Nonviral Cytokine Gene Therapy on an Orthotopic Bladder Cancer Model

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ABSTRACT

Purpose: The purpose is to assess cytokine gene transfection in tumor cells and its therapeutic efficacy in an orthotopic mouse bladder cancer model after liposome-mediated gene transfer.

Experimental Design: A total of \(1 \times 10^5\) MB49 cells was instilled into the bladder of C57BL/6 mice after electrocautery to establish the tumor model. The plasmids were constructed by inserting the coding sequences for murine IFN-\(\gamma\) and granulocyte macrophage colony-stimulating factor into a plasmid vector pBudCE4.1. Transient transfection was performed using a cationic lipid \(N\)-(1-(2,3-dioleoyloxyl)propyl)-N,N,N-trimethylammoniummethyl sulfate and methyl-\(\beta\)-cyclodextrin-solubilized cholesterol. The \textit{in vitro} expression of cytokines was checked by ELISA. The expression of the transgene \textit{in situ} was confirmed by immunohistochemistry and 5-bromo-4-chloro-3-indolyl-\(\beta\)-galactopyranoside staining. Mice bearing orthotopic tumors were treated with plasmid DNA/liposome complex by intravesical instillation twice a week for 3 weeks.

Results: Superficial bladder tumors were established by intravesical instillation of MB49 into cauterized bladders. The expression level of cytokines in transfected cell lines was increased significantly. \textit{In situ} gene transfer to bladder tumors was accomplished via intravesical instillation of plasmid DNA/\(N\)-(1-(2,3-dioleoyloxyl)propyl)-\(N\),\(N\),\(N\)-trimethylammoniummethyl sulfate/methyl-\(\beta\)-cyclodextrin-solubilized cholesterol after a single 2 h \textit{in situ} transfection. The tumor incidence in the treatment groups was dramatically decreased from 76.9% in the control group to 15.4–30.8% in the treatment groups.

Conclusions: We demonstrated in the orthotopic mouse bladder cancer model that successful inhibition of tumor cell growth could be obtained with cytokine gene therapy. The results suggest that our liposome transfection system appears to be a promising method for gene therapy of bladder cancer \textit{in vivo}.

INTRODUCTION

According to WHO statistics for 2000, bladder cancer is the seventh most commonly occurring cancer among men in the world. The American Cancer Society estimates that in 2003 there will be \(\sim 57,400\) new cases of bladder cancer and \(12,500\) deaths from bladder cancer in the United States (1). TCC\(^2\) accounts for \(>90\%\) of bladder cancer cases. The majority of TCC tumors are superficial at the time of diagnosis and most of these (60–70%) have a propensity for recurrence after initial transurethral resection of bladder tumor. Some 15–25% of patients are at high risk for progression to invasive bladder cancer. Intravesical administration of BCG after transurethral resection is by far the most effective treatment for superficial bladder cancer (2, 3). However, side effects of BCG therapy are common, and approximately one third of patients fail to respond. On the basis of the fact that BCG activates the local immune response through the induction of cytokines (4), intravesical administration of recombinant cytokines such as IFN-\(\alpha\), tumor necrosis factor \(\alpha\), GM-CSF, and interleukin 2 have been used in a number of clinical trials with encouraging results (5–8).

However, the use of recombinant cytokines is costly and requires repeated large dose applications because the cytokines are unstable, with a short half-life in urine. Moreover, because of the lack of posttranslational modification processes in bacteria, the effects of recombinant cytokines are diminished. One solution to these limitations lies in the direct transfection of the bladder urothelium as well as cancer cells with cytokine genes. This would permit a sustained and prolonged local release of cytokines at the tumor site, which may be important as some studies have implied that high cytokine levels in the vicinity of tumor cells is crucial (9).

