action of several mitogens (*2, 17*), AII may be implicated in fetal growth. In support of this possibility, AII has been shown to act as a mitogen in cultured bovine adrenal cells (*18*) and to stimulate growth and induce the expression of c-*myc* and c-*fos* in cultured smooth muscle cells (*4, 5*). In addition, injection of AII in 18-day rat fetuses in utero caused a 20% increase in ^{3}H-labeled amino acid incorporation into proteins in the skin, but not in the brain, measured 5 hours later (*19*).

AII may modulate growth through its effect on the calcium-phospholipid pathway directly or through the regulation of the expression of other growth factors or proto-oncogenes. The latter is supported by the finding that AII causes an increase in PDGF mRNA in cultured smooth muscle cells (*5*). In addition, our findings on the ontogeny and localization of AII receptors are similar to those reported for insulin-like growth factor II in the rat fetus (*20*). Although the exact physiological function of AII in the fetus remains to be demonstrated, the prominent and transient expression of functional AII receptors at unique sites in the fetus strongly suggests a role for AII in fetal development.

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Recombinant Gene Expression *in Vivo* Within Endothelial Cells of the Arterial Wall

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A technique for the transfer of endothelial cells and expression of recombinant genes in vivo could allow the introduction of proteins of therapeutic value in the management of cardiovascular diseases. Porcine endothelial cells expressing recombinant β-galactosidase from a murine amphotropic retroviral vector were introduced with a catheter into denuded iliofemoral arteries of syngeneic animals. Arterial segments explanted 2 to 4 weeks later contained endothelial cells expressing β-galactosidase, an indication that they were successfully implanted on the vessel wall.

REDUCTIONS IN BLOOD FLOW TO the heart and other organs are often caused by fixed atherosclerotic obstructions, which can become critical when accompanied by superimposed narrowings due to thrombus formation or vessel constriction. Vascular endothelial cells contribute to the pathogenesis of these lesions by (i) altering the thrombogenic properties of the vessel lumen (*1*), (ii) inducing smooth muscle cell proliferation (*2*), and (iii) regulating vascular smooth muscle tone (*3*). Genetically altered endothelial cells could transmit recombinant DNA products that would provide anticoagulant, vasodilatory, angiogenic, or growth factors to a localized segment of vessel. We show that endothelial cells can be stably implanted in situ on the arterial wall and express a recombinant marker protein, β-galactosidase, in vivo.

Because atherogenesis in swine has similarities to humans, we used an inbred pig strain, the Yucatan minipig (Charles River Laboratories), as an animal model (*4*). We established a primary endothelial cell line from the internal jugular vein of an 8-month-old female minipig and confirmed the endothelial cell identity of this line (*5*). Two independent β-galactosidase–expressing endothelial cell lines were isolated (*6*) after they were infected with a murine amphotropic β-galactosidase–transducing retroviral vector (BAG), which is replication-defective and contains both β-galactosidase and neomycin resistance genes (*7*).

Endothelial cells derived from this inbred strain, being syngeneic, were applicable for study in more than one minipig, and were tested in nine different experimental subjects. The animals were anesthetized, the femoral and iliac arteries were exposed, and a catheter was introduced into the vessel through a small branch (Fig. 1). Intimal tissues of the arterial wall were denuded mechanically by forceful passage of a partially inflated balloon catheter within the vessel. The artery was rinsed with heparinized saline and incubated with a neutral protease dispase (50 U/ml), which removed any remaining luminal endothelial cells. Residual enzyme was presumably inactivated by α_2 globulin in plasma (*8*) on deflating the catheter balloons and allowing blood to flow through the vessel segment. The cultured endothelial cells, which expressed β-galactosidase (Fig. 2, A and B), were introduced by means of a specially designed arterial catheter (C. R. Bard Inc., Billerica, MA) that contained two balloons and a central instillation port (Fig. 1). When these balloons were inflated, a protected space was created within the artery into which cells were instilled through the central port. These β-galactosidase–expressing endothelial cells were allowed to incubate for 30 min to facilitate their attachment to the denuded vessel. The catheter was then removed, the arterial side branch ligated, and the incision closed. Labeling with ^{51}Cr indicated that, of the 2×10^5 endothelial cells instilled in the central space, 2 to 11% successfully attached to the denuded arterial wall.

