Proc. Natl. Acad. Sci. USA
Vol. 90, 4645–4649, May 1993
Genetics

Immunotherapy of malignancy by *in vivo* gene transfer into tumors

(gene therapy/major histocompatibility complex/cancer/T cells/adenocarcinoma)

GREGORY E. PLAUTZ*†, ZHI-YONG YANG*, BEI-YUE WU*, XIANG GAO‡, LEAF HUANG‡, AND GARY J. NABEL*§

*Howard Hughes Medical Institute, †University of Michigan Medical Center, Departments of Internal Medicine, Biological Chemistry, and Pediatrics and Communicable Diseases, Ann Arbor, MI 48109-0650; and ‡University of Pittsburgh, Department of Pharmacology, Pittsburgh, PA 15261

Communicated by James V. Neel, January 28, 1993

ABSTRACT **The immune system confers protection against a variety of pathogens and contributes to the surveillance and destruction of neoplastic cells. Several cell types participate in the recognition and lysis of tumors, and appropriate immune stimulation provides therapeutic effects in malignancy. Foreign major histocompatibility complex (MHC) proteins also serve as a potent stimulus to the immune system. In this report, a foreign MHC gene was introduced directly into malignant tumors *in vivo* in an effort to stimulate tumor rejection. In contrast to previous attempts to induce tumor immunity by cell-mediated gene transfer, the recombinant gene was introduced directly into tumors *in vivo*. Expression of the murine class I *H-2K*^{s} gene within the CT26 mouse colon adenocarcinoma (*H-2K*^{d}) or the MCA 106 fibrosarcoma (*H-2K*^{b}) induced a cytotoxic T-cell response to H-2K^s and, more importantly, to other antigens present on unmodified tumor cells. This immune response attenuated tumor growth and caused complete tumor regression in many cases. Direct gene transfer *in vivo* can therefore induce cell-mediated immunity against specific gene products, which provides an immunotherapeutic effect for malignancy, and potentially can be applied to the treatment of cancer and infectious diseases in man.**

The immune system has demonstrated the potential to play a protective role in malignancy, but the vast majority of malignancies arise in immunocompetent hosts. This observation suggests that tumor cells escape normal host defenses, and appropriate immune stimulation may provide therapeutic effects (1–25). Several approaches have been used previously to augment the naturally occurring immune response to malignancies. These include the stimulation of cytotoxic T cells that demonstrate class I major histocompatibility complex (MHC)-restricted lysis (5); tumor-infiltrating lymphocytes, which selectively lyse the tumor from which they were derived (6–8); natural killer cells (9, 10); and macrophages. In animal studies and human trials, attempts have been made to bolster the immune response by tumor immunization (11), administration of cytokines (12), or infusion of lymphoid cells sensitized to tumor-associated antigens (12–14). Others have attempted to introduce new antigens into tumor cells by drug treatment or virus infection (15–17). In addition, the expression of cytokine genes by genetically modified tumor cells induces an antitumor effect (18–22), mediated by lymphocytes or other inflammatory cells. Despite considerable progress, many cancers remain unresponsive to immunotherapy or other treatments.

Immunotherapy by gene transfer has traditionally relied upon genetic modification of cells *in vitro* and cell-mediated introduction of recombinant genes *in vivo*. Because this approach requires establishment of cells in tissue culture and clonal selection *in vitro*, its application to tumors *in vivo* and its adaptation to common clinical practice have remained difficult. The introduction of recombinant genes directly into malignant tumors *in vivo* could eliminate the need to establish cell lines from each patient in the laboratory and could minimize delays in the time to treatment. This advance facilitates application to human disease and will allow further insight into the mechanisms of immunosurveillance *in vivo*. We now report a method to induce tumor regression by transfer and expression of a recombinant gene directly in tumors *in vivo*.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Vector Production and Analysis. The Moloney murine leukemia virus-derived retroviral vector pLJ (26) was modified by insertion of a 1400-base-pair (bp) *Eco*RI cDNA fragment encoding H-2K^s provided by Larry Pease (Mayo Clinic, Rochester, MN) at the *Bam*HI cloning site by blunt-end ligation. The pLJ-H-2K^s plasmid was modified to produce an RNA transcript analogous to that of the retroviral vector, but the 3′ long terminal repeat and polyoma virus sequence were removed. This vector, designated pLTS-K^s, was prepared by digestion of pLJ-H-2K^s with *Nhe* I and treatment with the Klenow fragment, followed by ligation of a *Bgl* II–*Bam*HI fragment from Rous sarcoma virus β-globin DNA that had also been treated with the Klenow fragment. This modification results in replacement of the 3′ long terminal repeat and polyoma region with a fragment containing the 3′ untranslated and polyadenylylation sequences of the simian virus 40 early gene region. An in-frame deletion mutant of pLTS-K^s, pLTS-K^s(del-1–205) was prepared by digestion with *Apa* I, and incubation with phage T4 DNA polymerase with dNTPs, followed by religation; this removed nucleotides 63–679, which encode the α_1 and α_2 domains of the class I MHC protein.

