



Antitumoral effect of a nonviral interleukin-2 gene therapy is enhanced by combination with 5-fluorouracil

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Using a novel cationic lipid delivery system consisting of *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride and cholesterol, we delivered murine interleukin-2 (IL-2) cDNA directly into an established murine renal cell carcinoma (Renca). Production of IL-2 within the tumor induced rejection of established tumors (62% on average), whereas control plasmid had little or no effect (17% on average). Surviving animals treated with IL-2:lipid were highly resistant to Renca rechallenge, but not to cross-challenge with a syngeneic mammary adenocarcinoma. Experiments on selectively immunosuppressed animals indicated a requirement for CD8⁺ T, natural killer, and polymorphonuclear cells. By contrast, depletion of CD4⁺ T cells did not disrupt the ability of IL-2:lipid to induce tumor rejection. A combination of IL-2 gene therapy with 5-fluorouracil treatment increased the antitumoral efficacy and survival of mice bearing primary and metastatic Renca tumors (42% survival with IL-2:lipid compared with 94% survival with IL-2:lipid plus 5-fluorouracil). These data indicate that rejection of primary and metastatic tumors can be achieved after intratumoral delivery of a nonviral IL-2 gene therapy, and is increased in combination with systemic delivery of a conventional chemotherapeutic agent. **Cancer Gene Therapy (2000) 7, 1165–1171**

Key words: Interleukin-2; gene therapy; chemotherapy; 5-fluorouracil; immunotherapy; cancer.

Cytokine gene therapy is under intensive preclinical and clinical study to ultimately provide efficacious alternatives to current chemotherapeutic regimens in patients. Interleukin-2 (IL-2), a T-cell growth factor that enhances both nonspecific immune responses, such as activation of macrophages, natural killer (NK) cells, and lymphokine-activated killer cells, as well as major histocompatibility complex-restricted cytotoxic T-cell responses, has been studied extensively. Recombinant IL-2 (rIL-2) is the currently recommended immunotherapeutic agent for human renal cell carcinoma (RCC) by the US Food and Drug Administration.¹ IL-2 protein, however, has a short half-life in sera and requires repeated doses for antitumoral effects. This cytokine exhibits limited efficacy against certain cancers and has a number of well-documented toxic effects when given systemically, such as capillary leak syndrome, hypotension, arrhythmia, nausea, fever, vomiting, and liver and kidney damage.^{2–4} The deleterious effects of systemic administration of cytokines coupled with the cloning of cytokine genes have prompted research into the local expression of cytokines.

Implantation with *ex vivo* IL-2 gene-transfected tumor cells^{5,6} or fibroblasts^{7,8} in experimental animal models

has been shown to be effective in delaying tumor growth and causing tumor rejection. A number of *ex vivo* gene therapy strategies have gone into clinical trials, mainly in RCC and melanoma.⁹ However, *ex vivo* approaches are costly, time-consuming, and labor-intensive because they involve excision of tumor tissue, establishment of a cell culture, transduction of the cells in culture, selection and growth of the transduced cells, and reimplantation of the cells into the animal model or patient. Such drawbacks led to the development of methods for direct transfection/transduction of tumor cells *in vivo*. Previously, DNA encoding IL-2 has been used in direct administration gene therapy models using adenoviral,^{10,11} particle-mediated gene-gun^{12,13} and lipid-mediated delivery.^{14,15}

We have employed a gene therapy approach using a murine IL-2 (mIL-2)-expressing plasmid complexed with *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA):cholesterol (lipid) to deliver functional IL-2 cDNA by direct injection into established tumors. The specific objective of our study was to evaluate the antitumoral response induced by IL-2:lipid and to examine the effectiveness of combining IL-2 gene therapy with a conventional chemotherapeutic agent, 5-fluorouracil (5-FU). 5-FU is currently widely used clinically for the chemotherapy of a variety of tumors, including gastric, colon, stomach, and breast carcinomas. An active metabolite of 5-FU, 5-fluoro-2'-deoxyuridine-5'-monophosphate, inhibits thymidylate synthetase, decreasing the formation of thymidylate needed for DNA synthesis.

