IL-2 Plasmid Therapy of Murine Ovarian Carcinoma Inhibits the Growth of Tumor Ascites and Alters Its Cytokine Profile

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IL-2 Plasmid Therapy of Murine Ovarian Carcinoma Inhibits the Growth of Tumor Ascites and Alters Its Cytokine Profile

Holly M. Horton,1* Oliver Dorigo, † Pepe Hernandez, * Deborah Anderson, * Jonathan S. Berek, ‡ and Suezanee E. Parker*‡

We have evaluated whether i.p. murine ovarian tumors could be treated with an IL-2 plasmid DNA complexed with the cationic lipid, (±)-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide/dioleoylphosphatidylethanolamine (DMRIE/DOPE). Reporter gene studies were initially conducted in which mice bearing i.p. murine ovarian teratocarcinoma (MOT) were injected i.p. with reporter gene plasmid DNA (pDNA):DMRIE/DOPE. Histochemical analyses revealed that transfection occurred primarily in the tumor cells of the ascites, with only a minority of other ascitic cells or surrounding tissues transfected. IL-2 levels in the MOT ascites were determined after i.p. injection of either IL-2 pDNA:DMRIE/DOPE or recombinant IL-2 protein. IL-2 was detected in tumor ascites for up to 10 days after a single i.p. injection of IL-2 pDNA:DMRIE/DOPE, but was undetectable 24 h after a single i.p. injection of IL-2 protein. In an antitumor efficacy study, MOT tumor-bearing mice injected i.p. with IL-2 pDNA:DMRIE/DOPE on days 5, 8, and 11 after tumor cell implant had a significant inhibition of tumor growth (< 0.001) as well as a significant increase in survival (p < 0.008). A cytokine profile of the MOT tumor ascites revealed that mice treated with IL-2 pDNA:DMRIE/DOPE had an IL-2-specific increase in the levels of IFN-γ and GM-CSF. Taken together, these findings indicate that i.p. treatment of ovarian tumors with IL-2 pDNA:DMRIE/DOPE can lead to an increase in local IL-2 levels, a change in the cytokine profile of the tumor ascites, and a significant antitumor effect. The Journal of Immunology, 1999, 163: 6378–6385.

Ovarian cancer is the fourth leading cause of cancer death among women in the U.S. with ~27,000 new cases and 14,000 deaths annually (1). Due to the lack of effective screening and the fact that early-stage disease manifests few symptoms, most ovarian cancer cases are diagnosed at an advanced stage when the disease has metastasized to the peritoneal cavity (2). Current treatment options for ovarian cancer involve surgical debulking followed by chemotherapy. Although initial response rates are high, the development of resistance to chemotherapeutic agents is common, resulting in a 5-year survival rate of only 20% for advanced-stage disease (3).

Immunotherapy using rIL-2 protein has been used with some success in ovarian cancer clinical trials; however, the requirement for frequent administration of high doses of the protein often resulted in dose-limiting side-effects (4–11). In the present research, we evaluated whether treatment of ovarian cancer with IL-2 could be more effective if the cytokine was delivered by injection of an IL-2-expressing plasmid DNA (pDNA)2 (2). For these studies, we used a model of advanced disease in which C3H/HeN mice were injected i.p. with murine ovarian teratocarcinoma (MOT) cells to establish peritoneal tumors (12, 13). The MOT model shares many of the characteristics of advanced ovarian cancer, including production of malignant ascites, peritoneal spread, lymphatic obstruction, and resistance to chemotherapy (14, 15).

