Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 10732–10736, October 1994
Medical Sciences

In vivo suppression of injury-induced vascular smooth muscle cell accumulation using adenovirus-mediated transfer of the herpes simplex virus thymidine kinase gene

(gene therapy/restenosis)

RAUL J. GUZMAN*, EDWARD A. HIRSCHOWITZ†‡, STEVEN L. BRODY‡, RONALD G. CRYSTAL†‡, STEPHEN E. EPSTEIN*, AND TOREN FINKEL*§

*Cardiology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892; and †Division of Pulmonary and Critical Care Medicine, The New York Hospital–Cornell Medical Center, New York, NY 10021

Communicated by Eugene Braunwald, July 25, 1994 (received for review April 14, 1994)

ABSTRACT **Restenosis, a process characterized in part by excessive smooth muscle cell (SMC) proliferation in areas of vascular injury, occurs in up to 50% of patients undergoing balloon angioplasty. In an effort to develop a treatment strategy for restenosis, we constructed a replication-deficient recombinant adenovirus (AdMLP.HSTK) containing the herpes simplex virus thymidine kinase gene (HSV tk). This viral gene product phosphorylates the prodrug ganciclovir to form a nucleoside analog that inhibits DNA synthesis. Cultured primary rat SMCs infected with AdMLP.HSTK were completely growth-inhibited by incubation in ganciclovir-containing medium. In addition, when only a portion of the SMC population received the HSV tk transgene, an inhibitory effect on neighboring SMCs was evident. Evaluation of this strategy *in vivo* using a rat carotid balloon injury model demonstrated that local infection of injured arteries with AdMLP.HSTK followed by 2 weeks of systemic ganciclovir treatment significantly ($P < 0.01$) reduced injury-induced SMC accumulation. In contrast, there was no suppression of injury-induced SMC accumulation in animals infected with AdMLP.HSTK but not receiving ganciclovir or in those animals infected with a control adenovirus and either treated or not treated with ganciclovir. These results demonstrate the potential utility of adenovirus-mediated gene transfer for treatment of restenosis after balloon injury.**

It is estimated that in 1994, >400,000 patients in the United States will undergo percutaneous transluminal coronary balloon angioplasty (PTCA) for treatment of coronary artery disease. Although PTCA is initially successful in >95% of patients, recurrent narrowing of the successfully dilated coronary arteries leads to treatment failures in 30–50% of patients over the ensuing 3–6 months (1, 2). In addition, restenosis is largely responsible for the treatment failures of newer techniques, which include atherectomy, stent implantation, and laser angioplasty. Considerable effort has therefore been expended to determine the mechanisms involved in restenosis as well as to devise effective treatment strategies.

We and others have recently shown that adenovirus vectors can efficiently transfer genes into the cells of both injured and uninjured vessels (3–7). In addition, using a rat carotid injury model, we have shown that smooth muscle cells (SMCs) that form the neointimal layer can be selectively targeted for gene transfer (7). Using such strategies, the efficiency of adenovirus-mediated gene transfer into vascular cells appears to be significantly greater than previous methods with either liposomes or retroviruses (8, 9). In an effort to expand treatment options for restenosis, we have investigated the feasibility of targeting neointimal SMCs with genes whose products inhibit cellular proliferation.

Several investigators have previously demonstrated that cellular expression of the herpes simplex virus thymidine kinase (HSV tk) gene product along with concomitant administration of the prodrug ganciclovir produces a potent antiproliferative effect on neoplastic cells (10–13). This is thought to result from the ability of the HSV tk gene product to phosphorylate ganciclovir to form a nucleoside analog that in turn inhibits DNA replication (14). Moreover, because the nucleoside analog is diffusible, neighboring nontransfected cells may also be affected through a "bystander effect," thereby amplifying the antiproliferative actions (15, 16). Recent reports of experiments using such strategies have demonstrated that intratumor injection of fibroblasts engineered to produce HSV tk retroviral vectors, along with ganciclovir treatment, results in tumor regression in animals (15). This strategy is being evaluated in human subjects with brain tumors (17). In the present study, we show that we can use adenovirus vectors to deliver HSV tk into neointimal SMCs and, in the presence of ganciclovir, inhibit the development of injury-induced SMC accumulation.