The pLJ-H-2K^s expression vector was introduced into the ecotropic Moloney murine leukemia virus producer cell line ψCRE (27) by calcium phosphate transfection, and helper virus-free subclones producing viral titers of 10^5 G418-resistant colonies per ml were isolated. The murine colon adenocarcinoma line CT26 was infected with the ψCRE-H-2K^s virus, and G418-resistant cells were analyzed by fluorescence-activated cell sorting *in vivo*.

To introduce retroviral or DNA liposome vectors, tumor capsules (0.5- to 1-cm diameter) were exposed surgically, and multiple needle injections (2–10) were delivered to the parenchyma. With β-galactosidase reporter plasmids, recombinant gene expression could be readily detected after intratumor injection of DNA–liposome complexes (28) or retroviral vectors and was estimated to be ≈0.1–1% at the sites of injection (data not shown). Genomic DNA was isolated 2 weeks after intratumor injection. Primers with the sequence

Abbreviation: MHC, major histocompatibility complex.

§To whom reprint requests should be addressed.

GACACGGAGTTCGTGCGCTTCGACAGCGAC (sense) and CGCCCTCTAGGTAGGCCCTGAATCTCTCTG (antisense) were used, which generated a 400-bp fragment when using a 72°C extension for 2 min and a 94°C melting step for 1 min for 35 cycles. The sensitivity of this method as determined by dilutional analysis is estimated to be between 1 copy per 10^3–10^5 genomes (29). CT26 and CT26 *H-2K*^{s} cell lines were maintained *in vitro* and served as negative and positive controls, respectively.

Cytolytic T-Cell Assay. Lymphocytes were incubated in a cytolytic assay with radiolabeled (^{51}Cr) CT26 or CT26 *H-2K*^{s} target cells at the indicated ratios for 4 hr at 37°C (see Fig. 2 *A–D*) as described (30). Specific lysis is defined as [(experimental − spontaneous cpm/(total − spontaneous cpm)] × 100. Antibodies to cell-surface glycoproteins to inhibit cytolysis were incubated at effector-to-target ratios of 25:1 (see Fig. 2*E*).

Transduction of Tumors *in Vivo*. Tumors were surgically exposed and injected several times (2–10) with 0.2 ml of the indicated vector. Retroviral vectors were concentrated 200-fold by centrifugation (4–8 × 10^6 plaque-forming units/ml) and mixed with polybrene prior to injection (31). DNA–liposome complexes were prepared by addition of *H-2K*^{s} or β-galactosidase reporter plasmid DNA (1 μg) into 0.2 ml of lactated Ringer's solution, followed by addition of 15 nmol of 3β-[*N*-(*N*′,*N*′-dimethylamino-ethane)carbamoyl]cholesterol liposome (28) and incubation at room temperature for 30 min prior to injection. Tumor diameter was measured in two perpendicular dimensions with calipers.

RESULTS

We have adapted previous methods with DNA–liposome complexes or retroviral vectors that deliver recombinant genes to specific sites *in vivo* (31). To express an allogeneic MHC antigen in poorly immunogenic transplantable tumors, CT26 (19, 32) and MCA 106 (11), a retroviral vector that encodes the product of *H-2K*^{s} gene was prepared. CT26 cells, transduced with this vector and selected for G418 resistance, showed a higher mean fluorescence intensity than did uninfected CT26 cells (Fig. 1*A*). When 10^6 CT26 cells that express H-2K^s protein were injected subcutaneously into BALB/c mice (*H-2K*^{d}) sensitized to this antigen, no tumors were observed over an 8-week period (0 of 5) in contrast to the unmodified CT26 (*H-2K*^{d}) tumor line, which routinely formed tumors at this dose (30 of 30). The immune response to H-2K^s therefore provided protection against CT26 cells bearing this antigen.

To determine whether protective effects could be achieved by introduction of H-2K^s protein in established growing CT26 tumors, the recombinant *H-2K*^{s} or a β-galactosidase control gene was introduced directly into tumors *in vivo* by using DNA–liposome complexes or a retroviral vector. Mice that received intratumor injections of the *H-2K*^{s} DNA–liposome complex or *H-2K*^{s} retroviral vector showed evidence of the recombinant DNA by PCR analysis in the tumor. When introduced by DNA–liposome transfection, it was occasionally also observed in other tissues (Fig. 1) but was present at low frequencies (<1 cell per 10^5). No evidence of inflammation or toxicity was detected in these organs pathologically (29).

