Inhibition of Vascular Smooth Muscle Cell Proliferation and Intimal Hyperplasia by Gene Transfer of β-Interferon

Dominique Stephan,[2] Hong San,[2] Zhi-yong Yang,[1,2] David Gordon,[4] Susan Goelz,[6] Gary J. Nabel,[1,2,3] and Elizabeth G. Nabel[2,5]

[1]Howard Hughes Medical Institute, University of Michigan Medical Center, Departments of [2]Internal Medicine, [3]Biological Chemistry, [4]Pathology, and [5]Physiology, Ann Arbor, Michigan, U.S.A., [6]Biogen, Fourteen Cambridge Center, Cambridge, Massachusetts 02142

ABSTRACT

Background: Balloon injury of the arterial wall induces increased vascular smooth cell proliferation, enhanced elastic recoil, and abnormalities in thrombosis, each of which contribute to regrowth of intima and the lesion of restenosis. Several gene transfer approaches have been used to inhibit such intimal smooth muscle cell growth. In this report, adenoviral gene transfer of β-interferon (β-IFN) was analyzed in a porcine model of balloon injury to determine whether a secreted growth inhibitory protein might affect the regrowth of vascular smooth muscle cells in vitro and in arteries.

Materials and Methods: An adenoviral vector encoding β-interferon (ADV-β-IFN) was prepared and used to infect porcine vascular smooth muscle cells in a porcine balloon injury model. Its antiproliferative effect was analyzed in vitro and in vivo.

Results: Expression of recombinant porcine β-IFN in vascular smooth muscle cells reduced cell proliferation significantly in vitro, and supernatants derived from the β-IFN vector inhibited vascular smooth muscle cell proliferation relative to controls. When introduced into porcine arteries after balloon injury, a reduction in cell proliferation was observed 7 days after gene transfer measured by BrdC incorporation (ADV-ΔE1 arteries 14.5 ± 1.2%, ADV-βIFN 6.8 ± 0.8%, $p < 0.05$, unpaired, two-tailed t-test). The intima-to-media area ratio was also reduced (nontransfected arteries, 0.70 ± 0.05; ADV-ΔE1 infected arteries, 0.69 ± 0.06; ADV-β-IFN infected arteries, 0.53 ± 0.03; $p < 0.05$, ANOVA with Dunnett t-test). No evidence of organ toxicity was observed, and regrowth of the endothelial cell surface was observed 3–6 weeks after balloon injury.

Conclusions: Gene transfer of an adenoviral vector encoding β-IFN into balloon-injured arteries reduced vascular smooth muscle proliferation and intimal formation. Expression of this gene product may have potential application for the treatment of vascular proliferative diseases.

INTRODUCTION

The proliferation of cells in response to vascular injury is regulated by different stimuli, including growth factors which induce cell proliferation, inflammatory cytokines which regulate inflammation, and other proteins which can act to limit cell proliferation, such as cyclin-dependent kinases expressed in vascular smooth muscle cells (vsmc). The role of individual gene products in intimal hyperplasia and restenosis after balloon injury is not completely understood, and an important goal is the definition of factors that can enhance or inhibit the formation of neointima. Although several factors have been described that either induce (1,2) or inhibit (3–6) intimal proliferation, there have been no descriptions to

Address correspondence and reprint requests to: Dr. Elizabeth G. Nabel, University of Michigan Medical Center, Departments of Internal Medicine and Physiology, 1150 West Medical Center Drive, 7301 MSRB III, Ann Arbor, MI 48109-0644, U.S.A. Phone: 313-763-5103; Fax: 313-763-4851; e-mail: enabel@umich.edu

date of secreted cytokines that may help to limit smooth muscle cell proliferation and neointima formation in vivo. Among the inflammatory cytokines that could potentially exert these effects, the interferons (IFNs) have the ability to inhibit cell proliferation in a variety of cells. Although some interferons (gamma and alpha) also induce inflammation, the β-IFNs, while able to exert antiviral effects, have a profound effect on cell proliferation without inducing a substantial inflammatory response (7–10). In this report, we address the role of β-IFN in limiting vascular smooth muscle cell proliferation after balloon injury in a porcine gene transfer model. Expression of β-IFN was achieved using an adenoviral vector after balloon injury and was compared to a control vector for its effect on smooth muscle proliferation in vitro and in vivo. These findings suggest that this secreted cytokine may act to inhibit the expansion of neointimal vascular smooth muscle cells after balloon injury and prove useful as a potential intervention for restenosis and atherosclerosis in vivo.