MATERIALS AND METHODS

Construction of AdMLP.HSTK. A recombinant replication-deficient adenovirus (AdMLP.HSTK) was constructed that contains the HSV tk gene downstream of the major late promoter of adenovirus (Fig. 1). The 1.8-kb cDNA encoding HSV tk was first ligated into an adenovirus shuttle plasmid. This shuttle plasmid based on pBluescript II SK contained a multiple cloning site flanked on the 5′ end by the left inverted terminal repeat of adenovirus, the E1A enhancer, along with the major late promoter and tripartite leader of adenovirus type 2. On the 3′ end of the multiple cloning site there was an ≈3-kb region homologous to adenovirus type 5. A linearized fragment of this recombinant plasmid was cotransfected into 293 cells (American Type Culture Collection) along with *Cla* I cut adenovirus DNA from the E3 deletion mutant Ad-dl327. Single plaques were amplified in 293 cells. Recombinant virus was screened by PCR amplification for the presence of HSV tk and the absence of the E1 region to demonstrate successful homologous recombination. A single PCR-positive plaque, AdMLP.HSTK, was amplified in 293 cells and subsequently purified on cesium gradients. Viral stocks in excess of 10^{10} plaque-forming units (pfu) of AdMLP.HSTK or AdRSV.βgal (a control vector containing the *Escherichia coli lacZ* gene

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.

Abbreviations: SMC, smooth muscle cell; HSV tk, herpes simplex virus thymidine kinase; pfu, plaque-forming unit(s); moi, multiplicity of infection.

§To whom reprint requests should be addressed.

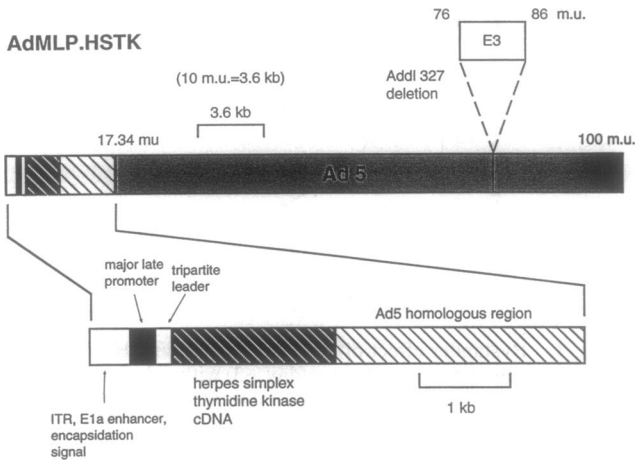

FIG. 1. Construction of AdMLP.HSTK vector. A recombinant replication-deficient adenovirus lacking the E1 and E3 region but containing HSV tk under control of the adenoviral major late promoter was constructed. m.u., Map units; Ad5, adenovirus type 5; ITR, inverted terminal repeat.

under control of the Rous sarcoma virus long terminal repeat) per ml were prepared and titered in 293 cells (18, 19).

***In Vitro* Assessment of AdMLP.HSTK on SMC Proliferation.** SMCs were obtained from rat thoracic aorta by the method of enzymatic digestion and used between passages 5 and 10. Cells were plated in M199 medium (GIBCO/BRL) containing 10% serum and allowed to grow to ≈30% confluence. Before infection they were made quiescent by serum starvation for 48 hr in medium containing 0.5% serum. Cells were then infected with the indicated multiplicity of infection (moi) of AdMLP.HSTK or AdRSV.βgal for 2 hr, at which point they were washed three times with phosphate-buffered saline (PBS) and returned to medium containing 0.5% serum for an additional 24 hr. For *in vitro* proliferation studies, with the exception of the cell mixing experiments, cells were then returned to medium containing 10% serum and the indicated concentrations of ganciclovir. Cells in triplicate wells were treated with trypsin and assayed with a Coulter Counter on days 2, 6, 10, and 14 for establishment of proliferation curves. Primary cultures of rat SMCs under the conditions used are not contact inhibited; however, the increase in cell density slows at $\approx 1 \times 10^5$ cells per cm^2.

To determine whether rat arterial SMCs were susceptible to the bystander effect, cells were infected with either AdMLP.-HSTK or the control virus AdRSV.βgal. After infection, the two groups of SMCs were maintained in low serum for 48 hr. Cells were then treated with trypsin, mixed in the indicated ratios, and replated in triplicate wells. Ganciclovir was added to the medium the following day (day 1).