Using the MOT model, reporter gene studies indicated that the i.p. ovarian tumor cells were targeted by i.p. injection of pDNA complexed with the cationic lipid DMRIE/DOPE, with minimal transfection of other cells in the ascites or of surrounding tissues. Using an IL-2-expressing pDNA:DMRIE/DOPE complex, we compared the levels of IL-2 after i.p. injection of either IL-2 pDNA:DMRIE/DOPE or rIL-2 protein. Although a single i.p. injection of IL-2 pDNA:DMRIE/DOPE resulted in sustained levels of IL-2 in the tumor ascites (up to 10 days), IL-2 levels after injection of IL-2 protein were short-lived (≤ 24 h). Therapy of MOT tumor-bearing mice with IL-2 pDNA:DMRIE/DOPE resulted in a significant antitumor effect as well as an IL-2-specific increase in the levels of IFN-γ and GM-CSF in the tumor ascites. The results of this research support the further evaluation of IL-2 pDNA:lipid therapy for treatment of advanced ovarian cancer.

Materials and Methods

Plasmids

The IL-2 pDNA used in these studies, VR1110, was constructed by cloning murine IL-2 DNA into the eukaryotic expression vector, VR1012, as previously described (16). VR1012 contains a bacterial kanamycin resistance gene and directs eukaryotic gene expression from a cassette containing the human CMV immediate early I gene promoter/enhancer and a transcriptional terminator region derived from the bovine growth hormone gene. The backbone pDNA, VR1012, served as the control pDNA for all studies. The pDNAs used in the reporter gene studies were VR1223, containing the luciferase gene cloned into VR1012 (17), and VR1412, containing the β-galactosidase (β-gal) gene cloned into VR1012 (18).

Plasmid DNA purification

Plasmid DNA was prepared by bacterial fermentation (17) and purified by standard double CsCl-ethidium bromide gradient ultracentrifugation followed by ethanol precipitation and dialysis. All plasmid preparations were free of detectable RNA, and endotoxin levels were < 0.06 endotoxin units.

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‡ Abbreviations used in this paper: pDNA, plasmid DNA; MOT, murine ovarian teratocarcinoma; RLU, relative light units; β-gal, β-galactosidase; DMRIE, (±)-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanaminium bromide; DOPE, dioleoylphosphatidylethanolamine.

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units/μg of plasmid DNA. The spectrophotometric A260/A280 ratios were between 1.75 and 2.0.

Cell lines

MOT cells were obtained from Drs. Robert Knapp and Robert C. Bast at the Dana-Farber Cancer Center (Boston, MA). The MOT cells were grown by serial i.p. transplantation of 10⁵ cells in C3H/HeN mice followed by collection of tumor ascites 14 days later. The cells were collected from ascites by centrifugation and resuspension of the cell pellet in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) with 40% FBS (HyClone, Logan, UT) and 10% DMSO (Sigma, St. Louis, MO). The MOT cells were cryopreserved in liquid nitrogen at a concentration of 10⁷ cells/ml.

MOT tumor model

C3H/HeN and nude (nu/nu) female mice between the ages of 7–10 wk were obtained from Harlan Sprague-Dawley (San Diego, CA). All animal experiments were conducted in accordance with Vical’s institutional animal care and use committee as well as the standards set forth in the National Research Council Guidelines concerning animal care and use.

To establish i.p. MOT tumors, C3H/HeN mice were injected i.p. with 10⁵ MOT cells. Mice bearing i.p. MOT tumors rapidly gain weight due to the accumulation of tumor ascites (15). Therefore, tumor growth was monitored by determining the body weight of the mice over time. The tumors bearing mice were also followed for survival. For the nude mouse study, athymic nude mice were injected i.p. with 10⁵ MOT cells followed by determination of mouse weight and survival over time.

Plasmid DNA:DMRIE/DOPE treatment of i.p. tumors

MOT tumors were in vivo transplanted using cationic lipid-based delivery. The lipid used in these studies, DMRIE/DOPE, consists of the cationic lipid DMRIE and the neutral lipid DOPE at a 1:1 mol/mol ratio (19). DMRIE/DOPE has been shown to be effective for both in vitro (19) and in vivo transfection (16, 20).

For the MOT tumor studies, 100 μg of mL-2 pDNA (VR1110) was diluted in 500 μl of 0.9% saline (Radix, Eau Claire, WI). DMRIE/DOPE lipoplexes were then mixed with 100 μg of either control pDNA:DMRIE/DOPE or luciferase pDNA without lipid according to the same regimen. Each treatment group consisted of 15 mice.

