T-lymphocyte unresponsiveness,[10] nullifies acquired tolerance to allogeneic cells,[11] and converts a stimulus causing unresponsiveness into one causing hypersensitivity.[12] In addition, in several murine models evaluating the immune response generated by genetically altered tumor cells, IL-2 stood out as an effective cytokine at inducing systemic immune responses against preestablished tumors and metastases.[7, 8]

The lack of a suitable preclinical immunocompetent animal model for ovarian carcinoma and the dismal prognosis for patients with advanced ovarian cancer provide a strong rationale for developing genetically altered tumor cells for vaccine trials in patients with this disease. Therefore we have generated and characterized a human ovarian carcinoma cell line genetically engineered to secrete high levels of IL-2 by retroviral-mediated gene transduction. This is the first report of such an IL-2–producing human ovarian carcinoma cell line. The potential use of these cells as a vaccine for women with advanced ovarian cancer will be discussed.

Material 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; it has been previously characterized.[13] UCI-107 cells were maintained at 37° C in 5% carbon dioxide in complete media containing RPMI 1640 with 10% fetal bovine serum.

Construction of retroviral vectors. The pLXSN plasmid was provided by Dr. A. Dusty Miller (Fred Hutchinson Cancer Center, Seattle, Wash.). This plasmid, derived from a Maloney murine leukemia virus, contains the neophosphotransferase gene whose constitutive expression is driven by the SV40 enhancer-promoter, and the 5′ retroviral long terminal repeat of the integrated vector drives the expression of an inserted gene.[14] The human IL-2 complementary deoxyribonucleic acid (DNA) was obtained from American Type Culture Collection (Rockville, Md.) in the Okaiama and Berg pCD cloning vector and was excised with *Bam*HI restriction enzyme. The complementary DNA was then cloned into the *Bam*HI restriction site in the multiple cloning region of pLXSN. Proper orientation of the complementary DNA was determined by diagnostic restriction endonuclease digests. Once constructed, retroviral plasmid DNA was then purified by cesium chloride gradient density centrifugation.

Retroviral transduction and selection of clones. Infectious replication incompetent retroviral vectors (LXSN/LSXN-IL-2) obtained from the PA317 packaging cell lines were used to transduce 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 culture media and incubated for 4 hours at 37° C in 5% carbon dioxide to allow adherence. After incubation the media were aspirated and replaced with 5 ml of 0.2% polybrene in phosphate-buffered saline solution, pH 7.3. After 30 minutes at 37° C in 5% carbon dioxide, 10 ml of retroviral supernatant was added, and retroviral-mediated gene

