Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 7024–7028, August 1993
Medical Sciences

Regression of established macroscopic liver metastases after *in situ* transduction of a suicide gene

(gene therapy/retroviral vector/thymidine kinase/nucleoside analogs/cancer)

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Communicated by Jean Dausset, April 27, 1993

ABSTRACT **The herpes simplex virus type 1 thymidine kinase (HSV1-TK) converts nontoxic nucleoside analogs such as ganciclovir into phosphorylated compounds that act as chain terminators and specifically kill dividing cells. This property could be exploited for the treatment of tumors that are made up of rapidly dividing cells invading a nonproliferating tissue. For this purpose, specific expression of the suicide gene into dividing tumor cells can be further targeted by using retroviral-mediated gene transfer. We investigated whether the direct intratumoral transduction of a suicide gene might induce the elimination of malignant solid tumors. Rats with established macroscopic liver metastases were given an intratumoral injection of packaging cells producing ejther HSV1-TK- or *lacZ*-expressing recombinant retroviral particles. All rats were next treated with ganciclovir. A dramatic regression of the tumor volume was observed in the HSV1-TK-treated animals. The residual tumors were mostly made up of a massive fibrotic reaction, with the mean cancer cell mass being reduced ≈60-fold compared to controls. In some animals, the residual tumors were devoid of cancer cells. This treatment efficacy appears in part due to a "bystander effect" in which phosphorylated ganciclovir could be transferred from cell to cell and to an active local immune reaction evidenced by massive infiltration of the tumors by macrophages and both CD4⁺ and CD8⁺ lymphocytes. This efficient therapeutic approach might be an ultimate treatment for disseminated liver metastases in humans and could also be applied to treatment of a large variety of solid tumors.**

Gene therapy offers more possibilities for the treatment of malignancies. Among the various options, gene transfer could be aimed at killing tumor cells either indirectly, by the induction or reinforcement of a host immune response (1), or directly, by the insertion of a "kill" or "suicide" gene (2). This latter approach might be particularly suitable for the treatment of tumors that are made up of rapidly dividing cells invading a nonproliferating tissue. First, tumor cells can be specifically targeted by using recombinant retroviruses, which integrate their genome and are expressed only in dividing cells (3). Second, the best studied and most used suicide gene, which codes for the herpes simplex virus type 1 thymidine kinase (HSV1-TK), induces killing of only dividing cells. Indeed, HSV1-TK can efficiently phosphorylate nucleoside analogs (NAs) such as acyclovir and ganciclovir (GCV) into monophosphorylated molecules. They are then converted into triphosphate substrates, which are incorporated into elongating DNA during cellular division, causing chain termination and cell death (4). Likewise NAs are toxic for dividing eukaryotic cells expressing HSV1-TK (5–7). Its versatility has since been exploited in many *in vitro* and *in vivo* systems. HSV1-TK tissue-specific expression has been used to produce transgenic animals depleted in specific cell populations, a process called thymidine kinase obliteration (8). HSV1-TK-regulated expression has been used to control the conditional suicide of "booby-trapped" cells carrying a human immunodeficiency virus-inducible HSV1-TK gene (9). Finally, HSV1-TK constitutive expression has been used in an attempt to eliminate tumor cells. Moolten and coworker (2, 10) reported that tumor cells transduced *in vitro* with HSV1-TK conventional expression vectors or with recombinant retroviruses were killed by NA treatment *in vitro* and after reimplantation in mice. Using a similar approach, Ezzedine *et al.* (11) then showed selective elimination of HSV1-TK-expressing glioma cells reimplanted in mice. This same group earlier reported that experimental glioma induced in rat brain could be efficiently transduced *in situ* by grafting a retrovirus packaging cell line (24). Combining these two approaches, Culver *et al.* (12) recently reported the elimination of microscopic brain tumors by stereotaxic injection of a packaging cell line making HSV1-TK recombinant retroviruses followed by NA treatment. This experiment indicates that a suicide gene delivered *in situ* by recombinant retroviruses could efficiently mediate tumor regression. However, because these brain tumors are rapidly lethal, the authors could not analyze the treatment of established tumors in their model. This is a serious limitation because, in patients, established tumors would be the main targets for such treatment. Since suicide gene transduction might be more difficult in the context of a large tumoral mass, the therapeutic efficacy of suicide gene transfer in this setting remains to be investigated.