Luciferase reporter gene assay

C3H/HeN mice were injected i.p. with 10⁵ MOT cells followed by i.p. injection of 100 μg of luciferase pDNA (VR1223) complexed with DMRIE/DOPE (1:1 pDNA:DMRIE mass ratio) on days 5 and 6 after tumor cell implant. Control MOT tumor-bearing mice received i.p. injection of control pDNA (VR1012, backbone plasmid without IL-2) complexed with DMRIE/DOPE. One day after the pDNA injection, luciferase activity was determined per mouse. The ascites samples were spun at 14,000 rpm for 2 min, and the supernatant was harvested. Blood was collected from the mice on the same day as the ascites collection, and the serum was separated from blood cells by allowing the blood to clot in serum separator tubes (Microtainer, Becton Dickinson, Franklin Lakes, NJ) followed by centrifugation at 14,000 rpm for 10 min and collection of the serum supernatant.

The IL-2 concentration (nanograms per milliliter) in the ascites and serum samples was determined using a murine IL-2 ELISA (R&D Systems). Since the volume of tumor ascites increases over time, the volume of ascites was also determined for each mouse. The total amount of IL-2 (nanograms) in the ascites was calculated using the formula: IL-2 (ng/ml) × ml of ascites = ng of IL-2. Serum IL-2 concentrations were reported as nanograms per milliliter.

Cytokine profile of MOT tumor ascites

C3H/HeN mice were injected i.p. with 10⁵ MOT cells. On days 5, 8, and 11 after tumor cell injection, the mice were injected i.p. with either control pDNA:DMRIE/DOPE or pDNA:DMRIE mass ratio) or received no treatment after the MOT tumor cell injection. Two days after each injection of pDNA:DMRIE/DOPE (days 7, 10, and 13 after tumor cell implant), five mice per group were sacrificed, and the tumor ascites was collected. The total volume of ascites was determined per mouse. The ascites samples were spun at 14,000 rpm for 2 min followed by collection of the supernatants. The ascites supernatants were assayed for the concentration of the cytokines, IL-2, IL-4, IL-6, IL-10, IL-12, GM-CSF, IFN-γ, and TNF-α, using ELISAs specific for each cytokine (R&D Systems). The concentration of naturally processed (not acid-activated in vitro) TGF-β in the ascites was assayed using the TGF-β2 Exma Immunoassay System (Promega). The total amount of cytokine (nanograms) in the ascites was calculated using the formula: (ng/ml cytokine × ml ascites) = ng of cytokine.

Statistical analyses

Mouse weight and ascitic cytokine levels were analyzed using the Mann-Whitney U nonparametric statistical test. Mouse survival was analyzed using a Kaplan-Meier survival plot followed by a log-rank (Mantel-Cox) test. Differences were considered statistically significant when p < 0.05.

Results

Plasmid DNA:DMRIE/DOPE targets ovarian tumor ascites

In initial studies it was of interest to determine the degree of reporter gene expression in ovarian tumor ascites and peritoneal tissues after i.p. injection of reporter gene pDNA:DMRIE/DOPE. For this particular study, on days 5 and 6 after MOT tumor cell implant, C3H/HeN mice were injected i.p. with 100 μg of luciferase-expressing pDNA:DMRIE/DOPE. One day after pDNA injection, tumor ascites and kidney, liver, spleen, diaphragm, intestine, and ovaries were collected from sacrificed mice. Tumor ascites had 8.8 × 10⁶ RLU of luciferase/mg, which was significantly higher than the luciferase levels found in the diaphragm (750 RLU/mg) or ovaries (186 RLU/mg; p < 0.05; Fig. 1). Kidney, liver, spleen, and intestinal tissue had no detectable luciferase activity. Luciferase activity in diaphragm and ovarian tissue was only found in MOT tumor-bearing mice injected with luciferase pDNA:DMRIE/DOPE.
Materials and Methods