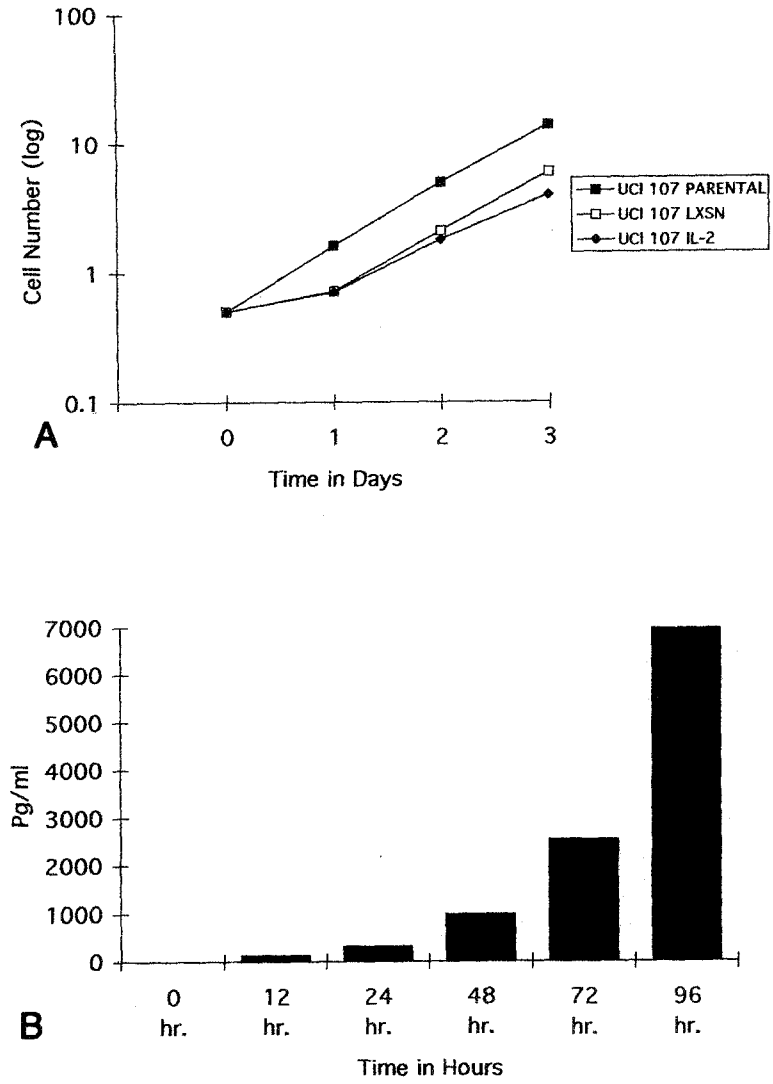

Fig. 2. A, Growth rates of UCI-107 parental, UCI-107 LXSN (vector), and UCI-107A IL-2 AS cells. Data are shown in semilog format. Cells were established in complete media at density of 0.5×10^6 cells/10 ml in 100 mm tissue culture–treated dishes. Cell counts were conducted every 12, 24, 48, and 72 hours, and total number of viable cells was determined by trypan blue exclusion. Results shown are average values from two different experiments. SEM was not >±10%. **B,** Kinetics of IL-2 production by UCI-107A IL-2 AS cells. These cells were established in complete media at 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 hours and leves of IL-2 measured by ELISA. Results of one experiment are shown and are representative of two separate studies.

transfer was accomplished by overnight incubation. Supernatants were then aspirated and replaced with culture media. After an additional 48-hour incubation in culture media at 37° C in 5% carbon dioxide, selection of transduced clones was accomplished by culture in culture media containing 0.075% G418. Clones were isolated after 14 days with sterile 8 × 8 mm cloning cylinders and expanded for 3 weeks in culture media containing G418.

In vitro growth characteristics. Cells were established in culture media 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 hours, and the number of viable cells was determined with trypan blue exclusion. Experiments were conducted to compare the growth of nontransduced (parental) and transduced tumor cell lines (UCI-107A IL-2 AS and vector control) and to evaluate the level of cytokine production over time. Supernatants were collected and frozen at −20° C (for subsequent enzyme-linked immunosorbent assay [ELISA] evaluation of cytokine levels) and culture dishes trypsinized to determine cell count and viability.

Analysis of human IL-2 secretion. Parental IL-2 transductants and vector control cells were seeded in 100 mm tissue culture dishes (Corning, Corning, N.Y.) at a density of 1×10^6 cells/ml in 10 ml of culture media. After a 48-hour incubation at 37° C in 5% carbon dioxide super-

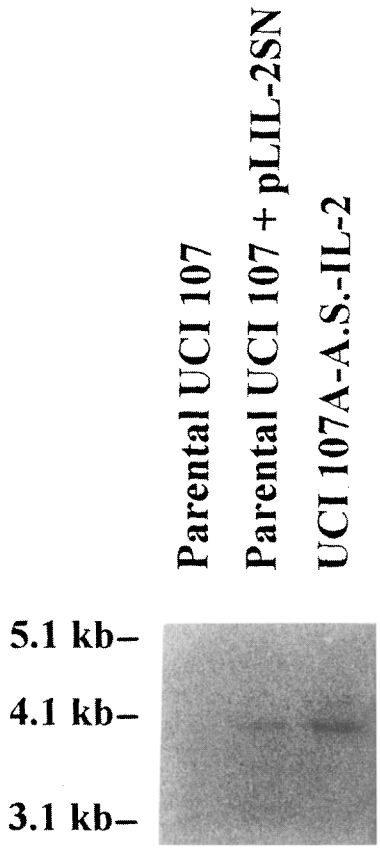

Fig. 3. Southern blot analysis of UCI-107 parental, parental UCI-107 + pLIL-2SN, and UCI-107A IL-2 AS. *Sst* I–digested DNAs were hybridized with random primer *neo* probe. Autoradiogram indicates expected 3.5 kb band in UCI-107A IL-2 AS cells and not parental UCI-107 cells. *Line* indicated as parental UCI-107 + pLIL-2SN represents 10 μg of *Sst* I–digested parental DNA mixed with 10 pg of *Sst* I–digested pLIL-2SN plasmid.