The popularity of adenoviruses for bladder gene therapy has waxed, waned, and waxed again as reports of its excellent transfection in animals (10) were followed by reports of the absence of CAR receptors on human bladder cancer cell lines (11, 12). More recent studies have reported better expression of CAR in human bladder cancers, but transfection is limited to the superficial layer, although CAR expression has been found in deeper layers of the urothelium (13, 14). There have been only a few reports on \textit{in vivo} transfection of orthotopic tumors using

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2 The abbreviations used are: TCC, transitional cell carcinoma; BCG, Bacillus Calmette-Guerin; GM-CSF, granulocyte macrophage colony-stimulating factor; DOTAP, \(N\)-(1-(2,3-dioleoyloxyl)propyl)-\(N\),\(N\),\(N\)-trimethylammoniummethyl sulfate; CAR, coxsackievirus adenovirus receptor; MBC, methyl-\(\beta\)-cyclodextrin-solubilized cholesterol; CMV, cytomegalovirus; mAb, monoclonal antibody; GAG, glycosaminoglycan.
liposomes (15, 16). The main reason for this is that the transfection efficiency of liposomes is generally lower than that of viral vectors. However, our group has developed a nonviral transfection system comprising of DOTAP and MBC that transfects urothelial cells with high efficiency after only 2 h of exposure (17).

This study extends our previous work by determining the efficacy of cytokine gene therapy using two cytokines, IFN-α and GM-CSF. IFN-α is a member of a family of glycoproteins that regulate cell growth and differentiation, inhibit the expression of oncogenes, up-regulate apoptosis, and activate lymphocytes, natural killer cells, and macrophages (18). Furthermore, IFN-α has been shown to down-regulate the expression of the proangiogenic molecules such as basic fibroblast growth factor, vascular endothelial growth factor, and matrix metalloproteinase 9 while increasing E-cadherin expression by human TCC, all of which results in the inhibition of tumor neoangiogenesis and growth (19, 20). GM-CSF is produced by many cell types, including B and T cells, macrophages, and endothelial cells, and it functions to stimulate the proliferation, maturation, and function of hematopoietic cells, including specialized antigen-presenting cells (21, 22).

In this study, we used an orthotopic mouse bladder cancer model that closely represents the physiological features of human bladder cancer. Our purpose was to assess the efficacy of cytokine gene transfection as a therapeutic tool.

MATERIALS AND METHODS

Tumor Cell Line. The murine transitional cell carcinoma cell line MB49 was obtained from Dr. Timothy Ratliff at the University of Iowa. The cell line were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 2 mM t-glutamine, 50 units/ml penicillin, and 0.05 mg/ml streptomycin (Sigma Chemical Company, St. Louis, MO) at 37°C and 5% CO₂

Plasmid Vectors. The β-galactosidase expression plasmid pCMV-LacZ (Clontech, Palo Alto, CA) was used to optimize gene transfer. The cytokine genes were cloned into pBudCE4.1 (Invitrogen, Carlsbad, CA), which contains two multiple cloning sites under the control of a CMV promoter and an elongation factor-1α promoter. The murine IFN-α 1 gene, a kind gift from Professor Ellen C. Zwarthoff (Erasmus University, Rotterdam, the Netherlands), was cloned behind the CMV promoter and GM-CSF were inserted behind the EF-1α promoter. The generated constructs were called pBud-IFN-α, pBud-GM-CSF and pBud-IFN-α-GM-CSF. All plasmid DNA was prepared using Qiagen Endofree Maxi Kits (Qiagen GmbH, Hilden, Germany).

In Vitro Cytokine Gene Transfection and Measurement of Expression Level by ELISA. A total of 2 × 10⁵ MB49 cells was plated on 6-well tissue culture plates 1 day before transfection. Briefly, cells were washed twice with PBS, and 1 ml of RPMI 1640 without serum was added. Next, 2.5 μg of plasmid complexed with 20 μg of DOTAP (Roche Diagnostics, Mannheim, Germany) and 40 μg of MBC (Sigma Chemical Company) were added to the well. The complexes were formed by making up the DNA and DOTAP/MBC to 162 μl with 20 mM HEPES buffer (Life Technologies, Inc., Rockville, MD) and allowing the mixture to stand for 10 min at room temperature before being incubated with cells for 2 h at 37°C. Then the liposome mixture was removed and replaced with 3 ml of fresh RPMI 1640. The pBudCE4.1 plasmid was used as vector control. After 48 h, the cell culture media were collected and frozen at −20°C. The ELISAs for murine IFN-α (Research Diagnostics, Inc., Flanders, NJ) and GM-CSF (R&D Systems, Minneapolis, MN) were performed according to the manufacturer’s direction.