Segments of experimental and control arteries were removed 2 to 4 weeks later. Staining of arterial specimens with X-gal chromagen revealed multiple areas of blue

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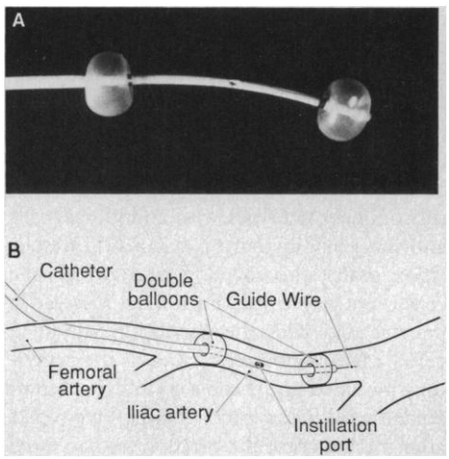

Fig. 1. Method of introduction of endothelial cells by catheterization. (**A**) Double balloon catheter used for instillation of endothelial cells. Proximal and distal balloon inflation isolates a central space, allowing for instillation of infected cells through the port into a discrete segment of the vessel. (**B**) Schematic representation of cell introduction by catheter. Female Yucatan minipigs were anesthetized with pentobarbital and underwent sterile surgical exposure of the iliofemoral arteries. The catheter was advanced into the iliac artery and the proximal balloon was partially inflated and passed to mechanically denude the endothelium. The catheter was positioned with the central space located in the region of denuded endothelium, and both balloons were inflated. The denuded segment was irrigated with heparinized saline, and residual adherent cells were removed by instillation of dispase (50 U/ml) for 10 min (*12*). After further irrigation, the BAG-infected endothelial cells were instilled for 30 min. The balloon catheter was subsequently removed, and antegrade blood flow was restored. The vessel segments were excised 2 to 4 weeks later. A portion of the artery was placed in 0.5% glutaraldehyde for 5 min and stored in phosphate-buffered saline, and another portion was mounted in a paraffin block for sectioning.