MATERIALS AND METHODS

Adenoviral Vector Construction and Purification

The recombinant adenoviral vector, ADV-β-IFN, was constructed by homologous recombination between sub360 genomic DNA, an Ad5 derivative with a deletion in the E3 region, and a porcine β-IFN expression plasmid, pAd-β-IFN, which had the left-hand sequence of Ad5 genome but not E1A and E1B (11). An adenoviral vector lacking β-IFN, ADV-ΔE1, was used as a negative control. The structure of these replication-defective viruses was confirmed by Southern blotting and prepared as previously described (11). The pCMV plasmid, also called VR1012, has been described previously (12) and pCMV-β-IFN vector was prepared by insertion of the porcine β-IFN cDNA into the SalI site of this vector.

Cell Culture, Infection, and Cell Cycle Analysis

Primary porcine vsmc were infected with ADV-β-IFN or ADV-ΔE1 for 1 hr in DMEM (GIBCO/BRL) and 2% fetal calf serum (FCS), and normal media was added after 1 hr. Additional control cells were uninfected and maintained in M199 with 10% fetal bovine serum (FBS). Twenty-four hours later, the cells were split into 6-well dishes at 6×10^4 cells per well. Cells were harvested on indicated days, and cell numbers were measured by a hemocytometer using Trypan blue exclusion.

For analysis of cell cycle, porcine vsmc were infected at a multiplicity of infection of 200/cell with the ADV-ΔE1 or ADV-β-IFN vectors, harvested 2 days after infection, washed with phosphate-buffered saline (PBS) twice, and fixed in 70% ethanol for 30 min at 4°C. Control cells were uninfected and maintained in M199 with 10% FBS prior to fixation. The cells were treated with 1 U DNase-free RNase in 1 ml of PBS for 30 min at 37°C, resuspended in 0.05 mg/ml propidium iodide, and analyzed by flow cytometry using a FACScan model (Becton Dickinson) (11).

Porcine Arterial Injury and In Vivo Gene Transfer

Iliofemoral arteries from Yorkshire pigs were injured with a double-balloon catheter (C. R. Bard) (3) for 5 min as described earlier (3), and animals were sacrificed 7 or 21 days (n = 40 arteries) after injury. Direct gene transfer was performed in injured iliofemoral arteries using a double-balloon catheter (3). In each animal, both right and left iliofemoral arteries were infected with the same vector at a titer of 1×10^{10} pfu/ml (dose of 7×10^9 pfu). In nontransfected control animals, the iliofemoral arteries were injured and saline was infused into the artery segment.

The artery segments infected with ADV-β-IFN (n = 14 arteries) and ADV-ΔE1 (n = 14 arteries) or nontransfected (n = 12) were excised 7 or 21 days later. Each artery was processed in an identical manner as previously described (3). To assess cell proliferation, animals sacrificed at 7 days received 5-bromo-2'-deoxycytosine (BrdC) (Sigma), and immunohistochemistry was performed using an anti-BrdU antibody as described (13). To assess the development of intimal thickening, intima and medial cross-sectional areas were measured 21 days after gene transfer in four sections from each artery which spanned the 1.5 cm arterial region (Image One Systems). The four intima-to-medial (I/M) area ratios were averaged to determined the I/M area ratio of each artery. All animal experiments were performed in accordance with NIH guidelines and with approval of the University of Michigan Committee in the Use and Care of Animals.