natant was aspirated and rendered cell free by centrifugation for subsequent evaluation by ELISA (Research & Diagnostic Systems, Minneapolis). The biologic activity of IL-2 was confirmed with a cell proliferation assay that used an IL-2–dependent murine cytotoxic cell line, CTLL-2.[15]

Southern blot analysis. Southern blot and hybridization analyses were performed as described by Strauss[16] and Tabor and Struhl,[17] respectively.

Cell surface antigen analysis. Cells were labeled with anti-HLA class I (monoclonal antibody W6/32, Accurate Chemical and Scientific Corporation, Westbury, N.Y.), anti-HLA class II (monoclonal antibody CR3-43, Accurate Chemical and Scientific Corporation), anti-ICAM-1 (monoclonal antibody LB-2, Becton-Dickinson, San Jose, Calif.), anti-CA 125 (monoclonal antibody OC125, Signet Laboratories, Dedham, Mass.), anti-HER-2/*neu* p185 (monoclonal antibodies TA-1, Oncogene Science, Uniondale, N.Y.) and analyzed for antigen expression with a fluorescence-activated cell sorter analyzer (Becton-Dickinson).

Irradiation of cell lines. UCI-107A IL-2 AS cells were irradiated in a 15 ml conical tube in culture media at room temperature with gamma rays (cesium 137) at a dose rate of 200 cGy/min. Cells were then seeded in a petri dish culture plate at a density of 1×10^6 cells in 10 ml of culture media. Test doses of 5000 and 10,000 cGy were applied. Every 48 hours culture supernatant was collected from the dish for cytokine production, and the number of viable cells was assessed by light microscopy with trypan blue exclusion. Irradiated cells cultured in separate T75 tissue culture flasks were harvested at day 2 for determination of surface antigen expression by fluorescence-activated cell sorter analysis.

Tumor formation in nude mice. UCI-107A IL-2 AS and UCI-107 LXSN vector control cells (10×10^6 cells in 0.5 ml of RPMI 1640 supplemented with 10% previously frozen cell-free ascites derived from mice bearing UCI-107 parental tumor) were injected intraperitoneally into 7-week-old female nude mice of Balb/C origin housed in an American Association of Laboratory Animal Science–approved facility. The UCI-107 parental cell line has already been reported to grow consistently when injected intraperitoneally in Balb/c nude mice at a dose of 10×10^6 cells.[13]

In vitro cytotoxicity assay

Chromium 51 release assay. Cytolytic activity of freshly isolated peripheral blood lymphoid cells collected from normal donors was measured in a 4- and 18-hour ^{51}Cr release microplate assay by methods described by Yamamoto et al.[18] The release of ^{51}Cr (experimental release, ER) was measured by the uptake of cell-free supernatants with a Titertek supernatant collection system (Skraton, Norway) and quantified in an automated biogamma counter (Beckman, Fullerton, Calif.). Total ^{51}Cr released (TR) was determined by lysing the cells with 100 μl of a 3% (wt/vol) sodium dodecyl sulfate solution. Spontaneous ^{51}Cr release (SR) was determined from target cells receiving culture media only and was found to be 1% to 2% per hour. Percent lysis was determined by the following formula:

$$\text{Specific lysis } (\%) = \frac{ER - SR}{TR - SR} \times 100$$

Long-term cytotoxicity assay. The antitumor potential of peripheral blood lymphoid cells from healthy donors was assessed with a previously described 72-hour long-term cytotoxicity assay that uses a crystal violet dye method.[19] The degree of tumor cell destruction mediated by peripheral blood lymphoid cell (PBL) was then quantified by the following formula:

$$\text{Cytotoxicity } (\%) = 1 - \frac{\text{Absorbance of tumor cells treated with PBL}}{\text{Absorbance of tumor cells in culture media}} \times 100$$

Statistical analysis. Significance analysis was performed by use of a paired Student *t* test. For survival statistical analysis, the BMDP statistical program was used (BMDP Statistical Software, Los Angeles).