A cellular immune response to the recombinant H-2K^s protein was evident in the animals injected with the *H-2K*^{s} DNA–liposome complex or the *H-2K*^{s} retroviral vector. Lymphocytes derived from mice bearing the *H-2K*^{s}-, but not the β-galactosidase reporter plasmid-transduced tumors, demonstrated a cytolytic response to H-2K^s, whether delivered by retroviral vectors or liposomes (Fig. 2). More importantly, lymphocytes derived from the *H-2K*^{s}-, but not β-galactosidase reporter plasmid-transduced animals, recog-

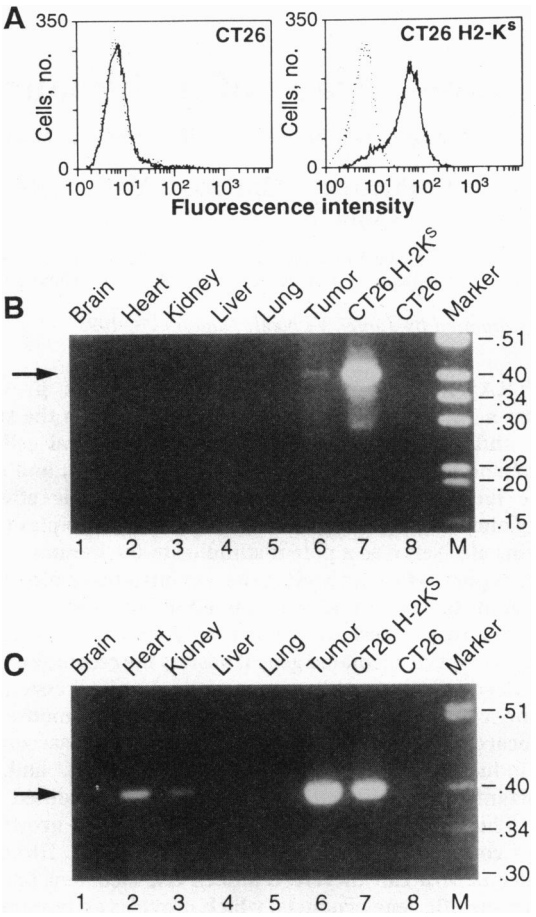

FIG. 1. Expression of H-2K^s glycoprotein on CT26 adenocarcinoma cell lines and distribution of the recombinant gene *in vivo*. (*A*) Cell-surface expression was analyzed by fluorescence-activated cell sorting with CT26 cells (*Left*) or with CT26 cells that express H-2K^s protein incubated with normal mouse serum (· · · ·) or alloantisera against H-2K^s (—) (*Right*). (*B* and *C*) Localization of vector DNA was determined *in vivo* by PCR. Genomic DNA was isolated from the indicated tumors or tissues from representative mice receiving intratumor injections of *H-2K*^{s} DNA–liposome, with the plasmid expression localized to tumor tissue alone (*B*) or with expression in the tumor and elsewhere (*C*).

nized and lysed unmodified CT26 cells (Fig. 2 *B* and *D*), suggesting that this stimulation induced immune reactivity against genetically unmodified tumor cells. This lysis was inhibited by an antibody against murine CD8 (Fig. 2*E*) which blocks cytolytic T cell but not lymphokine-activated killer or antibody dependent cell-mediated cytotoxicity cell killing (33). The CT26 and CT26 *H-2K*^{s} cell lines were appropriately lysed by *H-2K*^{s} cytotoxic T-lymphocytes and were equally sensitive to lymphokine-activated killer cells (data not shown). Antibodies against H-2K^s were not detected in mice that received intratumoral injection of *H-2K*^{s} (data not shown). Therefore, immune stimulation by H-2K^s induced specific T-cell recognition of tumor-associated antigens not normally seen by the immune system.