RT-PCR Analysis

Total RNA was prepared using Trizol reagents (GIBCO/BRL) according to the manufacturer's protocol. Polymerase chain reaction (PCR) for the β-IFN gene was performed in the presence or absence of reverse transcriptase (RT) with the primers: 5′-GGA ATG AAA CCG TCA TTA AGA CTA T-3′ (sense); and 5′-ACC ACA GAA GTA AGG TTC CTT CAC AAA GAT-3′ (antisense). The antisense primer was located in the 3′ region of the SV40 polyadenylation sequence and was specific for recombinant β-IFN RNA and not endogenous porcine β-IFN RNA. The expected length of the PCR product is 330 bp.

Statistical Analysis

All values are expressed as mean ± S.E.M. Each artery was considered as an individual observation. Comparisons of means between ADV-β-IFN, and ADV-ΔE1 and nontransfected samples were made by ANOVA with the Dunnett *t*-test. Comparisons between ADV-ΔE1 and ADV-β-IFN arteries were made by a two-tailed, unpaired *t*-test. Statistical significance was detected at the $p = 0.05$ level.

RESULTS

Inhibition of Vascular Smooth Muscle Cells following Gene Transfer of ADV-β-IFN

To study the effect of β-IFN on vascular cell growth and cell cycle progression, an adenoviral vector encoding this gene product was constructed. Because of the species specificity of IFN, the porcine IFN sequence was used, obtained by screening a cDNA expression library and subcloning the cDNA into an expression vector under the regulation of Rous sarcoma virus promoter. Porcine β-IFN is a glycoprotein that has >60% homology with human β-IFN (166 amino acids, 1N-linked complex carbohydrate). Similar to its human counterpart, it is encoded by a single intronless gene which can be induced in peripheral blood mononuclear cells (PBMCs) treated with viruses (14). This cDNA was incorporated into an adenoviral vector, and vsmc were infected in vitro. Transduction of these cells with the adenoviral vector suppressed cell proliferation (Fig. 1A). A slight reduction was observed after infection of these cells with the adenoviral vector alone, indicating some toxicity of

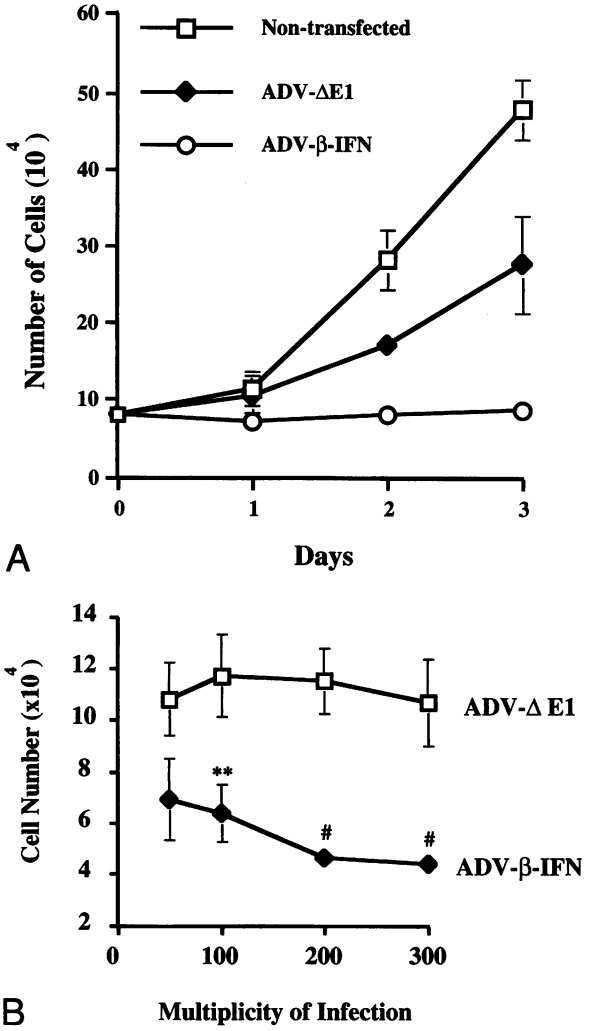

FIG. 1. Cell growth in vascular smooth muscle cells in response to β-IFN expression