***In Vivo* Assessment of AdMLP.HSTK on Neointimal Formation.** All animals were studied under protocols approved by the Animal Care and Use Committee of the National Heart, Lung, and Blood Institute and in accordance with the *Guide for the Care and Use of Laboratory Animals* (20). Rats underwent balloon injury of the left carotid artery as described by Clowes *et al.* (21). Seven days after injury, 100 μl of a solution containing 3×10^8 or 1×10^9 pfu of adenovirus was instilled into the vessel as described (7). After 50 min, the viral solution was withdrawn, the arteriotomy was closed, and the rats were allowed to recover. The following day treatment was started with either ganciclovir (30 mg/kg i.p. twice daily) or H_2O as a control. Treatment was continued for 14 days, at which time the animals were sacrificed and the carotid arteries were harvested for histologic assessment of the SMC proliferative response to injury (22). For histologic assessment, arteries were fixed by perfusing with 4% paraformaldehyde in PBS at physiologic pressure, divided into 2-mm segments, embedded in paraffin, and mounted in 5-μm sections.

To determine whether AdMLP.HSTK and ganciclovir caused actual neointimal regression, six animals were sacrificed 7 days after injury (the time of viral instillation in the remaining animals) and the neointimal/medial ratio was measured.

For detection of viral DNA in distal organs, DNA was isolated from the rat heart, liver, spleen, gonadal tissues, and infected carotid segment 48 hr after infection. Using 50 and 500 ng of DNA as a template, PCR was performed with specific primers for the HSV tk gene: 5′-GAC CTG TAT AAC GTG TTT GCC TGG-3′ and 5′-CAC GAA CCA TAA ACC ATT CC-3′.

RESULTS

***In Vitro* Inhibition of SMC Proliferation.** The ability of AdMLP.HSTK to inhibit SMC proliferation *in vitro* was dependent on both concentration of virus and concentration of ganciclovir (Fig. 2). Primary cultures of rat SMCs were infected with AdMLP.HSTK and incubated in medium containing various concentrations of ganciclovir. As shown in Fig. 2*A*, ganciclovir at 10 μg/ml completely inhibited SMC growth, while at 1 μg/ml the reduction in cell number was ≈70% at the end of the 2-week period. This same range of ganciclovir only modestly affected cell growth when SMCs were infected with the control adenovirus AdRSV.βgal (Fig. 2*B*). The antiproliferative effect of AdMLP.HSTK was also dependent on the moi in that growth inhibition of SMCs increased as the moi increased from 0 to 100 pfu per cell (Fig. 2*C*).

Previous experiments with transformed cells demonstrated a bystander effect wherein tumor cells expressing HSV tk exert an antiproliferative effect on neighboring cells that do not express HSV tk. To assess whether primary rat SMCs were also susceptible to the bystander effect, cells were infected with either AdMLP.HSTK or AdRSV.βGAL and then mixed and replated (day 0) in various proportions. Since we had previously shown *in vivo* that between 10% and 75% of neointimal cells were successfully transduced by adenoviral infection (7), similar ratios of HSV tk-infected and noninfected cells were prepared *in vitro*. The following day (day 1), ganciclovir (10 μg/ml) was added to the medium. At the end of 7 days, the -fold increase in cell number from day 1 was determined. As shown in Fig. 3, AdRSV.βgal-infected cells that were not mixed with AdMLP.HSTK-infected cells demonstrated an ≈20-fold increase in number. Cells infected with AdMLP.HSTK alone showed an almost complete inhibition of cell growth over the 7-day period. Although cells infected with AdMLP.HSTK alone had the greatest growth inhibition, a 50:50 mixture of AdMLP.HSTK-infected and AdRSV.βgal-infected cells were inhibited almost to the same degree, suggesting an effect on untransfected cells. Such results are similar in magnitude to the bystander effect observed in tumor cells (23).

These *in vitro* data indicate that adenovirus-mediated gene transfer of HSV tk can inhibit SMC proliferation and that this effect can be maintained for at least 2 weeks *in vitro* even though the adenovirus genome does not integrate into the host cell genome (24) and even in the absence of 100% HSV tk gene transfer. This time frame is important because experimental evidence indicates that most neointimal proliferation in the rat lesion occurs within the first few weeks after injury (21).