The aim of our work was to study the therapeutic effects of the retroviral-mediated transfer of a HSV1-TK gene on an established tumor. We chose to treat liver metastases of a primitive colon cancer for which we had previously established an experimental model in rat (13). This is a relevant model for the treatment of several types of malignancies with thymidine kinase obliteration. First, hepatocytes are quiescent in a normal adult liver. Second, liver is the most frequent site of metastases from digestive tract cancers. Once liver metastases have occurred, surgical excision by partial hepatectomy remains the best available therapeutical method, but <15% of the patients can benefit from surgery (14). The results of alternative therapeutics (i.e., local chemotherapy or immunotherapy) have been disappointing. Third, we had previously shown that retroviral-mediated gene transfer can

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Abbreviations: HSV1-TK, herpes simplex virus type 1 thymidine kinase; NA, nucleoside analog; GCV, ganciclovir; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

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be performed in the liver by perfusion during transient exclusion from the bloodstream (15). This could be an ultimate treatment when metastases are disseminated. In preliminary experiments, we had shown that tumor cells coinjected in the liver with cells producing HSV1-TK-expressing recombinant retroviral particles could be eliminated by NA treatment (16). We report here the successful treatment of macroscopic solid tumors after direct intratumoral injection of cells producing HSV1-TK-expressing recombinant retroviral particles.

MATERIALS AND METHODS

Retroviral Vector, Cell Culture, and Transfection. The HSV1-TK-expressing pMTK vector is derived from the pMA245 retroviral vector. pMA245 backbone is derived from Moloney murine leukemia virus (MuLV) and was constructed from fragments of MuLV proviruses cloned from mice of different Mov strains (Mov-3, -9, and -13) established by Jaenish *et al.* (17) by infection of preimplantation embryos. In this vector, HSV1-TK expression is directly controlled by the 5′ long terminal repeat.

ψCRIP (18) are amphotropic packaging cells, derived from the 3T3 fibroblastic cell line. We cotransfected them by the calcium phosphate procedure with the plasmids pWLNeo (Stratagene) (1 μg) and pMTK (19 μg). Two days after transfection, the cells were selected with G418 (500 μg/ml) until clones appeared. Each clone was then tested for its ability to confer hypoxanthine/aminopterine/thymidine resistance to TK^- mouse L cells. The M11 cell line used in this study is one of the 55 individual clones isolated and has a titer of 5×10^5 infectious particles per ml. The NB16 cell line (13) is also derived from the ψ CRIP cells and produced a virus with a titer of 5×10^5 focus-forming units/ml. 3T3-TK cells were generated by transfection of 3T3 cells with pMTK and pWLNeo. They express HSV1-TK but do not produce recombinant retroviruses. All the above cells were cultured in Dulbecco's modified Eagle's medium (GIBCO) containing 10% newborn calf serum (HyClone). DHDK12 is a colon carcinoma cell line originating from BDIX rats (19). They were maintained in F10 medium (GIBCO) supplemented with 10% fetal calf serum (HyClone).

Experimental Protocols. For all the experiments, we used adult BDIX male rats weighing 300–350 g. All the surgical procedures and the care given to the animals were in accordance with institutional guidelines.

Treatment of established tumors. At day 0, the rats were operated on under ether anesthesia, and a single liver tumor was induced by direct injection of 1.5×10^6 DHDK12 cells under the liver capsule. At day 5, operations on all rats were repeated. Randomization between the control and the treated group was performed before laparotomy. All rats were given intratumoral injection of 20×10^6 NB16 (control group) or M11 (treated group) cells. At day 10, all rats were given GCV at 150 mg per kg of body weight (Syntex) intraperitoneally twice daily for 5 days. All rats were sacrificed at the end of the GCV treatment, 15 days after tumor cell injection.

Coinjection of tumor and retrovirus-producing cells. At day 0, rats were coinjected with 1.5×10^6 DHDK12 cells together with an equal number of 3T3-TK, NB16, or M11 cells. After 5 days, all rats were treated with GCV as described above. The rats were sacrificed and autopsied on day 10.

Histological Evaluation, 5-Bromo-4-chloro-3-indolyl β-D-Galactoside (X-Gal) Staining, and Immunohistological Analysis. Tissue at the site of tumor cell inoculation was removed, cut in two parts, measured, and fixed in Bouin's solution. Specimens were then embedded in paraffin and stained with Masson trichrome. Cancer cell quantification was performed as described (20). Cancer cells were easily identified based on their morphology.