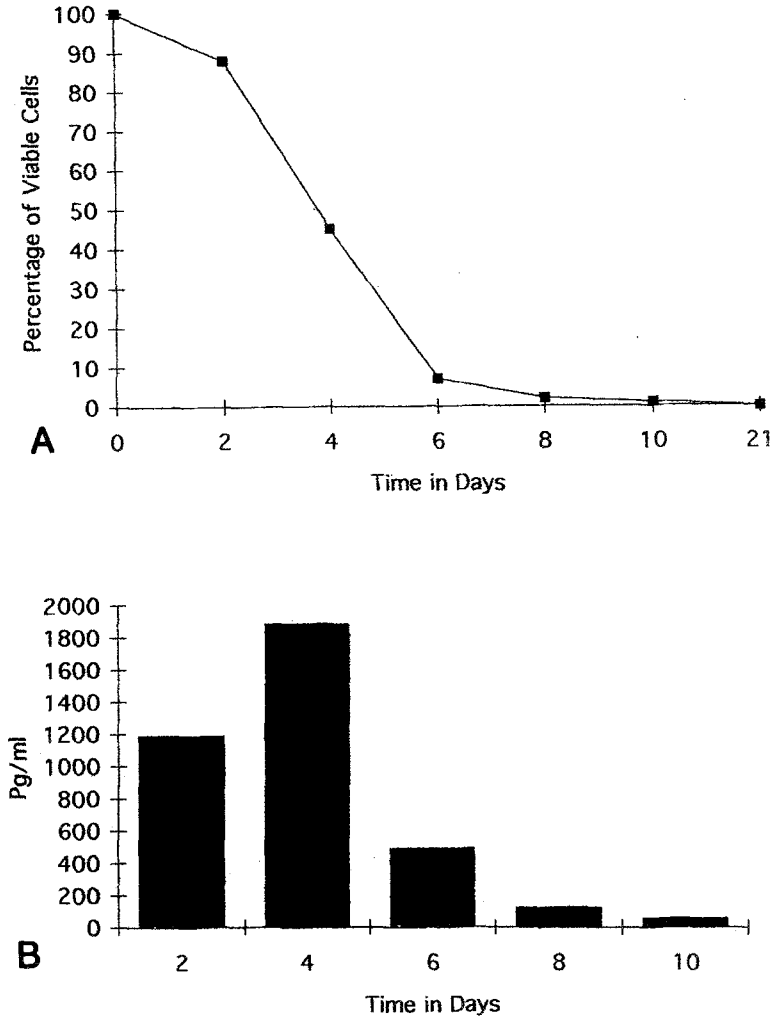

Fig. 4. A, Effects of gamma irradiation (10,000 cGy) on viability of UCI-107A IL-2 AS cells in vitro. Single cell suspension of UCI-107A IL-2 AS cells were irradiated and then established as monolayers as described in Material and Methods. Results of one experiment are shown, and they are representative of two different studies giving similar results. Difference between result of duplicate experiments was <10%. **B,** IL-2 production by UCI-107A IL-2 AS cells after irradiation at 10,000 cGy. Results are representative of two separate experiments giving similar results.

Results

Transduction and selection of a clone of UCI-107 cell line secreting high levels of IL-2. UCI-107 human ovarian carcinoma cells were transduced with LXSN and LXSN-IL-2 vectors, and clones were identified and expanded after selection in G418-containing media, as described in material methods. As expected, parental UCI-107 cells and cells transduced with the LXSN vector alone did not produce detectable levels of IL-2. Of 13 clones selected, the highest IL-2 producer, termed UCI-107A IL-2 AS, was shown to have a level of IL-2 production consistently in the range of 2000 to 2300 pg/ml/10^5 cells per 48 hours. This clone was expanded and used to form a master cell bank for further testing and characterization.

Morphologic and growth characteristics of UCI-107A IL-2 AS cells in vitro. The morphologic features of UCI-107 cells transduced with the LXSN vector were indistinguishable from those of parental UCI-107 cells. In contrast, UCI-107 cells containing the IL-2 gene exhibited significantly altered morphologic features, being much more spindle-shaped than were parental cells (Fig. 1). The doubling time of parental, vector control, and UCI-107A IL-2AS cells was determined to be 15.3, 15.7, and 19.2 hours, respectively (Fig. 2, *A*). The growth rates and morphologic characteristic observed in vitro have been stable for >55 passages and 8 months of culture.