To assess the protective effect of the immune response against H-2K^s *in vivo*, tumor growth was quantitated. When animals received no prior sensitization to H-2K^s, tumors transduced with *H-2K*^{s} showed attenuation of tumor growth that was not complete (Fig. 3*A*). In contrast, no antitumor effect was seen in unmodified or β-galactosidase reporter plasmid-transduced controls. Because these tumors were large at the time of initial injection and continued to grow as the primary immune response was generated, an attempt was

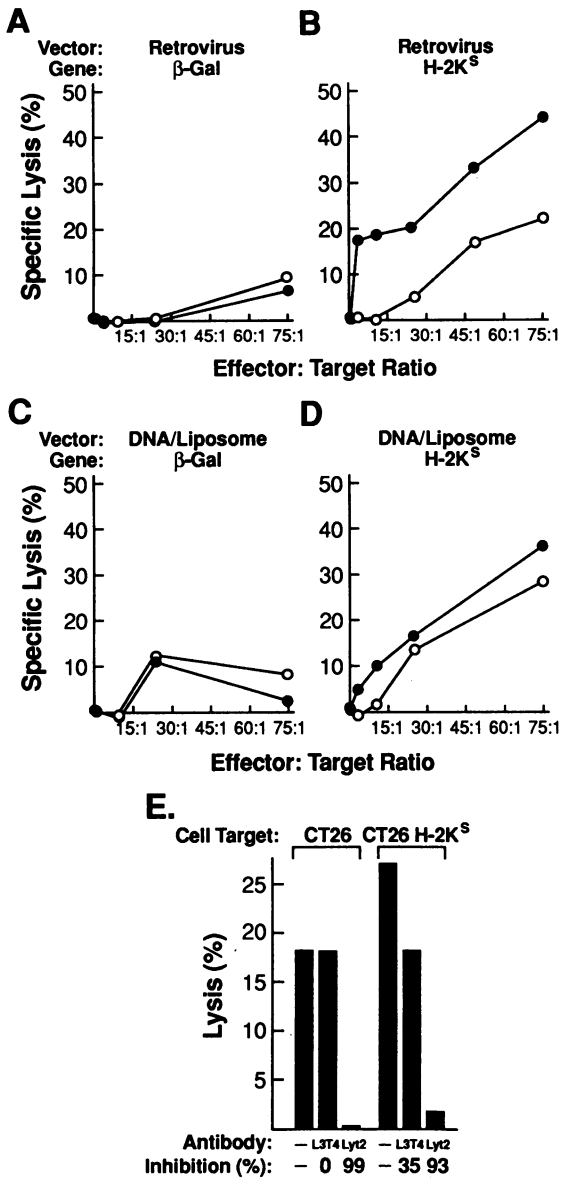

FIG. 2. Cytolytic T-cell response to H-2K^s and unmodified CT26 cells after direct *H-2K^s* gene transfer into tumors, and the CD8 phenotype of effector cells. Splenic lymphocytes were derived from nonimmune mice that received intratumoral injections with the retroviral vector carrying the β-galactosidase (β-Gal) gene (30) (*A*), the *H-2K^s* retroviral vector (*B*), the β-galactosidase plasmid DNA–liposome complex (*C*), or the pLJ-H-2K^s DNA–liposome complex (*D* and *E*). CT26 (○) or CT26 *H-2K^s* (●) cells radiolabeled with ^{51}Cr were added as target cells. The monoclonal antibodies to mouse CD4 (L3T4) and against mouse CD8 (Lyt-2) antigens were included in the cytolytic T-cell assay against CT26 and CT26 *H-2K^s* (*E*). Splenic lymphocytes were derived from nonimmune BALB/c mice bearing CT26 tumors injected with the retroviral vectors carrying the β-galactosidase reporter gene or *H-2K^s* on day 15 (*A* and *B*) or *H-2K^s* plasmid DNA–liposome complex at days 10, 14, and 17 (*C*, *D*, and *E*) after tumor inoculation. Results are representative of at least three independent experiments.