Received October 1, 1999; accepted April 15, 2000.

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By employing direct lipid-mediated gene transfer into established tumors, we demonstrated that the production of IL-2 within the tumor induces a strong antitumoral immune response and tumor-specific memory. The antitumoral activity induced by IL-2:lipid was efficacious against primary tumors and prevented the formation of lung metastases. Lastly, we present evidence that IL-2 gene therapy can be used in combination with a conventional chemotherapeutic agent to enhance the antitumoral activity against primary and metastatic tumors.

MATERIALS AND METHODS

Animals

Female BALB/c mice (6–8 weeks old, ~25 g body weight) were obtained from Charles River Laboratories (Raleigh, NC). Mice were provided with rodent feed and water *ad libitum* and kept at 23°C, 40–70% humidity, and a 12-hour/12-hour light-dark cycle. Animals were allowed an acclimation period of at least 4 days before the start of a study. Animal management conformed to an approved Institutional Animal Care and Use Committee protocol.

Tumors

Renca, a spontaneously arising murine RCC, was generously provided by Dr. Drew M. Pardoll (John Hopkins Hospital, Baltimore, Md). TS/A, a spontaneously arising moderately differentiated murine mammary adenocarcinoma,¹⁶ was generously provided by Dr. Guido Forni (University of Turin, Turin, Italy). Tumor cell cultures were maintained in sterile disposable flasks at 37°C in a humidified 5% CO₂ atmosphere using RPMI 1640 medium supplemented with 12.5% fetal bovine sera, 100 U/mL penicillin-G, 100 µg/mL streptomycin sulfate, and 2 mM L-glutamine (all from Life Technologies, Gaithersburg, Md).

Plasmid formulations

Plasmids were propagated in *Escherichia coli* strain DH5α and purified using alkaline lysis and column chromatography. Two plasmids were used in these studies, pIL0555A and pVC0612. pIL0555A (IL-2) is a *pUC ori*-based plasmid of 3452 bp coding for mIL-2 cDNA;¹⁷ this plasmid contains the cytomegalovirus promoter, cytomegalovirus 5' untranslated region, and a bovine growth hormone 3' polyadenylation signal and site. Plasmid pVC0612 (empty plasmid (EP)), which is a 2940-bp plasmid identical with pIL0555 except that it lacks the IL-2 cDNA sequence, was used as a control for nonspecific effects. Plasmid preparations were tested for endotoxin contamination by a Limulus ameobocyte lysate assay (BioWhittaker, Walkersville, Md). A single lot of pIL0555 with an endotoxin level of 6 international units of endotoxin per milligram of DNA was used for all experiments. Multiple lots of EP were used in these studies with an average endotoxin content of 10 international units per milligram of DNA.

Small unilamellar vesicles of a 1:1 molar ratio of DOTMA (Avanti Polar Lipids, Alabaster, Ala) and cholesterol (Avanti Polar Lipids) were prepared by microfluidization. Plasmid-lipid formulations were prepared by mixing purified plasmids with these liposomes under controlled conditions in a solution containing 10% lactose as an isotonic agent. The final DOTMA:cholesterol formulation had a DNA:lipid charge ratio of 1:0.5 (-/+).

Cytokine enzyme-linked immunosorbent assays (ELISAs)

To measure IL-2 production in tumors, female BALB/c mice were implanted with 7×10^5 Renca cells in a 30-µL volume subcutaneously (s.c.) in the left flank on day 0. On day 10, animals bearing tumors of 20–25 mm³ elliptical volume were randomized into groups of six and injected intratumorally (i.t.) with 6 µg of IL-2:lipid or EP:lipid. Tumors were harvested 24 hours after treatment, placed individually into the wells of 24-well tissue culture plates (Becton-Dickinson Labware, Lincoln Park, NJ), and cut into 10–12 pieces. Conditioned culture medium was collected after 24 hours of culture at 37°C/5% CO₂, centrifuged, and stored at -70°C until assayed for cytokines. The conditioned medium was assayed for the presence of the IL-2 gene product and a secondary cytokine, interferon-γ (IFN-γ), using commercial ELISA kits (mIL-2, R&D Systems, Minneapolis, Minn; murine IFN-γ, Genzyme, Cambridge, Mass) according to the manufacturer's instructions.