For X-Gal staining, tumor sections from the control group were fixed by immersion in a 4% paraformaldehyde solution for 2 hr. Next the tumors were immersed overnight in phosphate-buffered saline with 10% sucrose at 4°C, frozen in isopentane, and used to prepare 5-μm-thick cryostat sections. The slides were incubated for 3 hr at 37°C in X-Gal (Boehringer Mannheim) and counterstained with eosin. Immunohistological analyses were performed on 5-μm frozen sections. Monoclonal antibodies (all IgG1) were W3/25 (anti-CD4), OX8 (anti-CD8) (Sera-Lab), and the ED1 anti-monocyte antibody (21). They were revealed by the alkaline phosphatase/anti-alkaline phosphatase technique (DAKO). Sections were then counterstained with hematoxylin.

RESULTS

DHDK12 is an established transplantable colon carcinoma cell line originating from a chemically induced colon cancer in BDIX rats (19). We previously had proved that direct intraportal injection of DHDK12 cell aggregates into syngeneic rat livers induced solid and rapidly growing tumors (13). In an initial study, we showed that DHDK12 proliferating tumor cells can efficiently be infected *in vitro* and *in vivo* by amphotropic recombinant retroviruses (16). A single liver tumor was induced by direct injection of DHDK12 cells under the rat's liver capsule. At day 5, the liver tumors had a diameter ranging between 2 and 3 mm, as measured in an initial study, and contained $\approx 150 \times 10^6$ cells. At that time, the rats were given intratumoral injections of fibroblasts producing recombinant retroviruses. Animals from the control group received NB16 cells, fibroblasts that produce a *nls-lacZ*-expressing recombinant retrovirus. The *nls-lacZ* gene codes for an *Escherichia coli* β-galactosidase with a nuclear localization signal and could thus serve as an indicator gene for the study of tumor cell infection *in vivo*. In the treated group, animals were injected with M11 cells that produce an HSV1-TK-expressing recombinant retrovirus. After intratumoral injection of the packaging cells, a 5-day period was allowed for tumor cell transduction by the recombinant retroviruses. At day 10, all the rats were given GCV for 5 days. All the rats were sacrificed at the end of the GCV treatment, 15 days after tumor cell injection (Fig. 1).

At autopsy, in the control group ($n = 12$), all the rats had macroscopic liver tumors. The mean of the tumor's largest

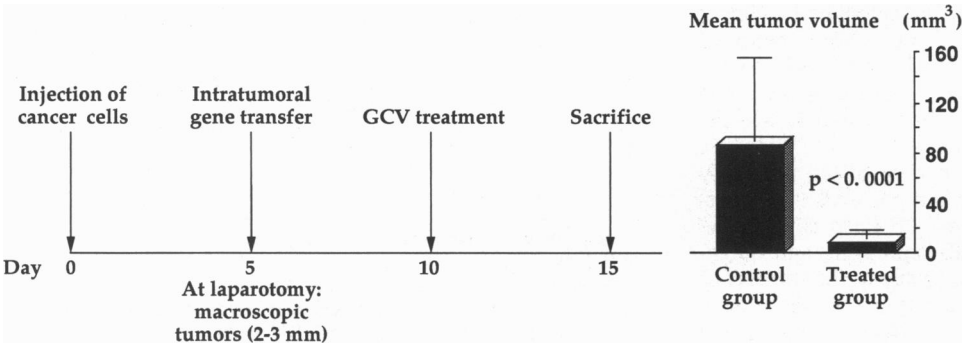

FIG. 1. Experimental protocol and GCV treatment effects on established tumors after *in situ* retroviral-mediated gene transfer. A dramatic reduction in tumor volume is noted in the treated ($n = 13$) compared to the control ($n = 12$) group (8.1 ± 6.7 and 86.3 ± 65.1 mm^3, respectively; $P < 0.0001$; Mann–Whitney test).