Kinetics and stability of IL-2 production by UCI-107A IL-2 AS cells in vitro. Supernatants were collected from UCI-107A IL-2 AS cell cultures at various intervals, and levels of IL-2 were measured by ELISA. The results in Fig. 2, *B,* show IL-2 production over a period of 96 hours. Stability studies were conducted over an 8-month period and a total of 55 passages. During this observation period levels of IL-2 production were consistently in the range of

Table I. Killing mediated by fresh allogeneic peripheral blood lymphoid cells against UCI-107 parental, UCI-107 LXSN, and UCI-107A IL-2 AS target cells in 4- and 18-hour ^{51}Cr release assays and 72-hour long-term cytotoxic assays

Target cell	*Effector/target ratio*	*4 hr ^{51}Cr release assay*	*18 hr ^{51}Cr release assay*	*72 hr long-term killing assay*
UCI-107, parental	25:1	28.0 ± 1.1	42.3 ± 2	44.5 ± 2.5
	10:1	2.0 ± 1.6	13.7 ± 3.1	11.0 ± 0
	5:1	2.7 ± 1.2	2.7 ± 1.2	9.0 ± 2.0
	2.5:1	0 ± 0	0 ± 0	ND
UCI-107 LXSN (vector control)	25:1	29.9 ± 2.0	42.7 ± 0.9	48.5 ± 5.5
	10:1	6.2 ± 1.0	10.8 ± 6.7	18.0 ± 6.5
	5:1	2.5 ± 0.6	1.6 ± 1.6	13.0 ± 6.0
	2.5:1	0 ± 0	0 ± 0	ND
UCI-107A IL-2 AS	25:1	0 ± 0	54.4 ± 10.4	76.0 ± 0
	10:1	0 ± 0	28.4 ± 1.6	75.0 ± 0
	5:1	0 ± 0	2.4 ± 2.4	69.5 ± 0.5
	2.5:1	0 ± 0	0 ± 0	ND

Values are mean ± SD. *ND,* Not done.

2000 to 2300 pg/ml per 10^5 cells per 48 hours (data not shown).

LXSN IL-2 vector is inserted into DNA of UCI-107A IL-2 AS cell line. The IL-2–secreting clone transduced with LXSN-IL-2 and selected in G418 was evaluated for successful gene insertion by Southern hybridization probing for the *neo* gene after 15 passages. Fig. 3 shows southern blot analysis of UCI-107A IL-2 AS and the parental UCI-107 cell DNA. The presence of the retroviral vector in the DNA of the transduced UCI-107 cells was confirmed.

Survival and stability of IL-2 secretion by UCI-107A IL-2 AS cells after irradiation in vitro. UCI-107A IL-2 AS cells received different levels of gamma irradiation and then were established as monolayers in culture media as described. 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 day 2 for surface antigen expression by fluorescence-activated cell sorter analysis. No effects on cell growth were observed at doses of <5000 cGy. Indeed, after receiving 5000 cGy, a few resistant clones were visible after 7 to 10 days and their levels of IL-2 production were identical to those of unirradiated control cells (data not shown). At higher doses (10,000 cGy) approximately 90% of the cells were viable 48 hours after irradiation, with 45% and 10% viability at 4 and 6 days, respectively. However, all the cells were dead after 3 weeks (Fig. 4, *A*). Secretion of IL-2 by cells irradiated with 10,000 cGy is shown in Fig. 4, *B*. As can be seen, IL-2 production was maintained at high levels for about 6 days, after which cytokine levels rapidly decreased parallel with decreasing viable cell number.

Expression of surface antigens on parental, vector-containing, and IL-2–secreting UCI-107 cells in vitro. Fluorescence-activated cell sorter analysis of parental and vector control cells showed that they express MHC class I antigens (Percentage of positive cells 87.5, mean channel fluorescence intensity 33.6, percentage of positive cells 94.8, mean channel fluorescence intensity 47.7, respectively, HER 2/*neu,* percentage of positive cells 95.8, mean channel fluorescence intensity 20.8, and percentage of positive cells 94.3, mean channel fluorescence 23.6, respectively) but did not express MHC class II determinants, CA 125 or ICAM-1 (data not shown). In contrast, UCI-107A IL-2 AS cells did not express any detectable surface antigens present on the parental or vector control cell lines (data not shown).