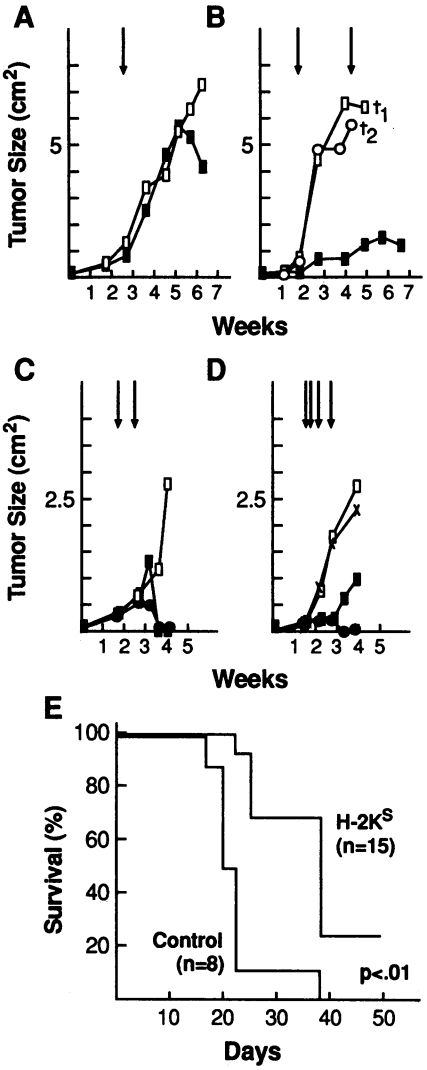

FIG. 3. Introduction of *H-2K^s* into CT26 or MCA 106 tumors *in vivo* inhibits tumor growth and prolongs survival. (*A*) Naive BALB/c mice bearing CT26 tumors were injected at the indicated times (↓) with the β-galactosidase reporter plasmid (□) or pLJ-H-2K^s (■) retroviral vector. DNA–liposome complexes with pLJ-H-2K^s (■, ●) or the β-galactosidase plasmid (□) were injected into CT26 tumors (*B* and *C*) preimmunized to H-2K^s in BALB/c mice, or these mice were uninjected (○). Preimmunized C57BL/6 mice bearing MCA 106 fibrosarcoma tumors were injected with the pLTS-K^s–liposome complex (■, ●) or the β-galactosidase plasmid DNA–liposome complex (□, ×) (*D*). (*E*) Survival of C57BL/6 mice inoculated with the MCA 106 tumor and injected with the *H-2K^s* DNA–liposome complex or the β-galactosidase DNA–liposome complex. Significance of the difference in survival was determined by the Wilcoxon rank sum test. Mice were naive (*A*) or preimmunized (*B–E*) by intraperitoneal injection with 10^6 irradiated CT26 *H-2K^s* cells 7–14 days prior to inoculation of 10^6 CT26 (*B* and *C*) or MCA 106 (*D* and *E*) cells subcutaneously into the hind flank. In *B*, mice that were treated with β-galactosidase DNA–liposome injection or were untreated expired on day 35 (t_1) and day 30 (t_2), respectively. In *C*, the experiment was terminated at 30 days for pathologic analysis of tissues.

made to optimize the antitumor response by preimmunization of mice with irradiated CT26 *H-2K^s* tumor cells and by earlier and/or more frequent injections of vector. Preimmunization and earlier treatment with the *H-2K^s* DNA–liposome complex improved survival and attenuated tumor growth in contrast to β-galactosidase reporter plasmid-transduced tumors, where there was no difference in growth rate compared with uninjected controls (Fig. 3*B*). Full tumor regression of CT26 was achieved by increasing the number of injections and by delivery of *H-2K^s* into tumors at an earlier stage (Fig. 3*C*). This treatment was not always curative, and residual tumor growth could be observed microscopically at the margins of some tumors, but it was nonetheless protective (Fig. 3 *B* and *E*). Presensitization with CT26 *H-2K^s*, followed by treatment with the *H-2K^s* DNA–liposome complex, also proved effective for an independent unrelated malignancy, the MCA 106 fibrosarcoma (Fig. 3*D*), showing that presentation of H-2K^s on a heterologous allogeneic cell line en-

hances protection against a syngeneic tumor transduced *in vivo* with *H-2K*^{s} DNA. A total of 12 BALB/c mice bearing CT26 tumors have been treated with intratumor injection of *H-2K*^{s}. In both unimmunized or preimmunized animals, statistically significant tumor regression was observed (0 of 14 vs. 9 of 12 responders for β-galactosidase plasmid- vs. *H-2K*^{s}-treated mice, $P \leq 0.015$). Additionally, >70% of C57BL/6 mice inoculated with the MCA 106 fibrosarcoma responded to treatment with *H-2K*^{s} (13 of 18), in contrast to β-galactosidase plasmid or *H-2K*^{s} deletion mutant controls (0 of 13) ($P = 0.01$). The average life span of mice whose tumors were transduced with *H-2K*^{s} was more than twice the control group ($P < 0.01$) (Fig. 3*E*). Cures were observed in ≈20% of animals (Fig. 4). In preliminary studies, injection of *H-2K*^{s} DNA–liposome complexes conferred a greater antitumor effect than injection of *H-2K*^{s}-modified cells (CT26 *H-2K*^{s}) into established tumors.