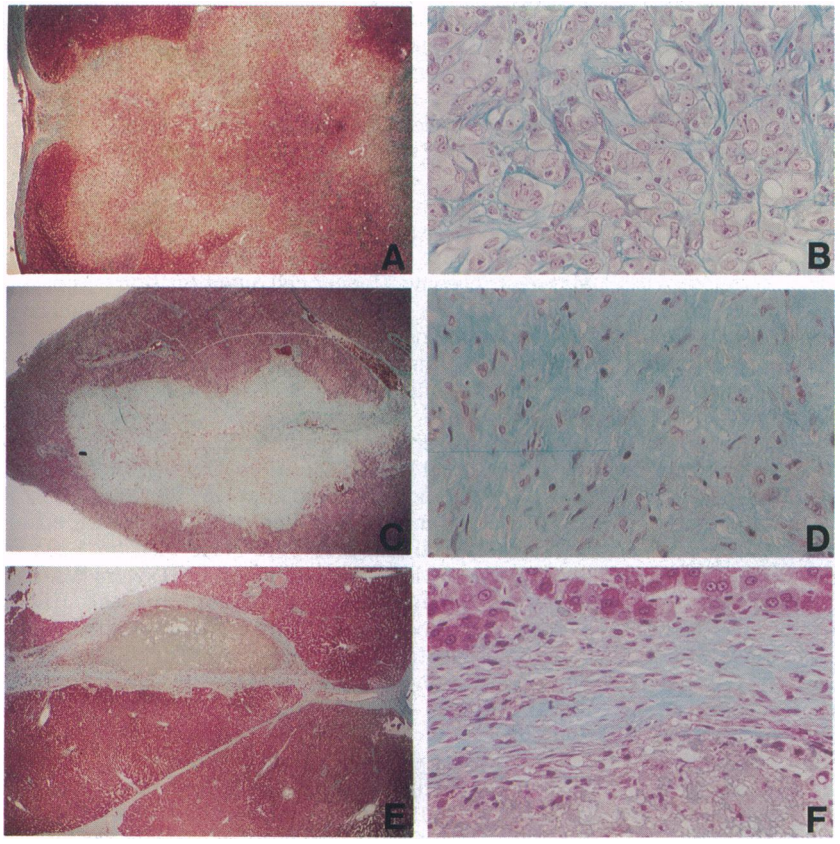

FIG. 2. Histological analysis of liver tumors from treated and control rats. (*A* and *B*) Sections of a tumor from a control rat. Note the florid aspect of the tumoral mass at low magnification (*A*) and the typical aspect of poorly differentiated adenocarcinoma consisting mainly of tumor cells organized in lobules or thick cell cords at higher magnification (*B*). The stroma is slightly fibrotic and is surrounded by a few mononuclear inflammatory cells. (*C* and *D*) Partial tumor regression in a treated rat. There is an important reduction of the cancer cell proportion, and these cells are poorly organized and necrotic. They are located in a dense fibrotic reaction surrounding inflammatory mononuclear cells. (*E* and *F*) Complete tumor regression. There is a massive necrosis of the tumor, which is surrounded by a fibrotic reaction infiltrated with inflammatory cells and devoid of cancer cells. (*A*, *C*, and *E*, ×40; *B*, *D*, and *F*, ×320.)

diameter was 6.5 ± 2.1 mm (range, 3–10 mm). Its volume, calculated according to the formula $V = A \times B^2/2$ (A = largest diameter; B = smallest diameter measured on the largest section of the tumor) (22) was 86.3 ± 65.1 mm³ (mean ± SD). Pathological examination of the tumors showed a typical aspect of poorly differentiated adenocarcinoma, consisting mainly of tumor cells organized in lobules or several thick cell cords. The stroma appeared slightly fibrotic and was surrounded by a few mononuclear inflammatory cells (Fig. 2 *A* and *B*). Nuclear β-galactosidase activity was detected on X-Gal-stained cryostat sections of all six control livers analyzed. Expression was restricted to the tumor cells. The fraction of infected tumor cells was difficult to assess in this type of experiment, but it was <10% of the cells.

In the treated group (n = 13), the mean of the tumor's largest diameter was 3 ± 1.1 mm (range, 2–4 mm), and the mean volume was 8.1 ± 6.7 mm³ (mean ± SD) ($P < 0.0001$; Mann–Whitney test) (Fig. 1). Pathological examination revealed that, in four animals, the residual tumors were actually fibrotic scars devoid of cancer cells (Fig. 2 *E* and *F*). In the other animals, there were few remaining cancer cells, which were poorly organized, often necrotic, and located in a dense fibrotic reaction surrounded by inflammatory mononuclear cells (Fig. 2 *C* and *D*). We determined the mean cancer cell mass in treated and control animals. We calculated a cancer cell index by counting cancer cells on random fields of Masson trichrome sections, using a classical procedure (20). The mean cancer cell mass, arbitrarily defined as the mean volume multiplied by the mean cancer cell index, was reduced by ≈60-fold in treated animals.