Effect of vector insertion and IL-2 production on tumorigenicity of UCI-107 cells in nude mice. Parental UCI-107 cells have been shown to consistently form tumors in Balb/c nude mice when injected intraperitoneally and animals die between 40 and 45 days.[13] Seven and eight 7-week-old female Balb/c nude mice, respectively, were injected intraperitoneally with 10×10^6 LXSN vector control or UCI-107A IL-2 AS cells, and the animals were followed up for 160 days to evaluate tumor formation. Vector control cells formed large solid tumor nodules within 3 weeks after injection, and all the animals died within 27 days (mean survival 25.2 days, range 22 to 27 days). Animals injected with UCI-107A IL-2 AS cells survived significantly longer than control animals did ($p \leq 0.0001$, mean survival 110.2 days, range 51 to 160 days) with 25% of the animals totally rejecting the tumor.

Cytotoxicity of peripheral blood lymphocytes in 4- and 18-hour ^{51}Cr release assays and a 72-hour long-term cytotoxicity assay. Susceptibility of tumors to lysis by freshly isolated normal allogeneic peripheral blood lymphoid cells in 4- and 18-hour ^{51}Cr release cytotoxicity assays and long-term cytotoxicity assay is shown in Table I. Cytotoxic activity against UCI-107 parental and vector control cells was evident at 4 hours. In contrast, the UCI-107A IL-2 AS cells were totally resistant. Killing of UCI-107A IL-2 AS was

evident at 18 hours and continued to increase up to 72 hours.

Cell line UCI-107A IL-2 AS is free of microorganisms. The UCI-107A IL-2 AS cell line was extensively tested for the presence of various microorganisms by our own and outside laboratories and was found to be free of mycoplasma, bacteria, DNA viruses (Epstein-Barr, human hepatitis B, human cytomegalovirus), and replication-competent retroviruses.

Comment

Immunotherapy by systemic administration of immune enhancing cytokines, with or without activated lymphoid cells, has resulted in dramatic clinical responses in some patients with advanced cancer.[20] In the majority of patients, however, systemic administration of high doses of cytokines has been associated with significant toxicity.[20] Local, continuous delivery of cytokines by tumor cells genetically engineered to produce these molecules provides an alternative approach for tumor immunotherapy that should have few, if any, side effects. Furthermore, this delivery system would also bypass the short half-life and the dilutional effect of systemically administered cytokines.

Therefore we initiated studies to develop genetically altered tumor cells from human ovarian carcinoma cell lines with the intention of using these lines as vaccines for treatment of advanced ovarian carcinoma. Our previous experience has shown that clones derived from the UCI-107 cell line were generally better cytokine producers than were clones from other human ovarian carcinoma cell lines (Santin et al., unpublished data). A clone of UCI-107 cells that produces high levels of IL-2, termed UCI-107A IL-2 AS, was selected and extensively characterized. UCI-107A IL-2 AS cells have the LXSN-IL-2 vector stably inserted into their DNA as determined by Southern blot analysis, and IL-2 production was a stable phenotype. However, these cells differed significantly from parental or LXSN vector control cells in morphologic characteristics, DNA content, and surface antigen expression. Although the parental UCI-107 cell line and LXSN vector control cells did express MHC class I and the tumor associated antigen HER2/*neu*, UCI-107A IL-2 AS cells did not express any of these surface antigens. It was interesting that neither irradiation with 5000 or 10,000 cGy nor a brief preincubation with tumor necrosis factor-α and interferon gamma induced the expression of these molecules. The basis for the loss of class I and HER2/*neu* antigens on UCI-107A IL-2 AS is unclear. We believe the loss of these antigens on UCI-107A IL-2 AS cells is not due to the production of IL-2 but rather to antigenic differences in the selected clone. It is of interest, however, that even if this IL-2 producing clone is aneuploid (1.4 × DNA content) compared with parental and vector control cells (1.1 × DNA content), the long-term stability of IL-2 production indicates that the IL-2 gene is stably inserted into the DNA.