(A) Effect of ADV-β-IFN on vsmc growth. Triplicate cultures of porcine vsmc were infected with ADV-β-IFN (○), ADV-ΔE1 (◆), or were uninfected (□), and then stimulated to proliferate with serum. Cells were harvested on the indicated days after infection and counted by Trypan blue staining. (B) Vsmc were infected at the indicated multiplicities with ADV-ΔE1 (□) or ADV-β-IFN (◆). Cell counts were obtained 2 days after infection as in A.

the vector. In contrast, cell proliferation was completely inhibited after infection with ADV-β-IFN. This effect, observed over a range of multiplicities of infection (MOIs), was significantly greater than the effects of ADV-ΔE1 alone at all MOIs ≥ 100 (Fig. 1B), suggesting that expression of porcine β-IFN in porcine cells revealed antiproliferative effects characteristic of β-interferon, as observed in other species and cell types (9,15).

To determine whether β-IFN was secreted into the media and was able to inhibit proliferation of nontransfected cells, supernatants from vsmcs transduced with ADV-ΔE1 or ADV-β-IFN were analyzed. Conditioned media from ADV-β-IFN, but not ADV-ΔE1, infected cells conferred an antiproliferative effect on vsmcs at concentrations of ~10%, even during continuous serum stimulation of porcine vsmcs (Fig. 2A). This effect was manifested by a decrease in the number of viable cells, as judged by trypan blue exclusion. Of the remaining viable cells, the expression of β-IFN did not induce growth arrest at a specific phase of the cell cycle but was cell-cycle independent (Fig. 2B), in contrast to other gene products recently described which show cell cycle–specific effect, such as a mutant retinoblastoma protein (4), or cyclin-dependent kinase inhibitors (CKIs) (6,13). This cytotoxicity did not appear to be mediated through apoptosis, as judged by a DNA laddering analysis (data not shown). These findings, consistent with those reported previously for interferons in other cells (16), suggest a mechanism of action that is independent of cell cycle regulation. Finally, the synthesis of β-IFN was confirmed by Western blot analysis in transfected 293 cells (Fig. 2C), and the growth inhibitory effects of these supernatants on vascular smooth muscle cells were abolished by the addition of a β-IFN neutralizing antibody. Activity of β-IFN was further confirmed by its ability to stimulate class I MHC expression (data not shown).

Overexpression of Interferon-β in Injured Arteries Limits Development of Intimal Hyperplasia

Because β-interferon inhibited vsmc proliferation and was secreted into the media, these findings raise the possibility that it may act on cells other than those directly expressing the gene. Thus, expression of this antiproliferative gene product in injured arteries could potentially limit the degree of intimal expansion following injury. To assess its effect in vivo, ADV-β-IFN was introduced into porcine arteries immediately following balloon catheter injury. As previously described (3), porcine arteries were injured for 5 min and infected with adenoviral vectors (0.7×10^{10} pfu). Expression of ADV-β-IFN was confirmed by using an RT-PCR assay. Gene transfer was demonstrated in iliofemoral arteries 7 days after infection by RT-PCR, at which time β-IFN RNA was readily detected in infected but not uninfected porcine arteries (Fig. 3).

The effect of β-IFN expression on vsmc growth in vivo was assessed by two endpoints: measurements of intimal cell proliferation 7 days after gene transfer assessed by BrdC incorporation, and comparisons of intima-to-media area ratios 21 days after gene transfer using quantitative morphometry (Fig. 4). At 7 days after gene transfer, cell proliferation in ADV-βIFN arteries was significantly reduced, compared with ADV-ΔE1 arteries ($n = 4$ arteries in each group) (ADV-βIFN: 6.8 ± 0.8%, ADV-ΔE1 14.5 ± 1.2%, $p < 0.05$, unpaired two-tailed t-test). A parallel decrease in intima-to-media area ratio was also present in ADV-β-IFN arteries compared with arteries from the two control groups (ADV-βIFN: I, 1.65 ± 0.56 mm^2; M, 3.12 ± 0.92 mm^2; I/M = 0.53 ± 0.03, $n = 10$ arteries; ADV-ΔE1: I, 1.93 ± 0.67 mm^2; M, 2.81 ± 0.87 mm^2; I/M 0.69 ± 0.064, $n = 10$ arteries; nontransfected: I, 2.05 ± 0.62 mm^2; M, 2.93 ± 0.96 mm^2; I/M 0.70 ± 0.05, $n = 8$ arteries; $p < 0.05$, ANOVA with Dunnett t-test). These results suggest that β-IFN expression at the time of balloon injury results in an inhibition of vascular cell proliferation and subsequent intimal lesion formation.