***In Vivo* Inhibition of Neointimal Formation.** We next assessed whether local infection of AdMLP.HSTK and concomitant ganciclovir administration inhibits vascular SMC proliferation *in vivo*. Six groups consisting of a total of 73 Sprague–Dawley rats were analyzed. Four control groups were treated with (*i*) vehicle only, no ganciclovir; (*ii*) AdRSV.βgal (1×10^9 pfu), no ganciclovir; (*iii*) AdMLP.HSTK (1×10^9 pfu), no ganciclovir;

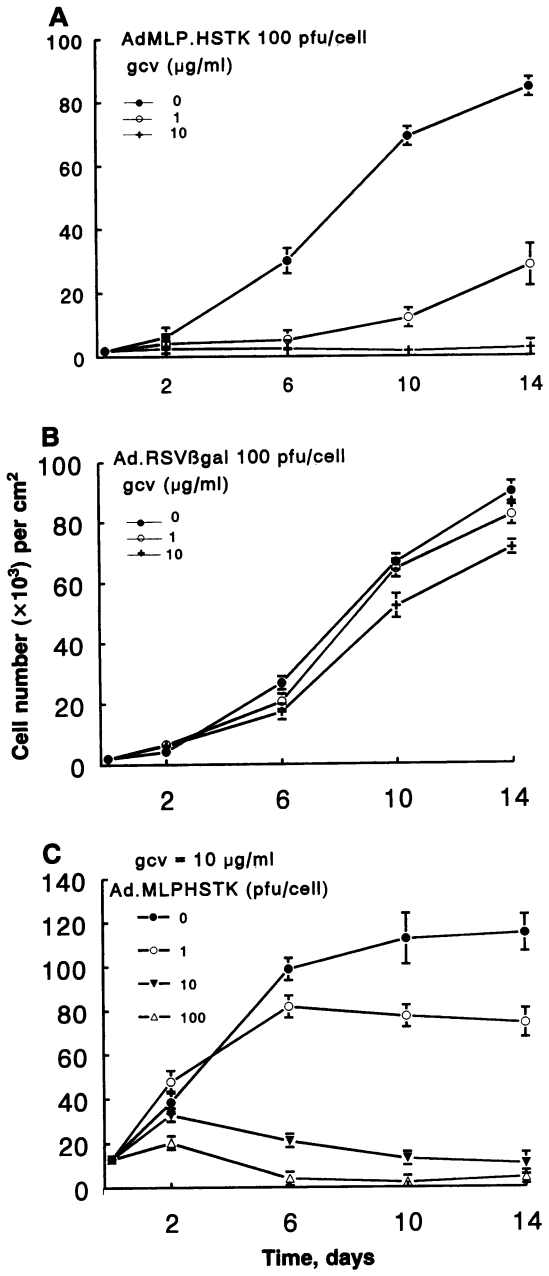

FIG. 2. Representative experiments from one of three similar experiments are shown. Values are plotted as means ± SEM with each point performed in triplicate. (*A*) Growth curves of AdMLP.HSTK-infected cells (100 pfu per cell) treated with increasing concentrations of ganciclovir (gcv). (*B*) Growth curves of cells infected with the control adenovirus vector AdRSV.βgal (100 pfu per cell) treated with increasing concentrations of ganciclovir. (*C*) Growth curves of cells infected with increasing concentrations of AdMLP.HSTK and treated with ganciclovir (10 μg/ml).

and (*iv*) AdRSV.βgal (1×10^9 pfu), with ganciclovir. The two treatment groups consisted of AdMLP.HSTK (3×10^8 pfu), with ganciclovir, or AdMLP.HSTK (1×10^9 pfu), with ganciclovir.

Visual examination of histological sections from animals in the four control groups all showed appreciable neointimal formation when compared to uninjured arteries (Fig. 4 *A–D*). In contrast, the neointimal mass appeared qualitatively reduced in those animals infected with AdMLP.HSTK and treated with ganciclovir (Fig. 4 *E* and *F*).

To quantify the apparent reduction in neointimal area seen in the two treatment groups, the ratio of neointimal/medial

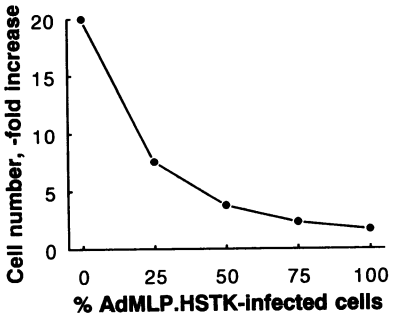

FIG. 3. Bystander effect of rat SMCs expressing HSV tk. Cells were separately infected at 100 moi (day −3) with either AdRSV.βgal or AdMLP.HSTK and then mixed in the proportions indicated (day 0). Ganciclovir treatment began on day 1. The fold increase in cell number from day 1 to day 7 is expressed as a function of the proportion of cells infected with AdMLP.HSTK. Values represent average triplicate determinations, each of which varied <10% from the mean.