To determine which cells in the ascites were transfectected after i.p. injection of pDNA:DMRIE/DOPE, mice were injected i.p. on days 5 and 6 after MOT tumor cell implant with 100 μg of β-gal pDNA complexed with DMRIE/DOPE lipid, β-gal pDNA without DMRIE/DOPE, or control pDNA:DMRIE/DOPE. Twenty-four hours after the final i.p. pDNA injection, the ascites was collected, followed by pelleting of the cells, fixation, sectioning, and X-gal staining. The ascites from mice injected with either control pDNA: DMRIE/DOPE or β-gal pDNA without lipid were negative for X-gal staining. In contrast, the ascites from mice injected with β-gal pDNA:DMRIE/DOPE had X-gal staining, with 0.1–1% of the tumor cells stained and only an occasional lymphocyte, macrophage, or mesothelial cell stained (data not shown). Neutrophils were negative for X-gal staining. Thus, i.p. injection of pDNA:DMRIE/DOPE appeared to preferentially target tumor cells in the peritoneal cavity with limited transfection of nontumor peritoneal cells and surrounding tissues.

IL-2 pDNA:DMRIE/DOPE leads to sustained IL-2 expression in ovarian tumor ascites

The levels of IL-2 in ascites and serum after a single i.p. injection of either rIL-2 protein or IL-2 pDNA:DMRIE/DOPE was determined for mice bearing i.p. MOT tumors. On day 5 after MOT cell implant, C3H/HeN mice were injected i.p. with 1 μg of IL-2 protein or with either 100 μg of IL-2 pDNA:DMRIE/DOPE or control pDNA (backbone plasmid without IL-2):DMRIE/DOPE. Ascites and serum were collected from sacrificed mice at 4 h or on days 1, 2, 3, 6, and 10 after protein or pDNA injection. Since the volume of tumor ascites can increase as much as 20-fold during the collection period, the nanograms of IL-2 per total mouse ascites were determined as described in Materials and Methods.

Mice injected i.p. with IL-2 protein had 10 ng of IL-2 in ascites by 4 h after protein injection, followed by a 10,000-fold reduction in IL-2 1 day later (0.009 ng; Fig. 2A). In contrast, mice injected with IL-2 pDNA:DMRIE/DOPE had 64 ng of IL-2 in ascites 2 days after pDNA injection and only a 2.6-fold reduction in IL-2 levels by 10 days after pDNA injection (25 ng). Although sampling of ascites at time points earlier than 4 h may have revealed even higher levels of IL-2 after protein injection, the duration of IL-2 in ascites was markedly different for the protein-treated vs the pDNA-treated mice (24 h for the IL-2 protein-treated mice vs 10

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days for the IL-2 pDNA:DMRIE/DOPE-treated mice, respectively). Mice injected i.p. with control pDNA with lipid had negligible IL-2 in the tumor ascites (0–0.03 ng). Similar to the results in the reporter gene study in which lipid was required for effective in vivo transfection of MOT tumors, mice injected with IL-2 pDNA without lipid had background levels of IL-2 in the ascites (data not shown).

Serum levels of IL-2 after either i.p. IL-2 protein or IL-2 pDNA: DMRIE/DOPE injection reflected a pattern similar to that found in tumor ascites; however, serum IL-2 levels were relatively short-lived for both groups. Four hours after protein injection, serum IL-2 was 2.4 ng/ml and undetectable 1 day later (Fig. 2B). Mice injected with IL-2 pDNA:DMRIE/DOPE had serum IL-2 levels of 1 ng/ml by 1 day after injection and 0.4 ng/ml by 3 days after injection. Although more sustained levels of IL-2 were found in the serum after i.p. injection of IL-2 pDNA:DMRIE/DOPE compared with IL-2 protein, the levels were <10% of the IL-2 levels found in the ascites, suggesting localization of IL-2 in the peritoneal cavity after i.p. injection of IL-2 pDNA:DMRIE/DOPE.