The efficiency of the treatment, in view of the relatively small proportion of transduced cells, emphasizes the importance of the "metabolic cooperation" (2) (also called "bystander effect") between HSV1-TK-expressing and neighboring cells. This phenomenon is likely due to the transfer of phosphorylated GCV. To more precisely assess the importance of this phenomenon in treatment efficacy, we analyzed the therapeutic effect of a cell line, 3T3-TK, which expresses HSV1-TK without producing recombinant retroviruses. Since metabolic cooperation requires cell contact with HSV1-TK-expressing cells (2), it was likely that this phenomenon would be best observed when increasing their proportion in the tumor. For this purpose, we used a different experimental protocol in which the DHDK12 tumor cells are coinjected with NB16, M11, or 3T3-TK cells, using a 1:1 ratio (16). Five days after cell injection, rats were treated with GCV for 5 days, sacrificed, and autopsied. As shown in Table 1, there is a dramatic effect of the GCV treatment on tumor growth in rats receiving M11 cells. The efficacy of tumor cell transduction was 10–20% as evaluated by X-Gal staining (Fig. 3*A*). The mean tumor volume in rats receiving the 3T3-TK cells is also markedly reduced compared to the control rats receiving the NB16 cells. However, compared to the control tumors, the residual cancer cell mass is much less reduced in rats injected with 3T3-TK cells than in those injected with M11 cells (Table 1; Fig. 3 *B–D*).

We next analyzed whether the destruction of tumor cells after GCV treatment was associated with modification of the phenotype and number of immune system cells infiltrating the tumor. We observed an inflammatory reaction with macrophages at the periphery of the tumor in both treated and

Table 1. Role of *in situ* recombinant retrovirus production for GCV treatment efficacy

	Cancer cell index	Tumor volume, mm³
NB16	100	45
3T3-TK	61	9
M11	20	7

Rats received DHDK12 cells together with an equal number of NB16 (n = 4), M11 (n = 5), or 3T3-TK (n = 4) cells. Mean tumor volume and cancer cell index are shown. Cancer cell index was calculated by counting cancer cells on random fields (20) and was arbitrarily normalized to 100 for NB16 cells.

control animals, while there was a moderate increase in the proportion of macrophages within the tumoral mass of treated animals (Fig. 3 *E* and *F*). Interestingly, although $CD4^+$ lymphocytes were present in very low numbers in controls, there was a dramatic tumor infiltration by these cells in the treated rats (Fig. 3 *G* and *H*). This was associated with an important increase in $CD8^+$ lymphocytes in the same tumors (Fig. 3 *I* and *J*). Similar, but less intense, modifications in tumor-infiltrating cells were observed in rats receiving 3T3-TK cells (data not shown).

DISCUSSION

Our study shows the regression of an established tumor after *in vivo* transfer of a suicide gene. The treatment is strikingly efficient in view of the small fraction of tumor cells that can be transduced with recombinant retroviruses. Using *nls-lacZ*-expressing retroviruses as a control, we analyzed the efficacy of the *in vivo* infection of our solid liver tumors. *nls-lacZ* activity was detected in all tumors. However, the true efficiency of transduction in solid tumors was difficult to assess in view of the uncertain distance between the tumor sections and the site of fibroblast injection. This efficiency was better assessed from studies in which cancer cells and retroviruses producing cells were coinjected. From these experiments, we estimated that with a cell line producing a virus with a titer of 5×10^5 infectious particles per ml, 10–20% of the cancer cells are infected. Nevertheless, treatment with GCV produced major to complete tumor destruction. A similar treatment efficacy was observed during the treatment of microscopic brain tumors by the same therapeutic strategy (12). The mechanism accounting for this general antitumor effect is believed to be the transfer of phosphorylated GCV to TK^-