Orthotopic Tumor Implantation. Four to 6-week-old female C57BL/6 mice were anesthetized and put on their back on the ground plate of the Aaron 800EU high frequency desiccator (Aaron Medical Industries, St. Petersburg, FL) after shaving the fur on their back to ensure better contact. A 24-gauge i.v. catheter was introduced into the bladder through the urethra. Next, a copper wire electrode with a diameter of 0.1 mm was inserted into the bladder via the catheter until the resistance of the bladder wall was felt. The monopolar coagulation function was applied for 1 s at 0.2 W. The electrode was withdrawn, and 0.1 ml of a single-cell suspension of MB49 cells (1 × 10⁶ cells/ml in RPMI 1640 without serum or antibiotics) were instilled into the bladder.

Histological Confirmation. The mice were sacrificed on day 7 to day 14 after implantation. The bladders were removed, formalin fixed, and embedded in paraffin. Six-μm sections were cut for H&E staining. The sections were inspected for tumor incidence, size, location, and histological stage.

Intravesical Gene Transfection. Seven days after tumor cell implantation, the animals were anesthetized and catheterized with 24-gauge i.v. catheter. The transfection complex was prepared in the same manner as for in vitro transfection, and 0.1 ml of the mixture were introduced into the bladder through the catheter and left for 2 h before being flushed out with PBS. After 48 h, the bladders were harvested. The bladders were snap frozen in liquid nitrogen, sectioned (6 μm) and fixed in 0.5% glutaraldehyde for 10 min, followed by overnight incubation with 1 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside solution and counterstained with hematoxylin.

Immunohistochemistry. Forty-eight h after transfection with pBudCE4.1 or pBud-IFN-α, the bladders were harvested. Six μm sections were cut from snap-frozen block. The sections were washed with PBS after fixation and were incubated with 1 μg/ml mouse antimouse IFN-α mAb (Research Diagnostics, Inc.) for 1 h at room temperature, washed with PBS for 5 min. Then secondary tetramethylrhodamine isothiocyanate-conjugated rabbit antimouse IgG antibody (Sigma-Aldrich Corp., St. Louis, MO) was applied to the section and incubated for 30 min at room temperature. The sections were mounted and viewed under epifluorescent illumination using Leica Q550CW fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

Flow Cytometric Analysis of Cell Surface Markers. The cells were harvested 48 h after transfection and resuspended in 1% BSA in PBS (100 μl). Cells were stained by 2 μl of purified mouse antimouse H-2Kb/H-2Db MHC I mAb mouse antimouse I-Ag MHC II mAb, hamster antimouse B7.1 mAb (PharMingen, San Diego, CA), and the corresponding isotype-matched control antibodies for 20 min at 4°C. After washing, the cells were then stained by 2 μl of rabbit antimouse IgG-FITC (Dako, Glostrup, Denmark) or antihamster IgG-FITC.
Nonviral Cytokine Gene Therapy and Bladder Cancer

Fisher's exact test. The tumor incidence and survival data were analyzed with statistical analysis of the antiproliferative effect, cell surface
iment was repeated twice.
organisms were also inspected for potential metastasis. This exper-
m sections were cut for H&E staining. The sections
were used as controls. The experiment was repeated twice.

Cell Proliferation Assay. Briefly, 2 x 10^4 MB49 cells/well were seeded in 96-well plates. Twenty-four h after incubation, the cells were transfected as described previously. After incubation for 48 h, a colormetric cell proliferation assay was performed using CellTiter 96 AQueous One Solution (Promega, Madison, WI) according to the producer's instruction. The absorbance at 490 nm was determined using a microplate reader. The percentage of cell proliferation was calculated by the formula: absorbance of cells transfected with cytokines/absorbance of cells transfected with control plasmid vector.

Experimental Cytokine Gene Therapy. All animal studies were approved by the Institutional Review Board of our university. The tumor implantation was performed on day 0 on all animals. On day 2, the mice were randomly divided into a control and three treatment groups of 6 mice each. They were treated with intravesical instillation of pBudCE4.1, pBud-IFN-α, pBud-GM-CSF, and pBud-IFN-α-GM-CSF plasmid DNA complexed with DOTAP and MBC, respectively. From day 2 onwards, each agent was administered via a 24-gauge i.v. catheter twice a week in a volume of 0.1 ml for 3 consecutive weeks. After the sixth instillation, the animals were observed for 1 more week. On day 27, all surviving mice were sacrificed. The bladders were removed, formalin-fixed, and embedded in paraffin. Six-μm sections were cut for H&E staining. The sections were inspected for tumor incidence, size, location, and histological stage. In addition, all bladders were weighed, and other organs were also inspected for potential metastasis. This experiment was repeated twice.