area was determined by digital planimetry as described (22). The mean neointimal/medial ratios in the four control groups were statistically indistinguishable (Fig. 5). In contrast, administration of AdMLP.HSTK with a fixed dose of ganciclovir significantly reduced the mean neointimal/medial ratio ($P = 0.027$). The observed reduction in neointimal/medial ratio was entirely dependent on reduction of the neointimal area, as the medial area did not differ significantly among the six groups ($P = 0.38$; data not shown).

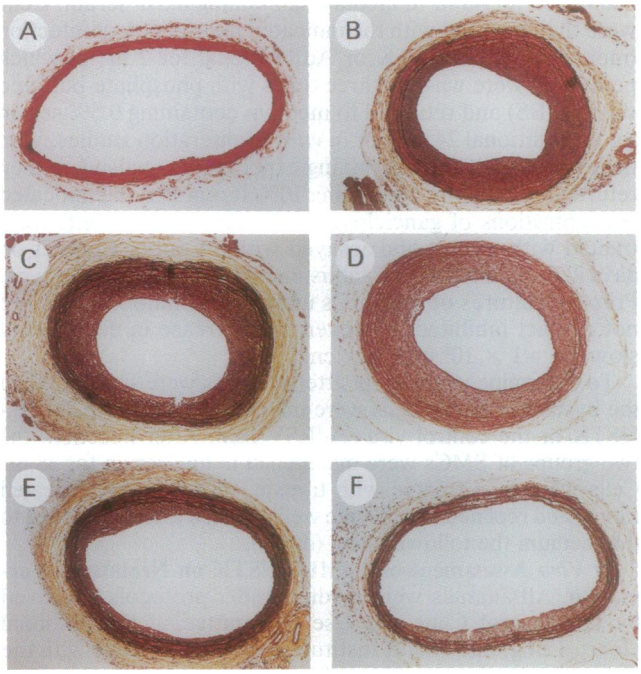

FIG. 4. Histological sections from representative arterial segments. All specimens (except in *A*) were obtained 21 days after balloon injury. The planimeterized ratio of neointimal/medial area (*B–E*) represents specimens that closely approximated the mean values for their respective groups. (*A*) Uninjured carotid artery. (*B*) Arterial segment exposed to vehicle only. (*C*) Arterial segment infected with 1×10^9 pfu of AdRSV.βgal with systemic ganciclovir treatment (30 mg/kg twice daily). (*D*) Arterial segment infected with 1×10^9 pfu of AdMLP.HSTK without ganciclovir treatment. (*E*) Arterial segment infected with 1×10^9 pfu of AdMLP.HSTK with systemic ganciclovir treatment (30 mg/kg twice daily). (*F*) Additional arterial segment infected with 1×10^9 pfu of AdMLP.HSTK and treated with systemic ganciclovir (30 mg/kg twice daily). (×45.)

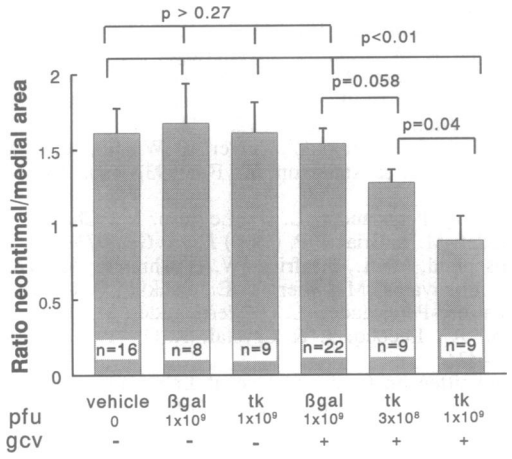

FIG. 5. Average ratio of neointimal/medial areas of balloon injured rat carotid arteries. Neointimal and medial areas were determined in a blinded fashion 21 days after injury. Six groups of animals described in the text were studied, with the number of animals studied in each group shown in the relevant bar. Average ratio ± SEM is shown. ANOVA of the six groups was significant ($P = 0.027$). Unpaired Bonferroni corrected t tests between groups are shown above. tk, AdMLP.HSTK; βgal, AdRSV.βgal; gcv, ganciclovir; n, number of individual animals.