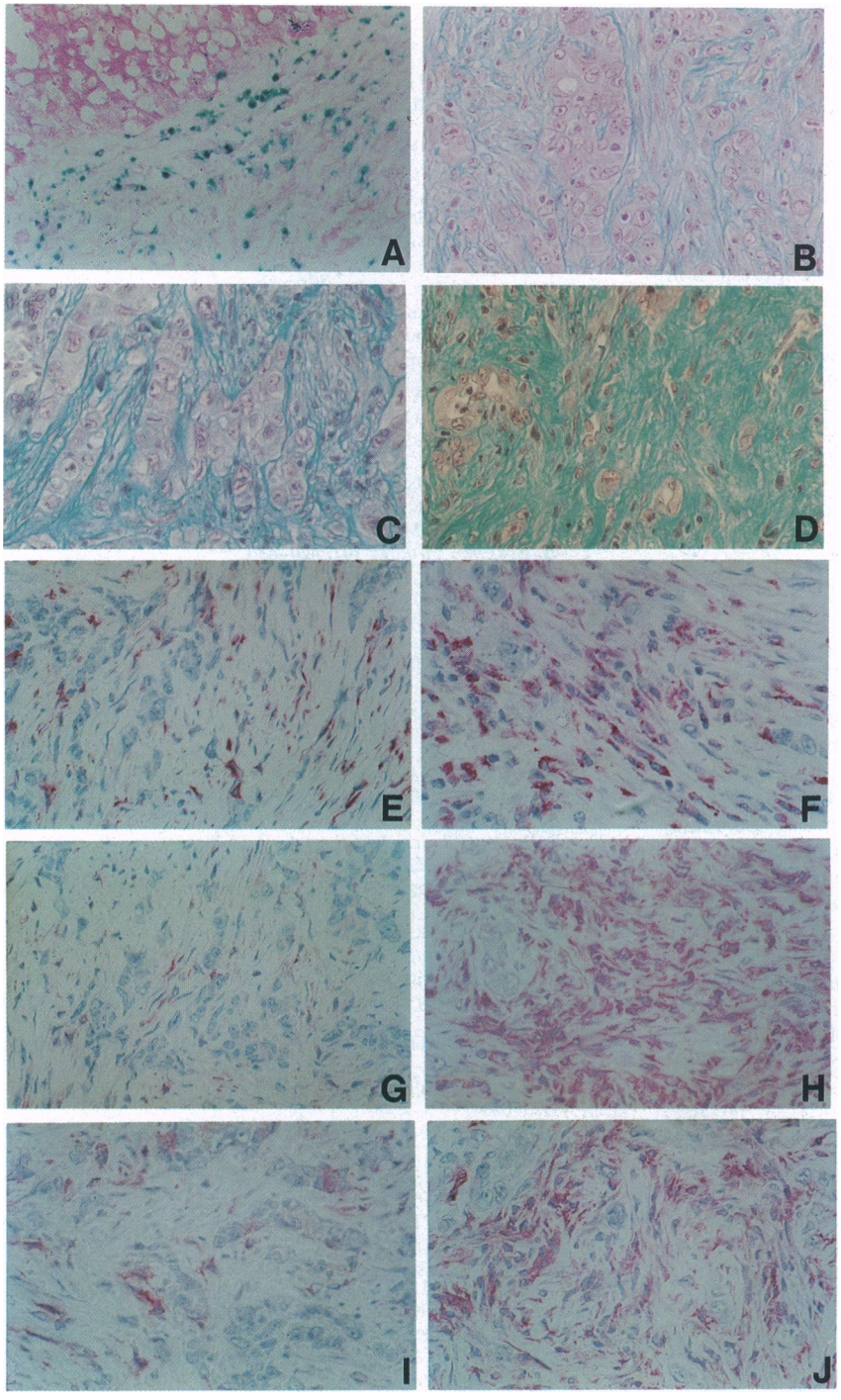

FIG. 3. Role of *in situ* recombinant retrovirus production and immune reaction for GCV treatment efficacy. (*A*) Example of a liver tumor from a rat that received NB16 cells stained with X-Gal and counterstained with eosin (Hoffman modulation contrast system). Hepatocytes are seen in the upper part of the picture. (*B–D*) Masson trichrome staining of tumor sections from rat coinjected with DHDK12 and NB16, 3T3-TK, or M11 cells, respectively. Note that the proportion of cancer cells is only slightly decreased in rats receiving 3T3-TK. Immunohistological analysis of tumor-infiltrating cells. Frozen liver sections from rats receiving DHDK12 alone (*E*, *G*, and *I*) or with M11 cells (*F*, *H*, and *J*) were stained with anti-monocyte (*E* and *F*), anti-CD4 (*G* and *H*), or anti-CD8 (*I* and *J*) monoclonal antibodies. Control stainings in the absence of primary antibody were negative. In control rats, there is significant macrophage infiltration, mostly located in the stroma (*E*) and rare $CD4^+$ (*G*) or $CD8^+$ (*I*) lymphocytes. In treated rats, there is a significant increase in macrophage numbers (*F*) and a dramatic infiltration by $CD4^+$ (*G*) and $CD8^+$ (*J*) cells. These cells are often seen in contact with tumoral cells. (×320.)