DISCUSSION

Expanded neointimal hyperplasia occurs as a response to balloon injury and represents a complex process resulting from vascular proliferative and thrombotic events, with local accumulation of smooth muscle cells and matrix deposition, often causing reocclusion of arteries. A number of gene transfer approaches have been pursued to prevent the recurrence of this process following balloon injury in a variety of different animal models (3–6,13). In this study, we have evaluated the effects of a naturally occurring antiproliferative gene that has favorable features. The β-IFNs have been well characterized for their antiviral and antiproliferative effects in a variety of different species and cell lines. In addition to their effect on cell proliferation, these proteins have the additional capacity to be secreted and thus to act on a large number of cells following gene transfer into a minority of the subpopulation. Thus, the ability of gene products to be secreted and cause cytostatic effects reduces the need to transduce all dividing cells in the injured artery and increases its potential to inhibit local proliferation.

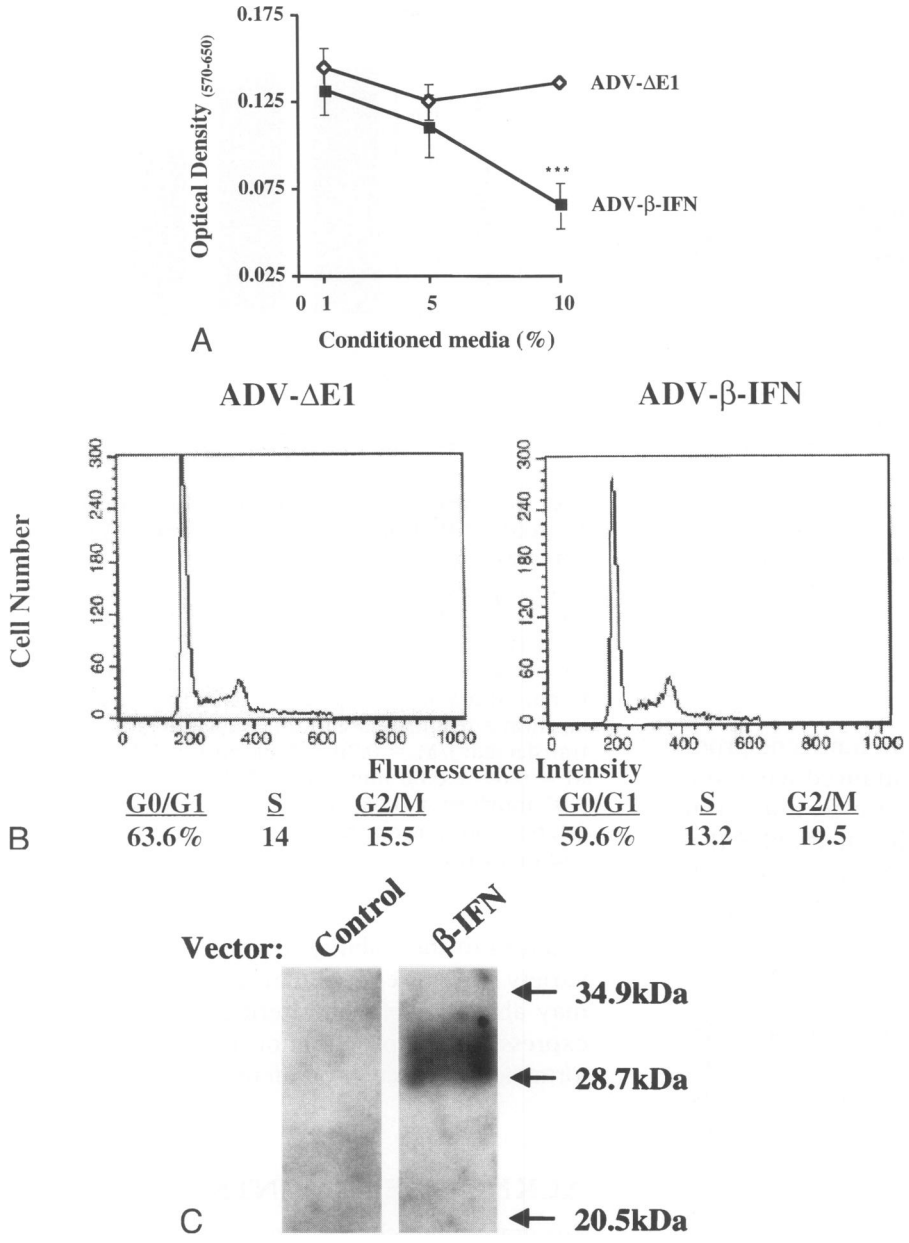

FIG. 2. The effect of secreted porcine β-IFN on cell cycle progression

(A) Supernatants from vsmc infected with ADV-β-IFN (■) or ADV-ΔE1 (◆) were analyzed for antiproliferative effects at the indicated concentrations of conditioned media. Cell proliferation was measured using the MTT colorimetric assay, detecting optical density at 570–650 to determine relative rates of proliferation as described previously (3). (B) Propidium iodide staining was used to determine cell cycle distribution of vsmc after infection using the indicated antiviral vectors. The percentage of cells in G0/G1, S, and G2/M are shown for each vector, respectively. (C) Western blot analysis for β-IFN protein in control (pCMV) or β-IFN (pCMV-β-IFN) transfected 293 cells. Western blots were performed as previously described (11,13) using a sheep anti-human β-IFN antibody (Biosource International, Camerillo, CA).