The magnitude of neointimal reduction in the treated groups was dependent on the amount of AdMLP.HSTK used (Fig. 5). Compared to the four control groups, animals infected with the lower dose of 3×10^8 pfu of AdMLP.HSTK and treated with systemic ganciclovir had an ≈20% reduction of the neointimal/medial ratio, a reduction that approached statistical significance ($P = 0.058$). At the higher dose of 1×10^9 pfu of AdMLP.HSTK, the reduction of neointimal/medial ratio was 46% ($P < 0.01$). Linear regression analysis of percentage neointimal reduction versus adenovirus dose was performed with three groups (vehicle, low dose, and high dose AdMLP.HSTK). This analysis revealed a slope that was significantly different from 0 ($P = 0.004$), indicative of a dose–response relationship. A similar linear or near-linear relationship between *in vivo* adenovirus dose and gene expression has previously been observed in marker gene studies in the heart, vasculature, and lung (4, 25, 26).

Analysis of carotid arteries 7 days after balloon injury, the time corresponding to adenoviral infection, revealed a neointimal/medial ratio of 0.27 ± 0.19 ($n = 6$). The neointimal/medial ratio at 21 days in the two AdMLP.HSTK-infected groups treated with ganciclovir was larger ($P < 0.05$), suggesting that treated lesions did not regress in size but rather that neointimal progression in the treated groups was inhibited. These results are similar to those obtained in the *in vitro* mixing experiments (Fig. 3) wherein some growth occurs when only 25–75% of cells contain the HSV tk gene. Whether the differences in the neointimal/medial ratio persist beyond 3 weeks is currently unknown.

Finally, since intravascular delivery of adenovirus has potential for widespread infection beyond the treated area, we assessed the presence of viral DNA in distal organs. DNA was extracted from the liver, spleen, myocardium, gonadal tissue, and infected carotid segment 48 hr after infection and used in the PCR. No adenoviral DNA was detected in any organ other than the infected carotid segment. This suggests that the methodology for local delivery of adenovirus that was used resulted in little distal infection.

DISCUSSION

Although various pharmacological interventions have been used in an effort to inhibit the proliferation of SMCs at the site of angioplasty, none has significantly affected the rate of restenosis in humans (27). Newer strategies have recently been evaluated, including antibodies directed against peptide growth factors (28, 29), and oligonucleotide-antisense molecules directed at transcription factors or cell-cycle regulatory molecules required for proliferation (30, 31). This study expands potential treatment options for restenosis by demonstrating that adenovirus-mediated transfer of a therapeutic gene into the vascular wall inhibits neointimal development.

Before these technologies can be contemplated for use in patients, other animal models need to be assessed to overcome the differences that exist between the rat model and more complex human lesions. While the rat lesion is composed of proliferating SMCs the kinetics of which are well described, the human lesion contains other cell types and includes contributing factors such as vessel remodeling, thrombus formation, and matrix secretion. Therefore, a strategy such as we described targeted solely at SMC proliferation may prove less effective in humans than in rats. However, it is currently thought that, in human lesions, neointimal SMCs produce much of the lesion's extracellular matrix and serve as a major source of chemoattractant factors. In such an event, it remains conceivable that the elimination of neointimal SMCs may have significant effects even in the more complex human lesion.

Perhaps because the rat model is primarily a model of SMC proliferation, and even though we have previously noted an inflammatory response to adenoviral infection in the myocardium (32), we noted little inflammation by light microscopy in infected vessels (Fig. 4). Indeed, except for the size of the neointima, sections from treated animals appeared histologically indistinguishable from sections of the control uninfected animals.

One possible limitation of using adenoviral vectors to deliver antiproliferative gene products is the potential to cause unwanted local or systemic toxicity. Locally, such therapy may impede endothelial function or regrowth. In the rat model, such possibilities are difficult to assess as little endothelial regrowth occurs after injury (33). We have confirmed these studies in our specific model by staining rat carotids with Evans blue dye 21 days after injury and noting that the lesion is predominantly not reendothelialized at this time. Whether endothelial regrowth occurs in other models or in human lesions and whether AdMLP.HSTK infection may impede this process remains untested.