We determined the levels of IL-2 after i.p. injection of a 5-fold lower dose of IL-2 pDNA:DMRIE/DOPE. Mice bearing MOT tumors were injected i.p. with only 10 μg of pDNA complexed with DMRIE/DOPE on days 5 and 6 after tumor cell implantation (for a total pDNA dose of 20 μg), and IL-2 levels in ascites were measured. Two days after IL-2 pDNA:DMRIE/DOPE injection, mice had 2.8 ng of IL-2 and only a 5.6-fold reduction in IL-2 by 10 days later (0.5 ng; data not shown). Thus, injection of tumor-bearing mice with a 5-fold lower dose of IL-2 pDNA:DMRIE/DOPE still resulted in sustained IL-2 levels in the ascites over a 10-day period.

**IL-2 pDNA:DMRIE/DOPE has antitumor effects in mice bearing ovarian tumors**

Since i.p. injection of IL-2 pDNA:DMRIE/DOPE in mice bearing i.p. ovarian tumors could result in sustained levels of IL-2 in the tumor ascites, the antitumor effect of this treatment was investigated. C3H/HeN mice bearing i.p. MOT tumor ascites were injected i.p. with 100 μg of IL-2 pDNA:DMRIE/DOPE or with control pDNA:DMRIE/DOPE on days 5, 8, and 11 after tumor cell implant. MOT tumors in the peritoneal cavity of C3H/HeN mice result in a rapid increase in tumor ascites, leading to a rapid gain in body weight. Thus, i.p. MOT tumor growth was determined by recording the body weight of the mice over time. Beginning on day 14 after tumor cell injection, mice treated with IL-2 pDNA:DMRIE/DOPE had a significant inhibition of tumor growth as measured by body weight (p < 0.05, from days 14 to 23) compared with that in mice treated with the control pDNA:DMRIE/DOPE (Fig. 4A). A significant increase in survival (p = 0.008) was also found for the IL-2 pDNA:DMRIE/DOPE-treated mice. The experiment was performed twice with replicates yielding consistent results.

**Dose-response of IL-2 pDNA:DMRIE/DOPE**

A dose-response study was conducted to determine the minimum dose of IL-2 pDNA:DMRIE/DOPE that would result in a significant antitumor effect. C3H/HeN mice were injected with 25, 50, or 100 μg of IL-2 pDNA:DMRIE/DOPE on days 5, 8, and 11 after MOT tumor cell injection. A control group of MOT tumor-bearing mice was injected with 100 μg of control pDNA:DMRIE/DOPE. By day 13 after tumor cell injection, mice treated with either the 50- or 100-μg dose of IL-2 pDNA:DMRIE/DOPE had a significant inhibition of tumor growth, as measured by body weight (p < 0.05, from days 13 to 17; Fig. 5A) and a significant increase in survival (p = 0.008; Fig. 5B) compared with mice treated with the control pDNA:DMRIE/DOPE. In contrast, tumor-bearing mice
treated with 25 μg of IL-2 pDNA:DMRIE/DOPE were not significantly different from control mice in either tumor growth or survival. By day 25 of the study, 0% of the control pDNA-treated and 6% of the 25 μg IL-2 pDNA:DMRIE/DOPE-treated mice were still alive, while 33 and 27% of the 100 and 50 μg IL-2 pDNA:DMRIE/DOPE-treated mice remained alive, respectively.

It should be noted that the median survival of the mice treated with 100 μg of IL-2 pDNA:DMRIE/DOPE occurred at 34 days in the IL-2 pDNA efficacy study (Fig. 3B) vs 23 days in the IL-2 pDNA dose-response study (Fig. 5B). This type of interstudy variability may be due to differences in the ages of the mice used for each study (which varied between 7–10 wk) or slight variations in the tumor cells injected on a particular day. However, when comparing groups within a single experiment, there was a consistent significant enhancement of survival for the 100 μg IL-2 pDNA:DMRIE/DOPE-treated group compared with the control pDNA:DMRIE/DOPE-treated mice (p = 0.008).