The T-cell dependence of this response and its immunologic specificity were also analyzed *in vivo*. Nude mice bearing CT26 or MCA 106 showed no reduction in tumor growth after *H-2K*^{s} injections compared with controls (Fig. 5 *A* and *B*). Furthermore, the presence of the polymorphic region of the class I MHC gene was required for this effect, since an in-frame deletion mutant plasmid did not provide an antitumor response (Fig. 5*C*). Finally, the specificity of tumor protection was evaluated in MCA 106 tumor-bearing mice treated with intratumoral injections of the *H-2K*^{s} DNA–liposome complexes. Secondary inoculation at distant sites with the parental MCA 106 tumor (10^5 cells) produced tumor growth in only one of five subjects, whereas the unrelated syngeneic tumor B16BL/6 melanoma (10^5 cells) grew in five of five animals after 3 weeks ($P \leq 0.02$). Similar results were obtained by inoculation of the syngeneic unrelated tumor MCA 205 ($P \leq 0.02$). The protective effect against secondary inoculation of the parental tumor line was observed when using up to 10^6 cells, whereas untreated animals readily form tumors with comparable numbers of cells. Mice cured of MCA 106 by *H-2K*^{s} gene transfer succumbed to B16 melanoma cells (10^5) within 2 weeks (data not shown), documenting the specificity of protection.

DISCUSSION

In this study, stimulation of the immune system by a foreign MHC antigen caused sensitization to otherwise unrecognized tumor antigens and provided a therapeutic effect for an established malignancy *in vivo*. These studies have relied upon the direct introduction of genes into tumors by *in vivo* transfection. Traditionally, gene-transfer approaches have focused on the modification of tumor cells *in vitro*, followed by transfer of modified cells. The introduction of recombinant genes directly into malignant tumors *in vivo* could eliminate the need to establish cell lines from patients in the laboratory, facilitating application to human disease.

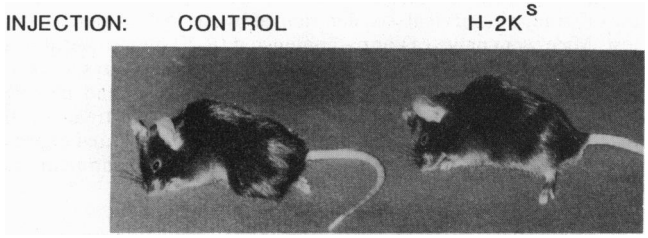

FIG. 4. Treatment of MCA 106 tumors with intratumoral injection of *H-2K*^{s} induces tumor regression. C57BL/6 mice, bearing MCA 106 tumors, were treated with intratumoral injections of DNA–liposome complexes with β-galactosidase DNA (control) or *H-2K*^{s} DNA. Mice were preimmunized by intraperitoneal injection with 10^6 irradiated CT26 *H-2K*^{s} cells 14 and 7 days prior to inoculation of 6×10^5 MCA 106 cells subcutaneously into the hind flank. Tumors were injected with DNA–liposome complexes on days 9, 12, 15, and 20 after inoculation. Photos were obtained on day 27.

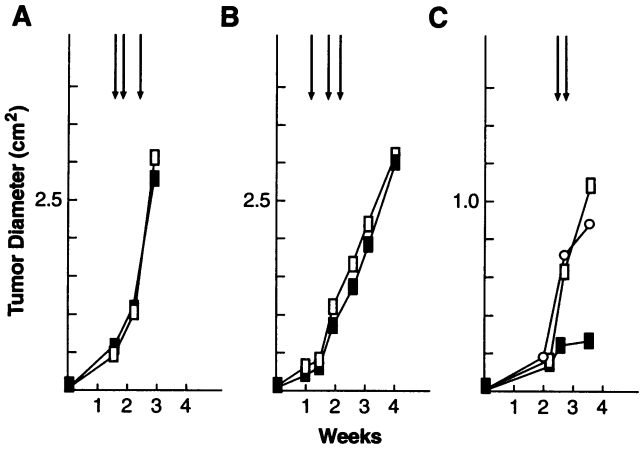

FIG. 5. The antitumor response to H-2K^s is T-cell dependent *in vivo* and requires the α_1 and α_2 domains of H-2K^s. CT26 (*A*) or MCA 106 (*B*) cells were injected subcutaneously into the flank of BALB/c *nu/nu* mice and injected with DNA–liposome complexes containing plasmids encoding full-length H-2K^s (pLTS-K^s) (■) or β-galactosidase (□). In *C*, C57BL/6 mice were inoculated with the MCA 106 fibrosarcoma and treated with these plasmids or an in-frame deletion mutant, pLTS-K^s(del 63–679) ($n = 3$) (○), respectively. Tumors were injected with DNA–liposome complexes (*A* and *B*) containing the β-galactosidase expression vector (□) or pLJ-H-2K^s (■) on days 7, 10, and 13 after tumor inoculation, and tumor diameter was measured in two perpendicular dimensions using calipers. Each group represents the average of five independent subjects. C57BL/6 mice were preimmunized (*C*) by intraperitoneal injection of 10^6 irradiated CT26 *H-2K*^{s} cells, and each group represents the average of at least three independent subjects. Fourteen days later, the MCA 106 fibrosarcoma was inoculated subcutaneously. The pLJ-H-2K^s plasmid was modified as described (*Materials and Methods*) to remove the 3′ long terminal repeat and polyoma sequences in the retroviral vector.