tumor cells, an effect reported *in vitro* by Moolten and called "metabolic cooperation" (2). When transfected HSV1-TK-expressing cells are plated with parental cells, both cell types are killed by GCV treatment. This phenomenon is observed only when cells are plated at high density such that contact between HSV1-TK-expressing and nonexpressing cells is ensured. This bystander effect has also been seen *in vivo* during the treatment of gliomas, sarcomas, or carcinomas, but its real contribution to treatment efficacy has been difficult to assess (12, 16). With our cell lines, we analyzed the bystander effect in an experimental setting designed to enhance its importance. We used the coinjection of DHDK12 cancer cells and HSV1-TK-expressing cells with a 1:1 ratio because this is likely to result in having the most DHDK12 cells in contact with HSV1-TK-producing cell. In this setting, 3T3-TK cells, which express HSV1-TK but do not produce retroviral particles, had a significant therapeutic effect as assessed by reduction of tumor volume. However, tumors treated with injection of 3T3-TK cells had only a mild reduction of their cancer cell content. These results are similar to those obtained by Culver *et al.* (12) with a cell line similar to 3T3-TK. They observed an early reduction of tumoral mass (≈80%), but the tumor finally developed after cessation of GCV treatment. Since the M11 recombinant retrovirus-producing cell line is itself expressing HSV1-TK, phosphorylated GCV metabolized by these cells and transferred to DHDK12 cells could contribute significantly to treatment efficacy. However, this cannot account for the overall treatment efficacy observed in our experiment in which established tumors are injected with M11 cells. In this setting, the ratio between M11 and DHDK12 cells at the time of injection is at best 1:5. Finally, it is possible that DHDK12 cells infected by the recombinant retrovirus in turn contribute to the overall bystander effect. Altogether, these results emphasize the therapeutic importance of the bystander effect and indicate that *in situ* transduction of the tumor cells by retroviral particles increases treatment efficacy.

A high rate of retrovirus integration can occur only in a tissue with active cell division. This limitation prevents the infection of a quiescent extratumoral normal tissue and becomes an advantage for treatment of actively proliferating tumor cells. In our study, gene transfer to other liver cell types did not seem to occur. With the exception of a single cell resembling a hepatocyte, all the *nls-lacZ*-expressing cells were located within the tumor and had a typical tumor morphology. No signs of hepatocyte necrosis could be detected. Retroviral gene delivery in nontargeted tissues was not assessed in this study.

During the three independent series of experiments performed, deaths occurred during GCV treatment, including rats that did not receive HSV1-TK-expressing cells, probably because of the high GCV doses administered. GCV dosage was chosen according to Moolten (2), who did not report any toxic effect of such treatment in mice. This GCV dosage has also been used by Culver *et al.* (12), who did not observe any systemic toxicity in their treated rats. We have undertaken dose/efficacy experiments to investigate this potential problem. Preliminary results indicate that this toxicity disappears when GCV dose is not more than 75 mg/kg twice daily. This point should be more precisely addressed in future safety studies.

Although the long-term efficacy of suicide gene transfer remains to be assessed in future studies, our observations support the potential application of this gene therapy procedure for treatment of liver metastases in humans. In 4 of 13 rats, there was complete elimination of cancer cells. In the others, the few remaining cancer cells were trapped in a dense fibrotic reaction and were surrounded by inflammatory cells, indicating a major therapeutic efficacy. The variations in treatment efficacy could be explained by the difficulty of precisely targeting the center of the tumor during fibroblast injection without the assistance of a stereotaxic frame. In most cases, at least some of the fibroblasts were injected in the vicinity of rather than inside the tumor. This problem could be overcome in patients by monitoring the injection by ultrasonography. Because metastases are usually disseminated into the liver, this gene therapy procedure could then require a technique of temporary perfusion of the isolated liver, as described for chemotherapy (22) or thermotherapy (23), and for retroviral-mediated gene transfer *in vivo* (15). In any case, generation of a specific immune response against the cancer cells should also be beneficial for treatment of multiple metastases that might not all be accessible to treatment. In this line, we observed that the treated tumors, in which cancer cell necrosis is observed, became invaded by macrophages and CD4 and CD8 lymphocytes, an optimal situation for initiating a specific immune response. The specificity of this immune reaction, which, if necessary, could be boosted by cytokines that can be released *in situ* for optimal efficacy, remains to be investigated. Finally, it is obvious that such a therapeutic approach could be applied to the treatment of a large variety of solid tumors.