In vivo studies

Female BALB/c mice were challenged s.c. with 7×10^5 Renca cells in the middle of the left flank. Treatments with EP:lipid or IL-2:lipid began 6 or 7 days later when the tumor size reached ~10 mm³; treatments were repeated at 3- to 4-day intervals for 2 weeks (two treatments per week). Groups of untreated tumor-bearing animals were included as controls. Tumor volume was measured with electronic calipers to determine two perpendicular diameters and depth. Measurements of the tumor masses were performed twice a week for 40–50 days. All mice bearing tumor masses of >1 cm³ volume or showing signs of severe ulceration of tumors or moribundity were sacrificed for humane reasons.

In rechallenge and cross-challenge experiments, animals that rejected the initial Renca tumor challenge were rechallenged with a higher inoculum of Renca cells (1×10^6 cells/30 µL) in the opposite (right) flank from the initial challenge. A group of naive animals was also challenged as a control for tumor-take. In the cross-challenge experiment, animals that rejected both the initial Renca challenge and the Renca rechallenge, as well as a naive group, were implanted with 2×10^5 TS/A cells also in the right flank. Animals were monitored for tumor establishment.

Antibody (Ab) depletions of CD4⁺, CD8⁺, NK, or polymorphonuclear (PMN) cells were done on days 6 and 13 after tumor implant. Tumors were treated twice per week for 2 weeks with IL-2:lipid or EP:lipid (6 µg/mouse) starting on day 7 after tumor challenge. CD4⁺ and CD8⁺ T cells were depleted using 500 µL of a 1/10 dilution of ascites fluid from GK1.5 (207-TIB) and 2.43 (210-TIB) hybridomas, respectively. (Hybridomas were obtained from American Type Culture Collection, Manassas, Va; ascites fluid was obtained from Charles River Laboratories.) NK cells were depleted using 50 µL/mouse of rabbit anti-mouse asialo-GM1 (Wako, Richmond, Va). PMN cells were depleted using 100 µg/mouse of Ab RB6-8C5 (PharMingen, San Diego, Calif). Abs were given by intraperitoneal injection at doses previously determined to be of sufficient concentration to maintain depletion of specific cell subsets for the duration of the experiment (data not shown). An isotype-matched Ab (R35-38, rat immunoglobulin G2b, κ; PharMingen) was used as a negative control at 100 µg/mouse. Tumors were monitored as described above.

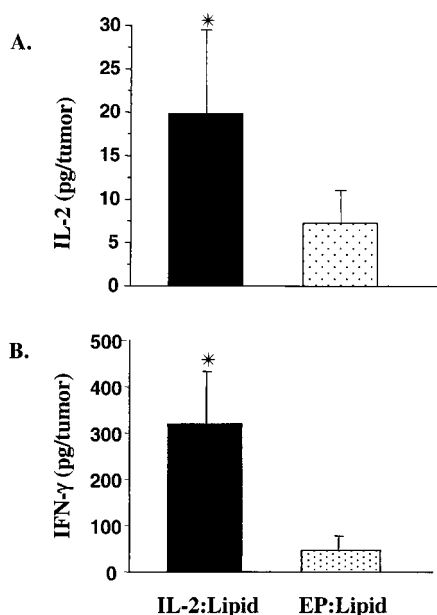


Figure 1. Cytokine production from cultured Renca tumor explants after IL-2:lipid treatment. BALB/c mice (six per group) were implanted s.c. in the flank with 7×10^5 Renca cells and treated 10 days later with $6 \mu\text{g}$ of IL-2:lipid or EP:lipid. Tumors were harvested 24 hours after treatment and cultured for 24 hours. Culture media were assayed for IL-2 (A) and IFN- γ (B) by ELISA. Data are presented as mean \pm SEM. *, $P < .05$.