Statistical Analysis. One-way ANOVA was performed for statistical analysis of the antiproliferative effect, cell surface marker expression, as well as animal body and bladder weight. The tumor incidence and survival data were analyzed with Fisher's exact test. P < 0.05 indicated statistical significance.

RESULTS

In Vitro Expression of GM-CSF and IFN-α. The ELISA results showed that in MB49 cells, the expression level of GM-CSF was elevated by transfection with pBud-GM-CSF or pBud-IFN-α-GM-CSF. A 130–180-fold increase was observed as compared with control cells. Similar results were observed after transfection with IFN-α alone (Table 1). Transfection with the vector alone did not cause a change in cytokine production.

Flow Cytometric Analysis of Cell Surface Markers. The tumorigenicity of cancer cells has been linked to the reduced expression of surface markers such as MHC class I and

Table 1 IFN-α and GM-CSF expression in MB49 cells

<table>
<thead>
<tr>
<th>IFN-α (pg/ml)</th>
<th>GM-CSF (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.9 ± 0.6</td>
</tr>
<tr>
<td>Vector</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>IFN</td>
<td>487 ± 35</td>
</tr>
<tr>
<td>IFN-GM-CSF</td>
<td>556 ± 28</td>
</tr>
</tbody>
</table>

The data are shown as mean ± SE of two experiments done each in duplicate.

Table 2 The expression level of cells surface molecules in MB49 cells

<table>
<thead>
<tr>
<th></th>
<th>MHC class I</th>
<th>MHC class II</th>
<th>B7.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>56.8 ± 2.6</td>
<td>3.2 ± 0.2</td>
<td>5.2 ± 1.2</td>
</tr>
<tr>
<td>Vector</td>
<td>55.0 ± 3.9</td>
<td>4.6 ± 0.5</td>
<td>3.1 ± 1.3</td>
</tr>
<tr>
<td>IFN</td>
<td>79.4 ± 3.0*</td>
<td>5.4 ± 1.1</td>
<td>18.0 ± 3.5*</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>77.3 ± 2.9*</td>
<td>5.8 ± 1.0</td>
<td>7.5 ± 1.0*</td>
</tr>
<tr>
<td>IFN-GM-CSF</td>
<td>82.1 ± 5.2*</td>
<td>3.8 ± 0.9</td>
<td>8.6 ± 0.8*</td>
</tr>
</tbody>
</table>

* The expression levels were significantly different from control vector-transfected cells, P < 0.05, one-way ANOVA analysis.

B7.1. MB49 cells have some MHC class I molecules, yet 48 h after transfection with GM-CSF and IFN-α singly, MHC class I expression was further increased. There was however only a small increase in B7.1 expression after transfection with IFN-α and GM-CSF. MHC class II expression did not change after transfection (Table 2).

Cell Proliferation Assay. To examine the effect of cytokines on the growth rate of the cells, a colormetric cell proliferation assay was used. The result showed that growth of the tumor cells transfected with pBud-GM-CSF, pBud-IFN-α, and pBud-IFN-α-GM-CSF were significantly inhibited by 21, 37, and 30%, respectively, with reference to cells transfected with pBudCE4.1, 48 h after transfection (Fig. 1).

In Vivo Expression of IFN-α in Bladder Epithelium. To confirm that normal bladder epithelial cells could be as easily transfected and express cytokines as well as MB49 cells, we transfected a normal mouse bladder with the pBud-IFN-α plasmid and harvested the bladder 2 days after transfection and carried out immunofluorescence staining for the presence of IFN-α. IFN-α expression was confined to the superficial epithelial cell layer of the normal mouse bladder (Fig. 2).