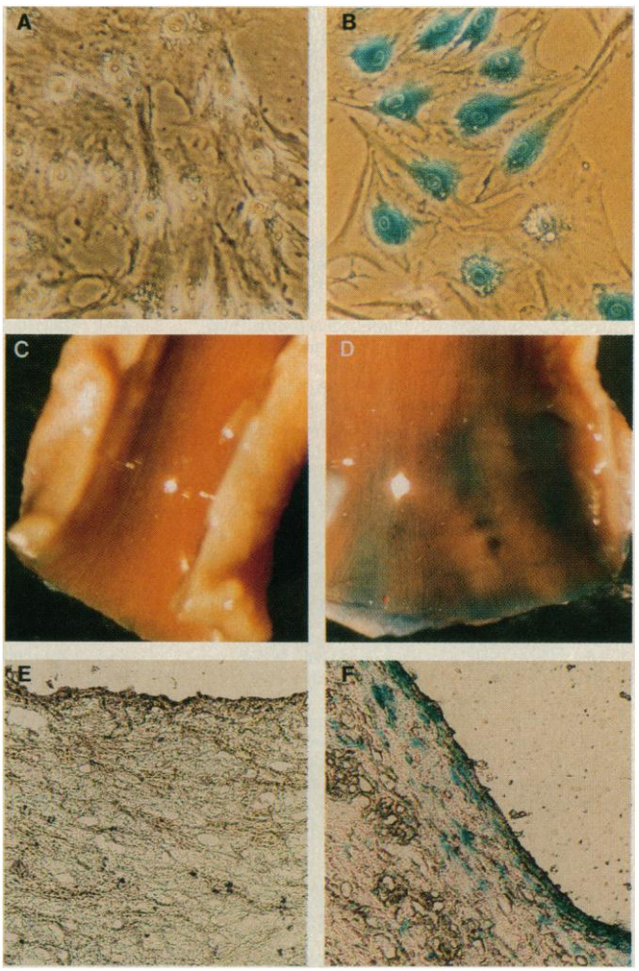

Fig. 2. Analysis of endothelial cells in vitro and in vivo. β-galactosidase activity documented by histochemical staining in (**A**) primary endothelial cells from the Yucatan minipig, (**B**) a subline derived by infection with the BAG retroviral vector, (**C**) a segment of normal control artery, (**D**) a segment of artery instilled with endothelium infected with the BAG retroviral vector, (**E**) microscopic cross section of normal control artery, and (**F**) microscopic cross section of artery instilled with endothelium infected with the BAG retroviral vector. Endothelial cells in tissue culture were fixed in 0.5% glutaraldehyde before histochemical staining (*13*). The enzymatic activity of the *Escherichia coli* β-galactosidase protein was used to identify infected endothelial cells in vitro and in vivo. Endothelial cells at two-thirds confluence were infected with BAG (7) retrovirus (10^4 to 10^5 G418-resistant colony-forming units per milliliter) and incubated for 12 hours in Dulbecco's minimum essential medium with 10% calf serum at 37°C in 5% CO_2 in the presence of polybrene (8 μg/ml). Cells were maintained in medium 199 with 10% fetal calf serum, endothelial cell growth supplement (50 μg/ml), and endothelial cell–conditioned medium (20%) for an additional 24 to 48 hours before selection in G418 (0.7 mg/ml of a 50% racemic mixture). G418-resistant cells were isolated and analyzed for β-galactosidase expression with a standard histochemical stain (*13*). Cells stably expressing the β-galactosidase enzyme were maintained in continuous culture for use as needed. Frozen samples were stored in liquid nitrogen.

coloration indicative of β-galactosidase activity in the arteries seeded with infected endothelium, estimated to represent 20 to 100% of the cells that attached initially. Arteries seeded with uninfected endothelium exhibited no blue staining (Fig. 2, C and D). Light microscopy revealed β-galactosidase staining primarily in endothelial cells of the intima in experimentally seeded vessels. No evidence of similar staining was observed in control segments (Fig. 2, E and F). β-Galactosidase staining was occasionally evident in deeper intimal tissues and was suggestive of entrapment or migration of seeded endothelium within the previously injured vessel wall (Fig. 2F). Local thrombosis was observed in the first two experimental subjects. This complication was minimized in subsequent studies by administering acetylsalicylic acid before the endothelial cell transfer procedure and by using heparin anticoagulation at the time of innoculation. In instances of thrombus formation, β-galactosidase staining was seen in endothelial cells extending from the vessel wall to the surface of the thrombus.

A major concern of gene transplantation in vivo is the production of a replication-competent retrovirus from genetically engineered cells. This potential problem was minimized through the use of a replication-defective retrovirus. No helper virus was detectable among these lines after 20 passages in vitro. Although defective retroviruses were used in this study because of their high rate of infectivity and their stable integration into the host cell genome (*9*), this approach to gene transfer is adaptable to other viral vectors. A second concern involves the longevity of expression of recombinant genes in vivo. Endothelial cell expression of β-galactosidase appeared constant in vessels examined up to 4 weeks after surgery.

Thus, genetically altered endothelial cells can be introduced into the vascular wall of the Yucatan minipig by arterial catheterization. A major complication of current interventions for vascular disease, such as balloon angioplasty or endarterectomy, is disruption of the atherosclerotic plaque and thrombus formation at sites of local tissue trauma (*10*). In part, this is mediated by endothelial cell injury (*11*). Genetically altered or normal endothelial cells introduced at the time of intervention could minimize local thrombosis. This technology could be used also for the treatment of myocardial or tissue ischemia by introducing cells expressing thrombolytic, angiogenic, or growth factor genes. Finally, this approach might be used to treat systemic or inherited diseases requiring secretion of gene products directly into the circulation.

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5. The endothelial cells exhibited growth characteristics and morphology typical of porcine endothelium in tissue culture. They also expressed receptors for the acetylated form of low density lipoprotein (AcLDL), in contrast to fibroblasts and other mesenchymal cells [R. E. Pitos, T. L. Innerarity, J. N. Weinstein, R. W. Mahley, *Arteriosclerosis* **1**, 177 (1981); T. J. C. Van Berkel, J. F. Nagelkerke, J. K. Kruijt, *FEBS Lett.* **132**, 61 (1981); J. M. Wilson, D. E. Johnston, D. M. Jefferson, R. C. Mulligan, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4421 (1988)]. More than 95% of the cultured cells contained this receptor, as judged by fluorescent AcLDL uptake. Cell cultures were incubated with (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbacyanine perchlorate) (Dil) AcLDL (Biomedical Technologies) (10 μg/ml) for 4 to 6 hours at 37°C, followed by three rinses with phosphate-buffered saline containing 1% paraformaldehyde and visualized by phase-contrast and fluorescent microscopy to detect AcLDL uptake. These cells also contained von Willebrand factor, detected in cDNA by the polymerase chain reaction.
6. Endothelial cells containing this vector were selected for their ability to grow in the presence of G418. Greater than 90% of selected cells synthesized β-galactosidase by histochemical staining (Fig. 2B). The endothelial cell nature of these genetically altered cells was also confirmed by analysis of fluorescent AcLDL uptake and von Willebrand factor detected in cDNA by using the polymerase chain reaction. Infection by BAG retrovirus was further verified by DNA blot analysis, which revealed the presence of intact proviral DNA at approximately one copy per genome.
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Implantation of Vascular Grafts Lined with Genetically Modified Endothelial Cells