In addition to local effects, it remains possible that such treatment could also lead to systemic toxic effects. Although we noted no viral DNA by PCR in distal organs, this is probably a reflection of the particular technique we used for viral instillation into the injured rat carotid artery. For such therapies to be adapted to human coronary arteries, a flow-through catheter equipped for local gene delivery would need to be used. Although such catheters currently exist, all produce some degree of leakage, which would inevitably increase the likelihood for distal viral infection. The extension of these results to the coronary arteries of larger animal models that may produce lesions more closely resembling those in humans, and in which catheter delivery systems similar to those employed in patients can be used, will more effectively address these issues. It should be noted that we infected the carotid artery with the adenoviral vector 7 days after injury, a time at which an appreciable neointima has already developed. One reason we adopted this strategy was to determine whether the transgene would be capable of inhibiting neointima development not in a perfectly normal vessel but in a vessel that was already diseased, as would exist in the clinical situation. Our results did confirm that treatment in the presence of a sizeable neointima was able to inhibit further expansion of the lesion.

Another question that needs to be addressed in the future is whether efficacy can be further enhanced. Since we demonstrated a dose–response relation between the amount of virus used and the degree of inhibition of neointimal development, it is possible that increasing the expression of HSV tk either by higher titers or by using a stronger promoter might increase efficacy. In addition, since the present insert capability of recombinant adenovirus is up to 7.5 kb (24), it is also possible that adenovirus vectors encoding HSV tk (or another gene whose product has potent antiproliferative effects) in combination with other therapeutic genes could be constructed. As such, a recombinant vector that encodes multiple gene products, some with antiproliferative effects and others with antithrombotic or antiplatelet effects, might ultimately prove to be the most clinically useful. Finally, because virus leakage will probably occur when delivered by a catheter-based system, use of a SMC-specific promoter may be desirable to achieve optimal safety.

It should be noted that previous applications with recombinant adenovirus vectors have primarily involved inherited genetic diseases such as cystic fibrosis, muscular dystrophy, or hemophilia (34–37). Such conditions require continuous stable gene expression, which presently cannot be achieved with nonintegrating adenoviral vectors. In contrast, the application of adenoviral gene transfer may be better suited to conditions such as restenosis, which presumably require short-term gene expression. As such, the present investigation should provide an impetus to further evaluate such strategies in the treatment of acquired cardiovascular disorders.