Combination of IL-2 gene therapy and chemotherapy

Mice bearing Renca tumors were treated with $6 \mu\text{g}$ of IL-2:lipid or EP:lipid twice per week for 2 weeks starting on day 7 after tumor challenge. After the first treatment, mice were also injected intravenously (i.v.) with 3×10^5 cells on day 9 after tumor challenge. Animals receiving 5-FU (Sigma, St. Louis, Mo) were dosed daily at 12 mg/kg (5 days per week for 2 weeks). Primary s.c. tumors were measured twice a week (see above). Animals with tumors of $>1 \text{ cm}^3$ or animals who had lost $>20\%$ of their body weight were sacrificed.

Statistical analyses

Tumor measurement data and cytokine ELISA data were analyzed using the nonparametric Mann-Whitney U test. Tumor rejection data were analyzed using the χ^2 test. In all cases, P values of <0.05 were considered statistically significant.

RESULTS

Expression of IL-2 after i.t. injection of IL-2:lipid complexes

The expression of IL-2 within the tumor after IL-2:lipid treatment was analyzed. Mice were challenged s.c. with 7×10^5 Renca cells; ten days later, when tumor size was $\sim 20\text{--}25 \text{ mm}^3$, $6 \mu\text{g}$ of IL-2:lipid or EP:lipid was injected i.t. Tumors were harvested 1 day later and cultured for 24 hours. The resulting conditioned medium was assayed for IL-2 by ELISA. Figure 1A shows that the IL-2 produced from tumors injected with IL-2:lipid was sig-

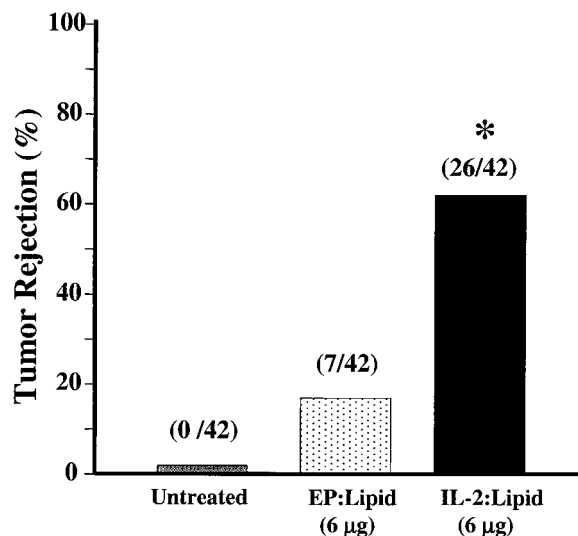


Figure 2. Antitumoral activity of IL-2 gene therapy. BALB/c mice were implanted s.c. with 7×10^5 Renca cells and treated 7 days later with EP:lipid or IL-2:lipid at 3- to 4-day intervals for 2 weeks (four treatments total). Groups of untreated tumor-bearing mice were also included. The percentage of total mice rejecting tumors in seven separate experiments is shown ($n = 6/\text{group/experiment}$). The average percentage of rejection across all experiments was 62% (26 of 42) for IL-2:lipid, 17% (7 of 42) for EP:lipid, and 0% (0 of 42) for untreated mice. Rejection of established Renca tumors with IL-2:lipid compared with EP:lipid and untreated tumors was $P < .001$.

nificantly increased over the EP:lipid treatment. One of the characteristics of bacterial-derived DNA is to induce cellular immune responses in mammalian due to unmethylated CpG dinucleotide motifs contained within the DNA sequence.^{18,19} The generation of secondary cytokines, including IFN- γ , that promote a T helper type 1-dependent cell response, is observed *in vivo* after treatment with bacterial DNA.²⁰ To determine whether there was a transgene-independent contribution of the DNA/lipid complex to the production of secondary cytokines, IFN- γ was analyzed by ELISA. Figure 1B shows that the production of IFN- γ by tumor explants was predominantly observed in mice treated with IL-2:lipid and not in the EP:lipid-treated group, suggesting an IL-2 transgene-dependent event.