We thank M. Martin for kindly providing the DHDK12 tumor cells and O. Danos and J. M. Heard for the NB16 cell line, M. Alizon for pMTK, and J. P. Camilieri for help in analyzing the pathological examination. We thank E. Brisson, D. Gentric, and C. Mandet for expert technical assistance, and Syntex France for providing GCV (Cimevan). This work was supported by grants from the Centre Nationale de la Recherche Scientifique, Agence Nationale de la Recherche sur le Sida, and Direction de la Recherche et des Etudes Doctorales. M.C. and S.G. are fellows from the Centre de Recherche Appliquée à la Chimiothérapie.

1. Rosenberg, S. A. (1991) *Cancer Res.* **51**, 5074s–5079s.
2. Moolten, F. L. (1986) *Cancer Res.* **46**, 5276–5281.
3. Miller, D. G., Adam, M. A. & Miller, A. D. (1990) *Mol. Cell. Biol.* **10**, 4239–4242.
4. Elion, G. B. (1983) *J. Antimicrob. Chemother.* **12**, 9–17.
5. Nishiyama, Y. & Rapp, F. (1979) *J. Gen. Virol.* **45**, 227–230.
6. Furman, P. A., McGuirt, P. V., Keller, P. M., Fyfe, J. A. & Elion, G. B. (1980) *Virology* **102**, 420–430.
7. Davidson, R. L., Kaufman, E. R., Crumpacker, C. S. & Schnipper, L. E. (1981) *Virology* **113**, 9–19.
8. Borrelli, E., Heyman, R., Hsi, M. & Evans, R. M. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7574–7576.
9. Caruso, M. & Klatzmann, D. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 182–186.
10. Moolten, F. L. & Wells, J. M. (1990) *J. Natl. Cancer Inst.* **82**, 297–300.
11. Ezzedine, Z. T., Martuza, R. L., Platika, D., Short, M. P., Malick, A., Choi, B. C. & Breakefield, X. O. (1991) *New Biol.* **3**, 608–614.
12. Culver, K. W., Ram, Z., Wallbridge, S., Ishii, H., Oldfield, E. H. & Blaese, R. M. (1992) *Science* **256**, 1550–1552.
13. Panis, Y., Ribeiro, J., Chrétien, Y. & Nordlinger, B. (1992) *Br. J. Surg.* **79**, 221–223.
14. Scheele, J., Stangl, R. & Altendorf-Hofmann, G. (1990) *Br. J. Surg.* **77**, 1241–1246.
15. Ferry, N., Duplessis, O., Houssin, D., Danos, O. & Heard, J.-M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8377–8381.
16. Panis, Y., Caruso, M., Houssin, D., Andreoletti, M., Khayat, D., Salzmann, J.-L. & Klatzmann, D. (1992) *C. R. Acad. Sci.* **315**, 541–544.
17. Jaenisch, R., Jähner, D., Nobis, P., Simon, I., Löhler, J., Harbers, K. & Grotkopp, D. (1981) *Cell* **24**, 519–529.
18. Danos, O. & Mulligan, R. C. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6460–6464.
19. Martin, M. S., Martin, F., Michel, M. F. & Hammann, A. (1991) *Virchows Arch. A Pathol. Anat.* **418**, 193–199.
20. Salzmann, J.-L., Peltier-Koch, F., Bloch, F., Petite, J.-P. & Camilleri, J.-P. (1989) *Lab. Invest.* **60**, 847–851.
21. Dijkstra, C. D., Döpp, E. A., Joling, P. & Kraal, G. (1985) *Immunology* **54**, 589–599.
22. Carlsson, G., Gullberg, B. & Hafström, L. (1983) *J. Cancer Res. Clin. Oncol.* **105**, 20–23.
23. Aigner, K., Walther, H., Tonn, J., Wenzl, A., Hechtel, R., Merker, G. & Schwemmle, K. (1983) *Recent Results Cancer Res.* **86**, 99–102.
24. Short, M. P., Choi, B. C., Lee, J. K., Malick, A., Breakefield, X. O. & Martuza, R. L. (1990) *J. Neurosci. Res.* **27**, 427–439.