The involvement of IFNs in a variety of other processes has also raised the possibility that they may affect cellular activity unrelated to smooth muscle cell proliferation, such as that exhibited by activated macrophages or lymphocytes (17,18). Thus, its effects may be exerted not only in acute proliferative responses, as might be observed in balloon catheter injury, but may also have potential to affect more chronic inflammation or proliferation as observed in atherosclerosis. The expression of β-IFNs and its ability to inhibit vascular smooth muscle proliferation would suggest that this gene product may be applicable to such vascular proliferative disorders.

Interestingly, despite its ability to inhibit cell proliferation, the effect of β-IFN was observed to be independent of cell cycle checkpoints in vitro. Thus, there appeared to be no specific accumulation of cells at different points in the cell cycle, which is consistent with previous data on this antiproliferative gene product (16). Recent studies have suggested that members of the Stat family, through which the IFNs act, may interact with transcriptional coactivators that may be coupled to growth arrest. This mechanism involves the p300/CBP coactivator protein (19) and may explain, in part, the ability of β-IFN not only to affect cell cycle progression but also to

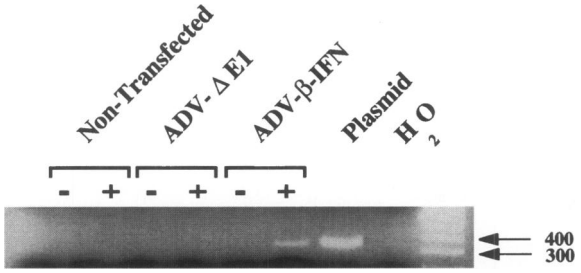

FIG. 3. Expression of recombinant β-IFN RNA in adenoviral vector-infected, balloon-injured arteries

β-IFN RNA was detected 7 days after injury and ADV-β-IFN gene transfer in porcine arteries using an RT-PCR technique. Total RNA was incubated in the presence (+) or absence (−) of RT and analyzed. Gene transfer with the indicated vectors or negative control are shown. For uninfected negative controls, carotid arteries from the same animals treated in the iliofemoral arteries with the ADV-β-IFN vector were analyzed.