Antitumoral activity of IL-2:lipid treatment

The antitumoral effect of IL-2:lipid was tested *in vivo* against established Renca tumors. Mice bearing 10-mm^3 tumors were treated i.t. with $6 \mu\text{g}$ of IL-2:lipid or EP:lipid. Each group consisting of six or eight tumor-bearing mice was treated at 3- to 4-day intervals (twice per week) for 2 weeks (four treatments total). Figure 2 shows the compiled result of seven independent experiments. A total of 62% of mice treated with IL-2:lipid (26 of 42) showed complete tumor rejection, whereas only a marginal tumor response (17%) was observed in mice treated with EP:lipid (7 of 42). No untreated animal went into spontaneous tumor remission (0 of 42).

**Table 1. Antitumoral Response Generated by Local IL-2: Lipid Treatments**

Challenging cells*	Treatment	Tumor rejection/ total mice	Surviving/total mice (RENCA rechallenge)†	Surviving/total mice (TS/A cross-challenge)‡
Renca	None	0/30 (0)§	—	—
	6 µg of EP:lipid	5/30 (17)	4/5 (80)	0/4 (0)
	6 µg of IL-2:lipid	21/30 (70)	21/21 (100)	0/21 (0)
	—	—	0/4 (0)	—
TS/A	—	—	—	0/6 (0)

*Mice were challenged in the left flank with 7×10^5 Renca cells.

†Mice were rechallenged or challenged for the first time (control group) in the right flank with 1×10^6 Renca cells.

‡Mice were cross-challenged or challenged for the first time (control group) in the right flank with 2×10^5 TS/A cells.

§Numbers in parentheses indicate percentage of survival.

Inhibition of Renca tumor growth after IL-2:lipid treatment is associated with a systemic immune response

Mice that rejected primary Renca tumors after treatment with IL-2:lipid were rechallenged with a greater number of Renca cells (1×10^6) in the opposite flank from the initial tumor site at 42–62 days after initial tumor challenge. Naive animals were also challenged for the first time as a control group. All IL-2:lipid-treated mice (21 of 21) that rejected the primary tumor also rejected the Renca rechallenge. A total of 80% (four of five) of animals treated with EP:lipid were protected against tumor rechallenge (Table 1). To determine the tumor specificity of the immune response, surviving mice from the IL-2:lipid and EP:lipid treatment groups were cross-challenged 53 days after Renca rechallenge with a different syngeneic tumor line, TS/A. An inoculum of 2×10^5 TS/A cells was implanted into the opposite flank from the initial Renca challenge. A group of six naive animals was challenged as a control group. None of the IL-2:lipid-treated mice (0 of 21), EP:lipid-treated mice

(0 of 4), or naive mice (0 of 6) rejected the TS/A cross-challenge (Table 1).

Antitumoral effects of IL-2:lipid in $CD4^+$, $CD8^+$, NK-, or PMN-depleted mice To evaluate the contribution of various immune cells to the antitumoral response induced by IL-2:lipid, tumor-bearing mice were selectively immunosuppressed using anti- $CD4$, anti- $CD8$, anti-PMN, or anti-NK Abs and then treated with IL-2:lipid. A depletion control group was treated with an irrelevant isotype-matched Ab before IL-2:lipid treatment. Depletion of $CD8^+$ and NK cells abrogated the antitumoral effects of IL-2:lipid (Fig 3). PMN depletion also significantly disrupted the antitumoral response to the IL-2 gene therapy ($P = 0.034$), but to a lesser degree. Treatment with the control Ab did not have a significant effect on tumor rejection. Interestingly, $CD4^+$ depletion did not disrupt the antitumoral activity induced by IL-2:lipid, but, on the contrary, increased the number of tumor rejections from 11 of 16 mice with IL-2:lipid to 16 of 16 mice in the $CD4^+$ -depleted group ($P = 0.0515$, not significant). The data suggest