In Vivo Expression of LacZ Reporter Gene in an Orthotopic Model of Bladder Cancer. Cauterization was used to damage the bladder wall before the instillation of tumor cells. As shown in Fig. 3A, the cauterization of the bladder mucosa produced a single point disruption of the epithelium layer of the

Fig. 1 Antiproliferative effect of cytokines on MB49 cells. Forty-eight h after transfection, the MB49 cell proliferation was inhibited from 21–37% compared with control. *, P < 0.05 versus control, one-way ANOVA analysis.
bladder, with edema in the lamina propria and muscle layer. Then the tumor cells will grow at the point where the epithelium has been damaged. We observed bladder tumors in as many as 90% of the animals tested. These tumors were single-focal and located exactly at the injured site of the bladder, normally on the posterior wall of the bladder. The tumor grew and protruded into the bladder lumen. This kind of growth pattern ensures the tumors are exclusively confined in the bladder, without i.p. dissemination or infiltration into the muscle layer of the bladder as shown in Fig. 3B.

Seven days after tumor implantation, the mouse bladder was transfected. Animals were sacrificed 2 days later. At the same time, a normal intact mouse bladder was also transfected. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside staining was performed on cryosections of the bladder. We observed positively stained superficial epithelial cells in the normal mouse bladder (Fig. 4A). Whereas in the bladder tumor, positive cells were found not only in the surface cells but also in the deeper layers of the tumor (Fig. 4B).

**Cytokine Gene Therapy.** All bladders in the cytokine gene experiment were evaluated by sectioning the whole bladder followed by H&E staining. The sections were then checked thoroughly under light microscope for any sign of microtumors.

In the control group, 69.2% (9 of 13) of the mice survived to the end of the scheduled study period in comparison with 76.9% (10 of 13), 92.3% (12 of 13), and 100% (13 of 13) in the groups treated with pBud-IFN-α, pBud-GM-CSF, and pBud-IFN-α-GM-CSF, respectively. The necropsy revealed that they usually died of renal failure caused by blockage of the urinary system or deterioration of general condition caused by extreme tumor burden. No lung metastasis was found in any of the animals confirming that the tumors were still confined to the bladders. The overall intravesical tumor incidence was significantly lower in the treatment groups compared with the control vector-treated group (P < 0.01). Histological examination showed that tumors were located at the dome of the bladder. They were single focal and remained superficial, although their sizes varied. Tumor incidence varied from 15.4 to 30.8% in the treatment groups, as compared with 76.9% in control group. (Table 3) The necropsies also revealed either bilateral or unilateral hydronephrosis in 6 animals with tumor, although no metastasis was found in the kidneys. This was probably because
of mechanical obstruction caused by the tumor. There was no significant difference in animal survival between the groups ($P > 0.05$). There was no body weight loss in the treatment group, which indicates that there was no obvious toxicity associated with our treatment.

**DISCUSSION**

Although the technique of orthotopic bladder tumor implantation has been used extensively by various investigators, the tumor incidence varies from 50 to 90% (23, 24). The techniques used include open surgery and intrabladder injection, which unfortunately, results in tumors in which growth is not restricted to the epithelium (25) and damaging the bladder epithelium with acid or trypsin, which results in multifocal tumors (26). In contrast electrocautery, first developed by Solomon et al. (23) and modified by others (27), consistently produces tumors located at the dome or the posterior wall of the bladder. Although it has been reported that up to 67% of animals may have pulmonary metastasis using electrocautery (24), we found no pulmonary metastasis, and only 1 of 56 mice had peritoneal metastasis, which was probably because of accidental puncturing of the bladder wall during the initial instillation or electrocautery. This lack of metastasis was probably because of the lower power of 0.2 W that was used for electrocautery, which ensured that only minimal damage was induced as shown in Fig. 3. The animals tolerated the implantation very well without any untoward side effects. This model produces superficial tumors that closely mimic the human disease.

The $\beta$-galactosidase reporter gene expression was found in the deeper layers of the tumor, but in the normal bladder, it was confined to the superficial epithelium. In contrast, with adenoviral transfection both in the normal bladder and tumor mass, $\beta$-galactosidase expression was confined to the superficial epithelial cells (14). This expression pattern was postulated to occur because fully differentiated epithelial cells might present a physical barrier to viral transfection; the presence of a GAG layer or the tight junctions between the urothelial cells (14). Although the GAG layer over tumor cells has a different composition from that over normal cells (28), it is likely that the deeper penetration of the Lac Z gene using our transfection system has more to do with the lack of proper organization of cells in the tumor mass rather than the GAG layer.