The introduction of recombinant genes into tumors also generates biologic responses that differ from those induced by coinoculation of tumor cell populations. For example, direct expression of H-2K^s proteins in tumors induces cytolytic cells to respond to previously unrecognized antigens present on unmodified tumor cells, whereas coinoculation of *H-2K*^{s}-positive and -negative cells does not confer tumor immunity. It is likely that this difference results from stimulation of the immune system within a mature tumor, which leads to increased local production of cytokines that act through several mechanisms. Such cytokines—for example, tumor necrosis factor α or γ interferon (IFN-γ)—can be directly toxic to malignant cells (34). In addition, IFN-γ can increase endogenous class I and class II MHC expression (35), which can present tumor antigens more effectively to lymphoid cells (36, 37). Despite the occasional presence of *H-2K*^{s} DNA within other organs (Fig. 1), no acute toxicity, long-term pathologic, or autoimmune changes were observed in the major organs of animals receiving intratumoral or intravenous DNA–liposome complexes (29, 38), suggesting that tumor-associated antigens were recognized by the immune system.

Finally, in contrast to most forms of immunotherapy, direct transfer of foreign MHC genes confers protection against established tumors. Its efficacy might be further improved by combination with other forms of treatment, including T-cell adoptive transfer or preimmunization. Preimmunization in the murine model was required to produce complete tumor regression because these tumor lines have been selected for rapid growth and minimal immunogenicity. However, most human tumors display more indolent growth;

therefore, preimmunization may not be necessary in man, and it could be performed easily if needed. Another potential improvement would be to deliver recombinant genes to specific sites by catheter *in vivo* in an effort to introduce gene products into metastatic lesions and into the tumor microcirculation. The direct transfer of a recombinant gene into tumors *in vivo* can thus induce a specific cell-mediated immune response to its product and provide a clinically relevant strategy for the treatment of established malignancy. At the same time that these approaches stimulate the recognition of otherwise undetected antigens and lead to protection against tumors, they may also facilitate efforts to identify tumor antigens that could serve as vaccines and to clone their corresponding cDNA. Such antigens could arise as a consequence of the genetic instability associated with the stepwise progression of mutations leading to malignancy. If such mutant proteins appear early and consistently, direct gene-transfer approaches could also play a role in the primary prevention of cancer or in immunization against infectious diseases.

We thank Drs. Elizabeth G. Nabel, Alfred Chang, Bernard Fox, Suyu Shu, and Mark Stewart for helpful comments and discussion; Xu Ling and Dr. Hong San for technical assistance; Dr. Larry Pease for kindly providing the H-2K^s cDNA and alloantisera; Dr. Suyu Shu for providing monoclonal antibodies, Lyt-2 and L3T4; Dr. Hans Schreiber for the hybridoma against H-2K^s; Eric Fearon for CT26 cells; and Donna Gschwend for typing the manuscript. This work was supported in part by a grant from the National Institutes of Health (AI-29179 to G.J.N.).