Cytokine profile of ovarian tumor ascites

Since i.p. injection of IL-2 pDNA:DMRIE/DOPE into mice bearing i.p. MOT tumors results in very high levels of IL-2 in tumor ascites, we were interested in determining whether the IL-2 pDNA therapy altered the expression of other cytokines in the ascites. Mice were injected i.p. with 100 μg of either IL-2 pDNA:DMRIE/DOPE or control pDNA:DMRIE/DOPE on days 5, 8, and 11 after MOT tumor cell implant. An additional group of MOT tumor-bearing mice received no treatment. Two days after each pDNA:DMRIE/DOPE injection (days 7, 10, and 13 after tumor cell injection), five mice per group were sacrificed, and the ascites was collected. The ascites samples were assayed by ELISA to determine the levels of IL-2, IL-4, IL-6, IL-10, IL-12, GM-CSF, IFN-γ, TGF-β, and TNF-α. As expected, tumor-bearing mice injected i.p. with IL-2 pDNA:DMRIE/DOPE had a significant increase in IL-2 levels (36, 30, and 50 ng on days 7, 10, and 13, respectively) compared with untreated tumor-bearing mice (0–0.05 ng) or mice injected with the control pDNA:DMRIE/DOPE (0–0.08 ng; p < 0.01 for all time points; Fig. 6A). Interestingly, IFN-γ and GM-CSF were both significantly elevated in the mice treated with IL-2 pDNA:DMRIE/DOPE compared with untreated mice or compared with mice treated with control pDNA:DMRIE/DOPE (Fig. 6, B and C). The IFN-γ and GM-CSF levels in the IL-2 pDNA:DMRIE/DOPE-treated mice increased most dramatically on days 10 and 13 (108 and 255 ng for IFN-γ and 3.4 and 5 ng for GM-CSF), suggesting that these two cytokines may be up-regulated by IL-2, which is very high by day 7 (36 ng). A less marked increase in IFN-γ and GM-CSF was also found in the ascites of tumor-bearing mice after injection of the control pDNA:DMRIE/DOPE (32 and 39 ng for IFN-γ and 0.3 and 0.36 ng for GM-CSF, on days 10 and 13, respectively), and these levels were significantly different from those in the untreated mice on days 10 and 13 for IFN-γ (0.003–0.08 ng; p < 0.03) and on day 13 for GM-CSF (0.04 ng; p = 0.02).

IL-6, TNF-α, and IL-10 were significantly increased in both the IL-2 pDNA:DMRIE/DOPE-treated groups as well as the control
pDNA:DMRIE/DOPE-treated groups compared with those in the untreated mice \( (p < 0.05; \text{Fig. 5, D–F}) \). However, there was no significant difference in levels of IL-6, TNF-\( \alpha \), and IL-10 when comparing the IL-2 pDNA:DMRIE/DOPE-treated group vs the control pDNA:DMRIE/DOPE-treated group. These results suggest that pDNA:DMRIE/DOPE complexes may nonspecifically stimulate the production of these particular cytokines. For both the IL-2 pDNA:DMRIE/DOPE-treated mice and the control pDNA:DMRIE/DOPE-treated mice, IL-6 ranged from 6–13 ng, TNF-\( \alpha \) ranged from 0.7–3 ng, and IL-10 ranged from 0.5–1.5 ng on days 10 and 13. In contrast, untreated mice had comparatively low levels of IL-6 (0.02–1.5 ng) and TNF-\( \alpha \) (0 – 0.03 ng) at any of the time points, while IL-10 in the untreated mice was low on days 7 and 10 (0.008–0.014 ng) and higher on day 13 (0.7 ng).

No differences were found for IL-4, IL-12, or naturally processed TGF-\( \beta \) in the ascites from any of the groups, and levels of these cytokines were low at all of the time points (0–0.17 ng for IL-4, 0–0.17 ng for IL-12, and 0.03–0.64 ng for TGF-\( \beta \); data not shown). For all the cytokines evaluated, mice treated with control pDNA without lipid, IL-2 pDNA without lipid, or lipid alone had cytokine levels similar to those in the untreated mice (data not shown).