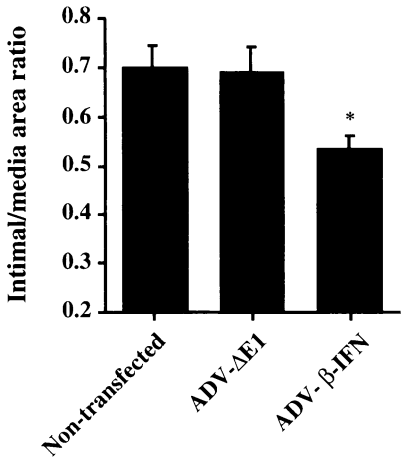

FIG. 4. Inhibition of intimal lesion formation by ADV-β-IFN infection after injury of porcine arteries

Injured porcine iliofemoral arteries were infected with ADV-β-IFN (n = 10 arteries), ADV-ΔE1 (n = 10 arteries) (7×10^9 pfu), or were not infected (n = 8 arteries). Twenty-one days later, cross-sectional intima and medial areas were measured, and an intima-to-media area ratio was derived (non-transfected: I/M = 0.70 ± 0.05 mm^2; ADV-ΔE1:I/M = 0.69 ± 0.06 mm^2; ADV-β-IFN:I/M = 0.53 ± 0.03 mm^2; *$p < 0.05$, ADV-β-IFN versus ADV-ΔE1 arteries and nontransfected arteries, ANOVA with Dunnett t-test).

regulate the transcription of different gene products that may be coordinately induced when the growth arrest occurs in response to interferon treatment. Finally, although the effects of β-IFN on smooth muscle proliferation are cytotoxic in vitro, it is likely that they are different, and possibly cytostatic, in vivo, accounting for the lack of necrosis or aneurysmal changes in ADV-β-IFN transduced arteries.

It is also important to assess the potential toxicities of gene transfer vectors that may have application to human disease. In these studies, no evidence of local inflammation was detected after gene transfer. Of equal importance, there were no signs of arterial dilatation or aneurysm formation at the site of arterial injury and viral infection. These findings are in agreement with previous studies using adenoviral vectors in porcine, rat, and rabbit models following balloon angioplasty (3,4,6,13,20,21). Although other studies have reported some inflammatory effects at the site of ADV gene transfer (22), it is not yet clear whether those toxicities were due to specific differences, contaminants in the ADV preparation, or the nature of the recombinant gene expressed in this vector. Although these studies represent a first step in exploring the potential utility of β-IFN for the treatment of vasculature proliferative disease, the findings suggest that the expression of this gene product in porcine arteries may help to limit excessive proliferation which may result from arterial balloon injury. In addition to their ability to affect expression of a variety of different cellular gene products, they may also be useful in altering patterns of gene expression and proliferation which may lead to formation of vascular proliferative lesions in vivo.

ACKNOWLEDGMENTS

We gratefully acknowledge L. Xu for technical expertise, and Donna Gschwend and Nancy Barrett for manuscript preparation. This study was supported in part by grants from the National Institutes of Health (HL-43757, E. G. Nabel; CA59327, G. J. Nabel; HL-42119, D. Gordon). E. G. Nabel is an Established Investigator of the American Heart Association. Dominique Stephan is the recipient of a 1995 grant from the French Society of Hypertension.

REFERENCES

1. Nabel EG, Yang Z, Liptay S, et al. (1993) Recombinant platelet-derived growth factor B gene expression in porcine arteries induces