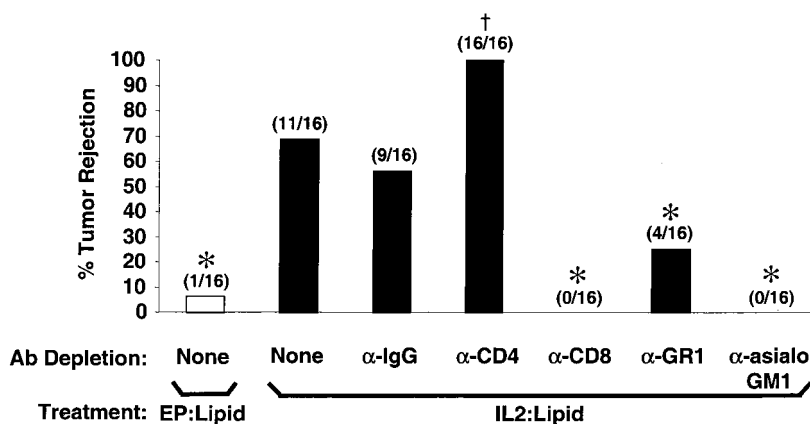


Figure 3. Tumor rejection in mice depleted of $CD4^+$, $CD8^+$, NK, and PMN cells after IL-2:lipid treatment. The pooled data from two replicate experiments with 8 mice per group (16 mice per treatment) are shown. Renca cells (7×10^5) were implanted s.c. in the left flank of BALB/c mice on day 0. Treatment with anti- $CD4^+$, anti- $CD8^+$, anti-GR1⁺ (PMN), anti-asialo-GM1⁺ (NK), or anti-immunoglobulin G2b, κ was performed on days 6 and 13 after tumor challenge (see *Materials and Methods*). Tumors were treated twice per week for 2 weeks with IL-2:lipid (6 µg/mouse/treatment) beginning 1 day after the initial depletion. The results are presented as the percentage of mice that rejected an established tumor. Numbers in parentheses represent the number of animals that rejected tumors out of the total number of mice per group. Tumor rejection was analyzed by χ^2 analysis (*, $P < .05$; †, $P = 0.0515$, versus IL-2:lipid group).

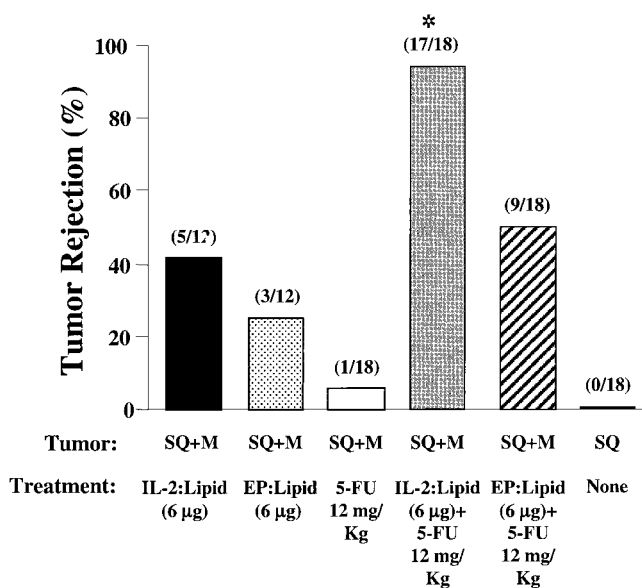


Figure 4. Combined antitumoral efficacy of IL-2 gene therapy and 5-FU. Tumor bearing-mice (s.c. = SQ) receiving IL-2:lipid or EP:lipid (6 µg/mouse/treatment) were treated as described in Figure 2. At 2 days after the first treatment, mice were injected i.v. with 3×10^5 Renca cells (M = metastases). Animals receiving 5-FU were dosed daily at 12 mg/kg for 5 days per week for 2 weeks. Mice that rejected primary tumors also survived lung metastases. Numbers in parentheses represent the number of animals that rejected tumors out of the total number of mice per group. *, $P < .05$ versus the IL-2:lipid and EP:lipid plus 5-FU groups.

a suppressive effect of at least some subset of CD4⁺ cells on antitumoral activity.