**Discussion**

One of the major limitations of immunotherapy with rIL-2 protein for ovarian cancer is that, due to its short half-life, frequent injections of high doses of the protein are required to sustain therapeutic levels, and this is often associated with serious side effects (21). Although i.v. bolus injection of IL-2 results in a serum half-life of ~7 min, i.p. infusion results in a peritoneal fluid half-life of ~22 h (22, 23). Recently, a promising 25% response rate was reported after treatment of advanced ovarian cancer patients with IL-2 protein delivered by either continuous 7-day i.p. infusion or weekly 24-h i.p. infusion (11). Constant delivery of the IL-2 protein over either 7 days or 24 h resulted in stable IL-2 levels in the tumor ascites during the infusion period. These results suggest that one improvement in IL-2 therapy for ovarian cancer may be administration of IL-2 in such a manner that the sharply fluctuating IL-2 levels common after bolus protein therapy are avoided.

In the present research we demonstrate that a single i.p. injection of an IL-2-encoding pDNA:DMRIE/DOPE in mice bearing i.p. ovarian tumors can lead to stable levels of the protein in tumor ascites for up to 10 days. Using the MOT ovarian cancer model, we compared the duration of IL-2 in tumor ascites after i.p. injection of either IL-2 protein or an IL-2 pDNA:DMRIE/DOPE complex. A single injection of IL-2 protein in this model resulted in a 10,000-fold decrease in IL-2 between 4 and 24 h after injection. In contrast, a single i.p. injection of IL-2 pDNA:DMRIE/DOPE resulted in only a 2.6-fold reduction in IL-2 from days 2–10 after injection. Thus, while the duration of IL-2 in tumor ascites after a single i.p. injection of IL-2 protein was only 24 h, IL-2 levels in the ascites after a single injection of IL-2 DNA:lipid lasted as long as 10 days. The rapid decline in IL-2 after protein injection vs the sustained IL-2 levels found after IL-2 pDNA:lipid injection most likely reflects the fact that cells transfected with the IL-2 gene may secrete IL-2 over an extended period of time. Thus, one advantage...
of delivery of IL-2 by i.p. injection of a plasmid encoding IL-2 is that the therapy may be better tolerated by the patient, because it would eliminate the requirement for frequent administration or prolonged infusion of the protein. Furthermore, IL-2-related side effects may be reduced by minimizing the marked fluctuations in IL-2 that normally occur with bolus injection of IL-2 protein (22, 23).

We also demonstrate that i.p. treatment of MOT tumor-bearing mice with IL-2 pDNA:DMRIE/DOPE results in a significant delay in tumor growth as well as a significant increase in survival. In a previous publication, MOT i.p. tumors were found to be sensitive to treatment with an IFN-γ-expressing pDNA:lipid complex; however, this therapy was not effective unless it was also combined with the chemotherapeutic agent, cisplatin (24). In contrast, we have found that IL-2 pDNA:lipid gene therapy in the same murine ovarian tumor model results in a significant antitumor effect without requiring chemotherapy. Because late-stage ovarian cancer patients often develop resistance to chemotherapeutic agents (3), a treatment that is effective in the absence of chemotherapy may be advantageous. In a related publication, mice immunized with IL-2-expressing fibroblasts were not significantly protected from MOT tumor challenge unless they were also immunized with MOT cells expressing antisense TGF-β (25). In the present research the IL-2 pDNA therapy alone was sufficient to generate a significant increase in survival, suggesting that IL-2 may be more effectively delivered via injection of IL-2 pDNA:lipid, rather than by injection of cells ex vivo transfected with the IL-2 gene.

In several reporter gene studies we found that ovarian tumor ascites is preferentially targeted by pDNA:lipid complexes. Peritoneal delivery of a luciferase-expressing pDNA:lipid complex into MOT tumor-bearing mice resulted in a 12,000-fold higher level of luciferase activity in tumor ascites compared with the surrounding tissues. In a histochemical analysis of MOT tumor ascites, peritoneal delivery of a β-gal-expressing pDNA:lipid complex resulted in transfection of primarily tumor cells, with only occasional transfection of lymphocytes, macrophages, or mesothelial cells. In previous studies of mice bearing i.p. tumors, peritoneal delivery of a reporter gene pDNA:lipid similarly resulted in reporter gene expression in either ascites or solid i.p. tumors (26–28). Because one of the major problems with many gene therapies is a lack of targeting, the tumor-associated transgene expression found after i.p. injection of pDNA:lipid supports the development of this type of therapy for patients with peritoneal tumors.