1. Leimgruber, P. P., Roubin, G. S., Hollman, J., Cotsonis, G. A., Meier, B., Douglas, J. S., King, S. B. & Gruentzig, A. R. (1986) *Circulation* **73,** 710–717.
2. Gruentzig, A. R., King, S. B., Schlumpf, M. & Siegenthaler, W. (1987) *N. Engl. J. Med.* **316,** 1127–1132.
3. Lemarchand, P., Jones, M., Yamada, I. & Crystal, R. G. (1993) *Circ. Res.* **72,** 1132–1138.
4. Lee, S. W., Trapnell, B. C., Rade, J. J., Virmani, R. & Dichek, D. A. (1993) *Circ. Res.* **73,** 797–807.
5. Willard, J. E., Jessen, M. E., Gerard, R. D. & Meidell, R. S. (1992) *Circulation* **86,** I-473.
6. Barr, E., Carroll, J., Kalynyc, A. M., Tripathy, S. K., Kozarsky, K., Wilson, J. M. & Leiden, J. M. (1994) *Gene Ther.* **1,** 51–58.
7. Guzman, R. J., Lemarchand, P., Crystal, R. G., Epstein, S. E. & Finkel, T. (1993) *Circulation* **88,** 2838–2848.
8. Flugelman, M. Y., Jaklitsch, M. T., Newman, K. D., Casscells, W., Bratthauer, G. L. & Dichek, D. A. (1992) *Circulation* **85,** 1110–1117.
9. Leclerc, G., Gal, D., Takeshita, S., Nikol, S., Weir, L. & Isner, J. M. (1992) *J. Clin. Invest.* **90,** 936–944.
10. Borrelli, E., Heyman, R., Hsi, M. & Evans, R. M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 7572–7576.
11. Moolten, F. L. (1986) *Cancer Res.* **46,** 5276–5281.
12. Moolten, F. L. & Wells, J. M. (1990) *J. Natl. Cancer Inst.* **82,** 297–300.
13. Plautz, G., Nabel, E. G. & Nabel, G. J. (1991) *New Biol.* **3,** 709–715.
14. Morse, G. D., Shelton, M. J. & O'Donnell, A. M. (1993) *Clin. Pharmacokinet.* **24,** 101–123.
15. Culver, K. W., Ram, Z., Wallbridge, S., Ishii, H., Oldfield, E. H. & Blaese, R. M. (1992) *Science* **256,** 1550–1552.
16. Bi, W. L., Parysek, L. M., Warnick, R. & Stambrook, P. J. (1993) *Hum. Gene Ther.* **6,** 725–732.
17. Oldfield, E. H., Ram, Z., Culver, K. W., Blaese, R. M., Devroom, H. L. & Anderson, W. F. (1993) *Hum. Gene Ther.* **4,** 39–69.
18. Stratford-Perricaudet, L. D., Levrero, M., Chasse, J., Perricaudet, M. & Briand, P. (1990) *Hum. Gene Ther.* **1,** 241–256.
19. Rosenfeld, M. A., Siegfried, W., Yoshimura, K., Yoneyama, K., Fukayama, M., Stier, L. E., Pääkkö, P. K., Gilardi, P., Stratford-Perricaudet, L. D., Perricaudet, M., Jallat, S., Pavirani, A., Lecocq, J. & Crystal, R. G. (1991) *Science* **252,** 431–434.
20. Committee on Care and Use of Laboratory Animals (1985) *Guide for the Care and Use of Laboratory Animals,* DHHS Publication No. [NIH] 86-23 (Natl. Inst. of Health, Bethesda, MD).
21. Clowes, A. W., Reidy, M. A. & Clowes, M. M. (1983) *Lab. Invest.* **49,** 327–333.
22. Banai, S., Shou, M., Correa, R., Jaklitsch, M. T., Douek, P. C., Bonner, R. F., Epstein, S. E. & Unger, E. F. (1991) *Circulation* **69,** 748–756.
23. Anderson, W. F. (1992) *Science* **256,** 808–813.
24. Berkner, K. L. (1992) *Current Topics in Microbiology and Immunology* (Springer, Berlin), pp. 39–66.
25. Kass-Eisler, A., Falck-Pedersen, E., Alvira, M., Rivera, J., Buttrick, P. M., Wittenberg, B. A., Cipriani, L. & Leinwand, L. A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 11498–11502.
26. Mastrangeli, A., Danel, C., Rosenfeld, M. A., Stratford-Perricaudet, L. D., Perricaudet, M., Pavirani, A., Lecocq, J. & Crystal, R. G. (1993) *J. Clin. Invest.* **91,** 225–234.
27. Schwartz, R. S., Holmes, D. R. & Topol, E. J. (1992) *J. Am. Coll. Cardiol.* **20,** 1284–1293.
28. Ferns, G. A. A., Raines, E. W., Sprugel, K. H., Motani, A. S., Reidy, M. A. & Ross, R. (1991) *Science* **253,** 1129–1132.
29. Lindner, V. & Reidy, M. A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 3739–3743.
30. Simons, M., Edelman, E. R., DeKayser, J. L., Langer, R. & Rosenberg, R. D. (1992) *Nature (London)* **359,** 67–70.
31. Morishita, R., Gibbons, G. H., Ellison, K. E., Nakanima, M., Zhang, L., Kaneda, Y., Ogihara, T. & Dzau, V. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 8474–8478.
32. Guzman, R. J., Lemarchand, P., Crystal, R. G., Epstein, S. E. & Finkel, T. (1993) *Circ. Res.* **73,** 1202–1207.
33. Reidy, M. A., Standaert, D. & Schwartz, S. M. (1983) *Lab. Invest.* **49,** 569–575.
34. Rosenfeld, M. A., Yoshimura, K., Trapnell, B. C., Yoneyama, K., Rosenthal, E., Dalemans, W., Fukayama, M., Bargon, J., Stier, L. E., Stratford-Perricaudet, L. D., Perricaudet, M., Guggino, W. B., Pavirani, A., Lecocq, J. & Crystal, R. G. (1992) *Cell* **68,** 143–155.
35. Zabner, J., Couture, L. A., Gregory, R. J., Graham, A. E., Smith, A. E. & Welsh, M. J. (1993) *Cell* **77,** 207–216.
36. Ragot, T., Vincent, N., Chafey, P., Vigne, E., Gilgenkrantz, H., Couton, D., Cartaud, J., Briand, P., Kaplan, J., Perricaudet, M. & Kahn, A. (1993) *Nature (London)* **361,** 647–650.
37. Smith, T. A. G., Mehaffey, M. G., Kayda, D. B., Saunders, J. M., Yei, S. Y., Trapnell, B. C., McClelland, A. & Kaleko, M. (1993) *Nat. Genet.* **5,** 397–402.