Combination of IL-2 gene therapy with 5-FU

We investigated whether the antitumoral activity induced by IL-2:lipid treatments could be increased by systemic administration of a conventional chemotherapeutic agent, 5-FU, in mice bearing primary tumor and lung metastases. Mice received primary tumor (s.c.) challenge followed by IL-2:lipid treatment 7 days later, when tumors were $\sim 10 \text{ mm}^3$. At 2 days after the first IL-2:lipid treatment, mice were also challenged i.v. with 3×10^5 Renca cells to induce lung metastases. Figure 4 shows tumor rejection as result of combining 5-FU treatment with IL-2 gene therapy. Mice that rejected primary tumors also survived lung metastases. A total of 42% of the mice treated with IL-2:lipid rejected their primary tumor (5 of 12) compared with 94% (17 of 18) of the mice receiving IL-2:lipid and 5-FU, suggesting an additive effect of gene therapy with 5-FU. The tumor rejection observed for the IL-2:lipid plus 5-FU groups was statistically different from either the IL-2:lipid or EP:lipid plus 5-FU groups.

DISCUSSION

In this study, we present evidence that delivery of IL-2 gene to established tumors by a nonviral vector system

induces a potent antitumoral response primarily mediated by CD8⁺ T lymphocytes, granulocytes, and NK cells. Combination of IL-2 gene therapy with 5-FU treatment increases the antitumoral efficacy and survival of mice bearing primary and metastatic tumors.

Cytokines naturally work in a paracrine fashion within a local microenvironment. Systemic administration of cytokines for cancer immunotherapy produces high concentrations of these proteins at sites distant from the antigen and often suboptimal levels in the tissues of interest.²¹ Continuous IL-2 production induced by local gene therapy will minimize IL-2 toxicity, overcome the problem of a short half-life, and produce IL-2 at the site of interest, inducing a local immune response. Direct viral IL-2 gene delivery studies mediated by adenovirus,¹⁰⁻¹¹ retrovirus,²² and vaccinia virus²³ have shown antitumoral efficacy in mouse models of primary cancer. However, concerns about viral replication and integration with viral vectors, along with additional manufacturing challenges, make these methods less attractive for widespread gene therapy.

By using a novel lipid delivery system to treat primary and metastatic tumors with the IL-2 gene, we first demonstrated detectable IL-2 production within the tumor after injection of IL-2:lipid complexes (Fig 1A). The detection of IL-2 produced from cultured mouse tumors may likely be underestimated due to trapping of cytokine within the tumor mass, receptor-mediated uptake by activated leukocytes, enzymatic degradation, and death of transfected producer cells during culture. IFN- γ was also detected in mice treated with IL-2:lipid and not in the EP:lipid-treated group, suggesting a direct IL-2 transgene event and its potential contribution to the overall antitumoral response induced by IL-2:lipid treatment. It is unlikely that the differences observed in IFN- γ induction were due to differences in contaminating levels of endotoxin in the plasmid preparations, because the EP DNA actually had slightly higher levels of endotoxin than the IL-2 DNA.

The 62% average rejection of Renca tumors with a 6- μg dose of IL-2:lipid versus only 17% tumor rejection with the same dose of EP:lipid demonstrates a specific effect of the IL-2 transgene. Parker et al¹⁵ used 1,2-dimyristyloxypropyl-*N,N*-dimethyl-*N*-hydroxyethylammonium bromide/dioleoylphosphatidylethanolamine (50- μg DNA dose, 5:1 DNA:lipid mass ratio) to transfect s.c. B16 melanoma tumors *in vivo*; however, they were not able to demonstrate a specific IL-2 effect, because both a control plasmid and an IL-2 plasmid slowed tumor growth. The nonspecific effect was DNA dose-dependent, and they suggested that the nonspecific effects could be overcome with higher IL-2 expression. Our experience suggests that lower DNA/lipid doses balance transgene expression, with minimization of nonspecific cytokine induction (data not shown). Animals that rejected an initial Renca challenge after IL-2:lipid treatment were rechallenged in the opposite flank with a higher cell inoculum. Treatment with IL-2 plasmid gave strong memory against rechallenge, with 100% (21 of 21) of the animals rejecting the rechallenge (Table 1).