Vaccines derived from allogeneic or autologous tumor cell lines require methods to block cell replication to prevent possible tumor formation at the immunization site. Such procedures should not reduce the capability of these cells to secrete high levels of the specific cytokine transduced or reduce their immunogenicity. High-dose gamma irradiation is one of the best methods to achieve this goal. Our studies showed that after high doses of gamma irradiation (10,000 cGy) UCI-107A IL-2 AS cells remained viable for about 10 days and all the cells were dead by 3 weeks. In contrast, cells irradiated with 5000 cGy recuperated and continued to proliferate and secrete high levels of IL-2. It is worth noting that parental UCI-107 and LXSN vector control cells were never able to recuperate after irradiation with 5000 cGy. Cytokine production remained high for about 6 days after 10,000 cGy and then decreased in a manner closely paralleling the number of viable cells. Collectively, these results indicate that UCI-107A IL-2 AS cells can be irradiated to effectively stop replication yet maintain production of IL-2 up to 10 days.

The effect of local cytokine release on tumor formation by xenografted UCI-107A IL-2 AS cells was examined in a nude mouse model. Intraperitoneal injection of LXSN vector control cells resulted in the death of 100% (7/7) of nude mice within 27 days. In contrast, animals injected with UCI-107A IL-2 AS cells had a highly significant longer survival than control animals ($p \leq 0.0001$), with 25% of the animals totally rejecting the tumor. Thus the induction of a nonspecific immune response by these IL-2–secreting tumor cells is capable of destroying the tumor and prolonging animal survival.

Finally, parental UCI-107, LXSN vector control and UCI-107A IL-2 AS cells were tested for their sensitivity to killing by fresh peripheral blood lymphoid cells in short- and long-term cytotoxic assays. Unlike UCI-107 and LXSN vector controls, UCI-107A IL-2 AS cells were found to be totally resistant to killing by peripheral blood lymphoid cells in short-term (4-hour) ^{51}Cr release assays. However, after 18 hours killing was detectable and continued to increase, reaching high levels by 72 hours. Because we have used allogeneic peripheral blood lymphoid cells, natural killer cell activity in the 4-hour assay and lymphokine-activated killer cell activation by the secreted IL-2 in the long-term assays are likely to be the most reasonable explanations of such results. The failure of UCI-107A IL-2 AS cells to express MHC antigens and ICAM-1 may explain in part its resistance to natural killer cell activity. However, the protracted killing of UCI-107A IL-2 AS cells as noted in these in vitro assays may provide an advantage to the use of these cells as vaccines. Because we have planned to use our transduced line as an adjuvant mixed with the irradiated patient's autologous tu-

mor cells, the lack of expression of MHC class I, class II, and ICAM-1 antigens should reduce a strong antiallogeneic response that would ordinarily quickly eliminate the vaccine cells. In contrast, UCI-107A IL-2 AS cells that lack MHC antigens would serve mainly as biologic pumps secreting IL-2 and may survive longer at the immunization site. In murine models with genetically altered tumor cells secreting IL-2 it has already been demonstrated that rapid destruction of the genetically altered tumor cells occur when high levels of IL-2 stimulate a too-powerful local immunologic reaction. In this setting little, if any, systemic immunity is developed.[7]

There are multiple reasons for testing an allogeneic IL-2–secreting tumor vaccine mixed with the patient's autologous tumor cells in women with advanced epithelial ovarian cancer. First, this allogeneic cell line is well characterized: (1) it has been shown to stably produce high levels of IL-2, (2) this production is resistant to high-dose gamma irradiation, (3) the vaccine is devoid of strong allogeneic signals, and (4) it is free of infectious agents including competent retrovirus. Second, the presence of autologous tumor antigens at the site of IL-2 secretion overcomes the necessity of sharing the MHC 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 IL-2–secreting allogeneic vaccine eliminates the need to culture each autologous cell line in an attempt to obtain an autologous transduced vaccine.

We believe the development of a cytokine-secreting allogeneic vaccine not expessing MHC molecules mixed with the patients autologous tumor for the treatment of cancer could be an attractive alternative to currently developed cytokine-transduced autologous vaccines. Allogeneic vaccines with such qualities will have the benefit of careful standardization and quality control and will offer high cytokine production to enhance host antitumor immunity. The future design and implementation of clinical trials will ultimately determine the validity of this approach.

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