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The possibility of using the vascular endothelial cell as a target for gene replacement therapy was explored. Recombinant retroviruses were used to transduce the *lacZ* gene into endothelial cells harvested from mongrel dogs. Prosthetic vascular grafts seeded with the genetically modified cells were implanted as carotid interposition grafts into the dogs from which the original cells were harvested. Analysis of the graft 5 weeks after implantation revealed genetically modified endothelial cells lining the luminal surface of the graft. This technology could be used in the treatment of atherosclerosis disease and the design of new drug delivery systems.

MYOCARDIAL INFARCTIONS, strokes, and amputations due to lower extremity occlusive disease are the clinical sequelae of atherosclerosis and the major causes of mortality in our society despite concerted attempts at prevention and treatment (*1*). One important clinical intervention in advanced disease states is the replacement of stenotic or occluded arteries with bypass grafts. In large diameter vessels, this can be accomplished through the use of autologous veins or arteries or by using prosthetic conduits composed of materials such as dacron or expanded polytetrafluoroethylene (*2*). Bypass of smaller diameter, low flow vessels (for example, coronary arteries and some peripheral arteries) usually requires the use of autologous vessels, since the performance of prosthetic vascular grafts is poor (*2*).

We have considered whether the performance and versatility of prosthetic vascular grafts might be improved by lining the luminal surface with endothelial cells genetically modified to promote repopulation, prevent thrombosis, or secrete therapeutic proteins. As a first step, we implanted into dogs vascular grafts seeded with autologous retrovirus-transduced endothelial cells by means of a technique that has been used in the study of graft thrombosis (*3*). External jugular veins harvested from adult mongrel dogs were used as a source of endothelial cells. The cells were plated in culture and expanded in vitro for 10 to 14 days. Cells from each animal were divided into two portions and infected with a replication-defective retrovirus or were mock-infected. Small diameter Dacron grafts were seeded at subconfluent densities with endothelial cells and surgically implanted as carotid interposition grafts into the dog from which the cells were harvested. Each dog received a graft seeded with the genetically modified cells (subsequently referred to as the genetically modified graft) and a contralateral graft seeded with mock-infected cells. Five weeks after implantation the grafts were harvested and analyzed.

Replication-defective retroviruses with amphotropic host range were used to introduce a reporter gene into the genomic DNA of the endothelial cells. The *Escherichia coli lacZ* gene was used as the reporter gene because its product of expression, β-galactosidase, can be detected in situ through the use of enzymic histochemical assays that stain the cell's cytoplasm blue (*4*). Two retroviral vectors that express the *lacZ* gene, BAG and BAL, were used in this study (*5*). Viruses derived from these vectors were used to infect primary cultures of endothelial cells derived from each dog. Approximately 5% to 15% of the endothelial cells exposed to the BAG virus were infected, whereas 40% to 60% of the endothelial cells exposed to BAL virus were infected (Table 1).

At the time of seeding, the cultures were analyzed for endothelial cell–specific function [uptake of acetylated low density lipoprotein (LDL) and the presence of von Willebrand's factor] and for the presence of antigens specific for smooth muscle cells (α- and γ-actin isoforms). These analyses indicated that >98% of the cells from each isolate were functional endothelial cells.

Dogs 1 to 3 received implants seeded with BAG-infected endothelial cells, whereas dogs 4 to 7 received implants seeded with BAL-infected endothelial cells. After 5 weeks, the grafts were explanted and divided longitudinally into thirds for analysis. The analyses included fixation and scanning electron microscopy, fixation and histochemical characterization for β-galactosidase–ex-

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