1. Khavari, P. (1987) *Yale J. Biol. Med.* **60,** 409–419.
2. Lotze, M. T., Chang, A. E., Seipp, C. A., Simpson, C., Vetto, J. T. & Rosenberg, S. A. (1986) *J. Am. Med. Assoc.* **256,** 3117–3124.
3. Osband, M. E. & Ross, S. (1990) *Immunol. Today* **11,** 193–195.
4. Russell, S. J. (1990) *Immunol. Today* **11,** 196–200.
5. Kern, O., Klarnet, J., Jensen, M. & Greenberg, P. J. (1986) *J. Immunol.* **136,** 4303–4310.
6. Rosenberg, S. A., Spiess, P. & Lafreniere, R. (1986) *Science* **223,** 1318–1321.
7. Muul, L. M., Spiess, P. J., Director, E. P. & Rosenberg, S. A. (1987) *J. Immunol.* **138,** 989–995.
8. Topalian, S. L., Solomon, D. & Rosenberg, S. A. (1989) *J. Immunol.* **142,** 3714–3725.
9. Oldham, R. K. (1983) *Cancer Metastasis Rev.* **2,** 323–336.
10. Herberman, R. B. (1985) *Concepts Immunopathol.* **1,** 96–132.
11. Shu, S. & Rosenberg, S. A. (1985) *J. Immunol.* **135,** 2895–2903.
12. Lafreniere, R. & Rosenberg, S. A. (1985) *Cancer Res.* **45,** 3735–3741.
13. Rosenberg, S. A., Packard, B. S., Aebersold, P. M., Solomon, D., Topalian, S. L., Toy, S. T., Simon, P., Lotze, M. T., Yang, J. C. & Seipp, C. A. (1988) *N. Engl. J. Med.* **319,** 1676–1680.
14. Yoshizawa, H., Sakai, K., Chang, A. E. & Shu, S. Y. (1991) *Cell. Immunol.* **134,** 473–479.
15. Itaya, T., Yamagiwa, S., Okada, F., Oikawa, T., Kuzumaki, N., Takeichi, N., Hosokawa, M. & Kobayashi, H. (1987) *Cancer Res.* **47,** 3136–3140.
16. Pucetti, P., Romani, L. & Fioretti, M. C. (1987) *Cancer Metastasis Rev.* **6,** 93–111.
17. Sugiura, C., Itaya, T., Kondoh, N., Oileawa, T., Kuzamaki, N., Takeichi, N., Hosokawa, M. & Kobayashi, H. (1988) *Jpn. J. Cancer Res.* **79,** 1259–1263.
18. Tepper, R. I., Pattengale, P. K. & Leder, P. (1989) *Cell* **57,** 503–512.
19. Fearon, E. R., Pardoll, D. M., Itaya, T., Golumbek, P., Levitsky, H. I., Simons, J. W., Karasuyama, H., Vogelstein, B. & Frost, P. (1990) *Cell* **60,** 397–403.
20. Gansbacher, B., Zier, K., Daniels, B., Cronin, K., Bannerji, R. & Gilboa, E. (1990) *J. Exp. Med.* **172,** 1217–1224.
21. Gansbacher, B., Bannerji, R., Daniels, B., Zier, K., Cronin, K. & Gilboa, E. (1990) *Cancer Res.* **50,** 7820–7825.
22. Esumi, N., Hunt, B., Itaya, T. & Frost, P. (1991) *Cancer Res.* **51,** 1185–1189.
23. Hui, K., Grosveld, F. & Festenstein, H. (1984) *Nature (London)* **311,** 750–752.
24. Wallich, R., Bulbuc, N., Hammerling, G. J., Katzav, S., Segal, S. & Feldman, M. (1985) *Nature (London)* **315,** 301–305.
25. Hui, K. M., Sim, T. F., Foo, T. T. & Oei, A. A. (1989) *J. Immunol.* **143,** 3835–3843.
26. Korman, A. J., Frantz, J. D., Strominger, J. L. & Mulligan, R. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2150–2154.
27. Danos, O. & Mulligan, R. C. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6460–6464.
28. Gao, X. & Huang, L. (1991) *Biochem. Biophys. Res. Commun.* **179,** 280–285.
29. Stewart, M. J., Plautz, G. E., Del Buono, L., Yang, Z. Y., Xu, L., Gao, X., Huang, L., Nabel, E. G. & Nabel, G. J. (1992) *Hum. Gene Ther.* **3,** 267–275.
30. Nabel, E. G., Plautz, G. & Nabel, G. J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5157–5161.
31. Nabel, E. G., Plautz, G. & Nabel, G. J. (1990) *Science* **249,** 1285–1288.
32. Brattain, M. G., Strobel-Stevens, J., Find, D., Webb, M. & Sarrif, A. M. (1980) *Cancer Res.* **40,** 2142–2146.
33. Sarmiento, M., Glasebrook, A. L. & Fitch, F. W. (1980) *J. Immunol.* **125,** 2665–2672.
34. Powell, M. B., Conta, B. S., Horowitz, M. & Ruddle, N. H. (1985) *Lymphokine Res.* **4,** 13–26.
35. Lindahl, P., Leary, P. & Gresser, I. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2785–2788.
36. Itaya, T., Fearon, E., Fiesinger, T., Hunt, B., Vogelstein, B. & Frost, P. (1991) *Cancer Immunol. Immunother.* **33,** 267–273.
37. Restifo, N. P., Spiess, P. J., Karp, S. E., Mule, J. J. & Rosenberg, S. A. (1991) *J. Exp. Med.* **175,** 1423–1431.
38. Nabel, E. G., Gordon, D., Yang, Z.-Y., Xu, L., San, H., Plautz, G. E., Wu, B.-Y., Gao, X., Huang, L. & Nabel, G. J. (1992) *Hum. Gene Ther.* **3,** 649–656.