intimal hyperplasia *in vivo*. *J. Clin. Invest.* **91:** 1822–1829.
2. Nabel EG, Shum L, Pompili VJ, et al. (1993) Direct gene transfer of transforming growth factor β1 into arteries stimulates fibrocellular hyperplasia. *Proc. Natl. Acad. Sci. U.S.A.* **90:** 10759–10763.
3. Ohno T, Gordon D, San H, et al. (1994) Gene therapy for vascular smooth muscle cell proliferation following arterial injury. *Science* **265:** 781–784.
4. Chang MW, Barr E, Seltzer J, et al. (1995) Cystostatic gene therapy for vascular proliferative disorders using a constitutively active form of Rb. *Science* **267:** 518–522.
5. Indolfi C, Avvedimento E, Rapacciuolo A, et al. (1995) Inhibition of cellular ras prevents smooth muscle cell proliferation after vascular injury *in vivo*. *Nat. Med.* **1:** 541–545.
6. Chang M, Barr E, Lu M, et al. (1996) Adenovirus-mediated overexpression of the cyclin/cyclin- dependent kinase inhibitor, p21 inhibits vascular smooth muscle cell proliferation and neointima formation in the rat carotid artery model of balloon antioplasty. *J. Clin. Invest.* **96:** 2260–2268.
7. Isaac A, Lindenmann J. (1975) Virus interference: The interferons. *Proc. R. Soc. Lond. B Biol. Sci.* **147:** 258–267.
8. David M. (1995) Transcription factors in interferon signaling. *Pharmacol. Ther.* **65:** 149–161.
9. Palmer H, Libby P. (1992) Interferon-β, a potential autocrine regulator of human vascular smooth muscle growth. *Lab. Invest.* **66:** 715–721.
10. Min W, Gosh S, Lengley P. (1995) The interferon-inducible p202 protein as a modulator of transcription: Inhibition of NF-kB, c-Fos, and c-Jun activities. *Mol. Cell. Biol.* **16:** 359–368.
11. Yang Z, Perkins ND, Ohno T, Nabel EG, Nabel GJ. (1995) The p21 cyclin-dependent kinase inhibitor suppresses tumorigenicity *in vivo*. *Nat. Med.* **1:** 1052–1056.
12. Stephan DJ, Yang Z, San H, et al. (1996) A new cationic liposome DNA complex enhances the efficiency of arterial gene transfer *in vivo*. *Hum. Gene Ther.* **7:** 1803–1812.
13. Yang Z, Simari RD, Perkins ND, et al. (1996) Role of the p21 cyclin-dependent kinase inhibitor in limiting intimal cell proliferation in response to arterial injury. *Proc. Natl. Acad. Sci. U.S.A.* **93:** 7905–7910.
14. Artursson K, Gobl A, Lindersson M, Johansson M, Alm G. (1992) Molecular cloning of a gene encoding porcine interferon-beta. *J. Interferon Res.* **12:** 153–160.
15. Fukumoto Y, Kawahara Y, Kariya KI, Araki SI, Fukuzaki II, Takai Y. (1988) Independent inhibition of DNA synthesis by protein kinase C, cyclic AMP and interferon α and β in rabbit aortic smooth muscle cells. *Biochem. Biophys. Res. Commun.* **157:** 337–345.
16. Ileyns A, Eldor A, Vlodavsky IK, N, Fridman R, Panet A. (1985) The antiproliferative effect of interferon and the mitogeneic activity of growth factors are independent cell cycle events. *Exp. Cell Res.* **161:** 297–306.
17. Kuo PT, Wilson AC, Goldstein RC, Schaub RG. (1984) Suppression of experimental atherosclerosis in rabbits by interferon-inducing agents. *J. Am. Coll. Cardiol.* **3:** 129–134.
18. Wilson ACS, Schaub RG, Goldstein RC, Kuo PT. (1984) Suppression of aortic atherosclerosis in cholesterol-fed rabbits by purified rabbit interferon. *Arteriosclerosis* **10:** 208–214.
19. Bhattacharya S, Eckner R, Grossman S, et al. (1996) Cooperation of Stat2 and p300/CBP in signalling induced by interferon-α. *Nature* **383:** 344–347.
20. French B, Mazur W, Ali NM, et al. (1994) Percutaneous transluminal *in vivo* gene transfer by recombinant adenovirus in normal porcine coronary arteries, atherosclerotic arteries, and two models of coronary restenosis. *Circulation* **90:** 2402–2413.
21. Barr E, Carroll J, Kalynych AM, et al. (1994) Efficient catheter-mediated gene transfer into the heart using replication-defective adenovirus. *Gene Ther.* **1:** 51–58.
22. Newman KD, Dunn PF, Owens JW, et al. (1995) Adenovirus-mediated gene transfer into normal rabbit arteries results in prolonged vascular cell activation, inflammation, and neointimal hyperplasia. *J. Clin. Invest.* **96:** 2955–2965.

Communicated by J. Leiden. Accepted June 26, 1997.