In a cytokine profile study we found that treatment of MOT tumor-bearing mice by i.p. injection of IL-2 pDNA:DMRIE/DOPE resulted in a significant increase in the Th1 cytokine, IFN-γ, with no increase in the Th2-type cytokine, IL-4. A 24-fold increase in IFN-γ occurred after the second IL-2 pDNA:lipid injection, and a 58-fold increase in IFN-γ occurred after the third IL-2 pDNA:lipid injection. Previous studies have found that IL-2 can specifically up-regulate the production of IFN-γ by T cells in vitro (29–31), and ovarian cancer patients treated with IL-2 protein therapy have also been found to have a marked increase in IFN-γ in tumor ascites (6, 32). In our studies nude mice bearing MOT tumors and treated with IL-2 pDNA:DMRIE/DOPE had only a modest and nonsignificant inhibition of tumor growth, suggesting that T cells are required for the IL-2-mediated effect. By increasing the levels of the Th1 cytokine, IFN-γ, after IL-2 pDNA:DMRIE/DOPE therapy, a more favorable environment may be established for a cell-mediated antitumor response.

Interestingly, while there was a significant increase in the ascitic levels of GM-CSF and IFN-γ after treatment with IL-2 pDNA:lipid compared with control pDNA:lipid, the increase in ascitic levels of IL-6, TNF-α, and IL-10 was not significantly different when comparing IL-2 pDNA:lipid vs control pDNA:lipid. The increase in the levels of some cytokines after injection of only the control pDNA:lipid suggests a possible nonspecific, adjuvant effect of the pDNA:lipid complex. This type of nonspecific effect has also been observed in several murine tumor studies (33, 34), yet the mechanism is not well understood. We did not observe an increase in any of the cytokines if the pDNA was injected without lipid or if lipid alone was injected, suggesting that it is the pDNA:lipid complex that is acting as an adjuvant. Recently, it was demonstrated that a pDNA:lipid complex injected i.v. was more immuno-stimulatory than either pDNA alone or lipid alone (35). In the latter study, if the pDNA was methylated to remove the effect of CpG motifs, the pDNA:lipid complex was much less immuno-stimulatory. Whether CpG motifs are involved in the nonspecific increase in certain cytokines in the MOT tumor model is unclear at this time and deserves further investigation.

High levels of IL-6 and IL-10 are commonly found in the tumor ascites of ovarian cancer patients (36, 37), and IL-6 may be a prognostic factor for ovarian tumors (38), while IL-10 can be immunosuppressive (39). In the MOT model, ovarian tumors are still responsive to the IL-2 pDNA:DMRIE/DOPE therapy despite increases in IL-6 and IL-10. This may be due to the marked increase in the two Th1 cytokines, IL-2 and IFN-γ, which can enhance a cell-mediated response. Studies are planned to selectively block the activity of specific cytokines in the tumor ascites to further delineate the roles of these cytokines in the IL-2-mediated antitumor effect.

In summary, sustained expression of IL-2 in tumor ascites after IL-2 pDNA:lipid injection represents a critical improvement over traditional i.p. protein therapy of ovarian cancer. The delivery of the IL-2 protein via IL-2 pDNA:lipid complexes may have several advantages: 1) a reduction in the frequency of hospital visits for IL-2 therapy by eliminating the necessity for frequent and extended infusion of IL-2 protein, and 2) an avoidance of marked fluctuations in IL-2 levels, which may result in fewer side effects of the IL-2 therapy. The results of this research indicate that this approach may be amenable for the treatment of advanced-stage ovarian cancer patients.

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