To test the specificity of the antitumoral immune memory induced in our system, animals surviving rechallenge for >50 days were cross-challenged with an unrelated syngeneic tumor cell implant. None of the animals were able to reject the cross-challenge, indicating that the immunological memory was indeed Renca-specific (Table 1). Animals that rejected tumors after EP:lipid treatment were also resistant to Renca rechallenge (80% survival) but not to cross-challenge. This indicates that although IL-2 production strongly induces tumor rejection that is associated with specific immune memory, such immunity could also be generated by EP:lipid treatment.

To assess the role of the effector cells involved in the immune response, animals were depleted of CD4⁺, CD8⁺, NK, or PMN cells before IL-2:lipid treatment. We showed that CD8⁺, NK, and PMN cells were necessary for antitumoral effects in our Renca model (Fig 3). Studies with IL-2-secreting TS/A tumors have shown previously that depleting CD8⁺ cells and granulocytes diminished the antitumoral response, whereas immunosuppression of NK and CD4⁺ cells had little effect.^{5,23-25} In our model, CD4⁺ depletion increased tumor rejection, indicating that some subpopulation of CD4⁺ cells may suppress antitumoral activity. Similar CD4⁺ suppression of antitumoral effects has been observed with systemic rIL-2 treatment in a murine model of advanced sarcoma.²⁶

Immunotherapy with rIL-2 has been shown to enhance the antitumoral activity of chemotherapeutic drugs in a variety of combinations in clinical studies.²⁷⁻³³ In a study of patients with metastatic colorectal cancer treated with IL-2 and 5-FU, 28% of patients showed >50% regression in the size of their tumors and 43% had stable disease over the course of the study.³³ These data suggest that the IL-2 and 5-FU combination therapy has promise, and that IL-2 gene therapy could be employed to give the benefits of IL-2 without the adverse effects associated with the systemic administration of this cytokine. In the absence of the toxic side effects of systemic IL-2, local production of IL-2 combined with 5-FU may be even more effective. We examined the effects of the combination of local IL-2 gene therapy and systemic 5-FU in mice bearing an established primary tumor and lung metastases. This model mimics clinical cases in which patients display primary tumors associated with established or undetected metastases. The 12-mg/kg dose of 5-FU used in these experiments was well tolerated in the mice and gave increased efficacy without immunosuppression. Our hypothesis is that the antimetabolite, 5-FU, acts to slow the growth of the tumors long enough to give the immune system a larger therapeutic window in which to work. Interestingly, the 42% of animals that rejected established primary flank tumors after IL-2:lipid treatment (Fig 4) also did not develop lung metastases. This is another indicator of the robustness of the IL-2:lipid response. The 94% of animals treated with IL-2:lipid and 5-FU that rejected the primary tumor also survived the lung metastases.

In this study, we present evidence that delivery of the IL-2 gene to established tumors by a nonviral vector system induces a potent antitumoral response primarily mediated by CD8⁺ T cells, granulocytes, and NK cells. We have also shown that a conventional chemotherapeutic agent, 5-FU, can be used in combination with IL-2 gene therapy to enhance the antitumoral activity. These data suggest that cytokine gene therapy may have a role as adjunct therapy to existing chemotherapeutic regimes. Clinical trials using a slightly modified human IL-2 plasmid formulated in DOTMA:cholesterol are currently underway.³⁴

ACKNOWLEDGMENTS

We thank Dr. Michael Fons for helpful discussions and critical review of the manuscript and the Integrated Manufacture and Analytics teams at Valentis, Inc. for supplying the plasmids. We also thank the Laboratory Animal Resource staff for animal care.

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