In our current study, using an optimized 2-h transfection protocol, we demonstrated $>100$-fold increase in the levels of both murine IFN-$\alpha$ and GM-CSF production after the in vitro transfection of MB49 cells. This level of IFN-$\alpha$ expression is comparable with that achieved by retroviral transfection of the highly metastatic $\alpha$/$\beta$-IFN-resistant Friend leukemia cells with IFN-$\beta$ (29). GM-CSF production by the MB49 cells after transfection was comparable with that achieved by particle-mediated gene transfer to human breast and melanoma cells (30). Immunofluorescence staining of a normal bladder transfected with IFN-$\alpha$ confirmed that in vivo and in vitro transfection efficiencies were similar. Moreover, in vivo transfection efficiency of IFN-$\alpha$ and GM-CSF in mouse bladder was also confirmed by reverse transcription-PCR (data not shown).

Cytokine gene transfection resulted in an antiproliferative effect of some 20–37%. This small decrease in cell numbers coupled with the known antiangiogenic properties of IFN-$\alpha$ (19) and immunostimulatory effects of GM-CSF (31) could result in a significant effect on tumor growth in vivo. As our transfection efficiency in vitro is $\sim50$–75% (17), the lower decrease in cell proliferation and B7.1 expression may be attributable to the fact that the MB49 tumor cell line is made up of a heterogeneous mixture of cells with varying mutational background.

In this study, we demonstrated that 69.2–84.6% of the animals were cured of bladder tumors with intravesical instilla-

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**Table 3** Effect of intravesical cytokine gene therapy

The data are recorded as mean ± SD.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean body weight (g)</th>
<th>Mean bladder weight (mg)</th>
<th>Tumor incidence</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>15.4 ± 2.5</td>
<td>30.5 ± 3.8</td>
<td>10/13</td>
<td>9/13</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>16.7 ± 2.1</td>
<td>24.6 ± 3.2</td>
<td>2/13*</td>
<td>12/13</td>
</tr>
<tr>
<td>IFN-$\alpha$</td>
<td>15.9 ± 1.9</td>
<td>30.1 ± 4.1</td>
<td>4/13*</td>
<td>10/13</td>
</tr>
<tr>
<td>IFN-$\alpha$-GM-CSF</td>
<td>17.0 ± 1.1</td>
<td>31.2 ± 4.6</td>
<td>2/13*</td>
<td>13/13</td>
</tr>
</tbody>
</table>

* $P < 0.01$ versus control, Fisher’s exact test.

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**Fig. 4** $\beta$-Galactosidase reporter gene transfection of a normal and tumor bearing bladder. A 2-h intravesical administration of DNA/plasmid complex resulted in transduction of the urothelial cells. A, in a normal mouse bladder transfected with pCMV-LucZ, the transgene expression was limited in the superficial layer of the urothelium. Original magnification, ×200. B, in contrast, in a bladder tumor the transgene expression was also observed in deeper layers of cells. Original magnification, ×100.
tion of plasmid-encoding cytokine genes but not control plasmid. These results strongly suggest that the treatment effects were attributable to local secretion of cytokines but not because of the inflammation caused by repeated instillation. In the control group, 10 of 13 mice had tumors, and 4 mice died from excessive tumor burden. Mice with bladders that were >50% occluded by tumor died prematurely. A similar pattern was observed in the GM-CSF- and IFN-α-treated groups, but in the group transfected with both cytokines, there were no deaths, although 2 mice developed tumors.

It has been previously reported that for tumor-bearing mice, body weights decrease at day 18 after tumor implantation (24). We did not find a dramatic difference in bladder weights between treatment groups, rather there was a difference in bladder weights between tumor-bearing and cured mice regardless of their treatment. The average bladder weight was 37.16 ± 3.79 mg in tumor-bearing mice compared with 25.01 ± 1.22 mg in cured mice. Overall, the bladder weights of our tumor-bearing mice were much lower than that reported by others (24, 32). This could be because of the age of the mice (4–6 weeks old) used in our experiments.

In summary, our transfection system transfects bladder epithelial cells in vivo with a significant level of transgene expression. Additional repeated intravesical instillations of cytokine genes were well tolerated and did result in a reduction of the tumor incidence. Future studies would include the study of cytokine combinations that could act synergistically. It is not clear from our studies if the two cytokines were acting synergistically.

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