Gene transfer is poised to become a clinical reality for gene therapy of vascular disease.1-3 Several important cardiovascular diseases appear to be excellent candidates for gene therapy: inhibiting angioplasty restenosis has already received much attention, and stimulation of collateral blood vessel growth is the focus of the first clinical trial of vascular gene therapy. Other targets include atherosclerosis, vein graft failure, and arterial thrombosis. Despite dramatic advances in gene transfer techniques, several fundamental obstacles to the widespread utility of vascular gene therapy remain to be overcome. We will briefly review the current status of vascular gene transfer, focusing on methods and techniques, on what has been attained, and on prospects for the future.

Gene transfer vectors
Gene transfer involves the delivery of genetic material (DNA) into target cells, where expression of the foreign gene (transgene) results in a biological effect. Numerous genes which might be suitable for therapeutic vascular gene transfer have been identified and cloned. These include genes targeting growth factors and their intracellular signalling cascades, and genes affecting production of biologically active molecules such as nitric oxide. The success of gene transfer relies upon an efficient delivery method. Whereas the uptake of naked DNA is inefficient, gene transfer vectors greatly enhance transgene uptake and expression. There are two broad categories of vector systems of current interest for vascular gene transfer: Plasmid DNA-liposome complexes enhance cellular uptake of DNA by facilitating passage across cell membranes. Liposomes are safe, cheap, and simple to produce but result in low transfection efficiency in the vascular wall (typically less than 1% of cells express the transgene). Nevertheless, for a secreted protein, even these very low efficiencies can result in adequate expression and a biological effect.4

Viral vectors—Viruses are highly adapted to entering a cell and using the host machinery to synthesise foreign proteins. Incorporation of the transgene into a genetically modified virus therefore allows higher transfection efficiency and enhanced gene expression.

Retroviral vectors insert the transgene into the host genome, resulting in prolonged transgene expression. The risk of insertional mutagenesis, however, raises important concerns about safety. In addition, retroviruses only infect proliferating cells and are difficult to purify in high titres. In contrast, recombinant adenoviruses have gained widespread popularity for vascular gene transfer (figure). Their advantages include high efficiency infection of a broad range of cells, the ability to infect quiescent cells, and a low risk of insertional mutagenesis. Unfortunately, these favourable characteristics are tempered by important problems that continue to limit the clinical utility of adenoviral vectors. High concentrations of virus are directly toxic to cells, and inflammation also results from host immunity. These factors, and the fact that the transgene is not integrated into host DNA, result in a short duration of transgene expression: typically days or at most a few weeks. Although a limitation for some applications, this short window of expression may nevertheless be adequate for gene therapy of angioplasty restenosis or for re-endothelialisation of an injured vessel.

Safety concerns will continue to temper enthusiasm for adenoviral gene therapy, but potential solutions to these problems are already emerging. Newer adenoviral vectors incorporate more extensive genetic modifications to reduce the expression of viral proteins, thus limiting host immune recognition of infected cells. Alternatively, co-administration of immunomodulators such as interleukin-12 may also limit the host immune response.5 Finally, higher efficiency vectors may allow significant reductions in the dose of virus required to produce a biological effect.

Progress in vascular gene transfer
What has already been attained in the field of vascular gene transfer? Following early landmark reports of low level in vivo arterial gene transfer using DNA-liposomes or retrovirus,6 the widespread use of adenovirus to attain higher transfection efficiencies spawned numerous studies of arterial gene transfer in various animal models. In vivo transfer of marker genes (usually β-galactosidase) has now been evaluated in arteries in several species7-9 in the setting of arterial injury, neointimal hyperplasia, or atherosclerosis.9-10 In uninjured arteries, adenoviral gene transfer results in very high endothelial transgene expression but little medial expression because of anatomical barriers.11 In contrast, arterial injury or pressure-assisted delivery directs gene transfer to medial smooth muscle cells.12 In an atherosclerotic artery, however, the efficiency of transfer may be markedly reduced.10 Venous bypass grafts are particularly attractive targets for gene therapy. Both early thrombosis and late proliferative occlusion could be targeted, and the excised vein is available for intraoperative gene transfer. Antisense RNA techniques have been successful in altering vein graft pathobiology.12 Adenoviral gene transfer to vein grafts has also yielded promising results; high-level expression was obtained after intraoperative transfer of both β-galactosidase and a soluble form of the adhesion molecule VCAM-1.13

Particular interest in vascular gene transfer has been directed at strategies for local delivery. Modified percutaneous balloon catheters deliver the vector to a particular segment of vessel, resulting in a high level of local transgene expression without significant systemic distribution.8 Different devices have varying delivery characteristics that target the transgene to particular cell types or regions in
High efficiency arterial gene transfer using adenovirus: A segment of rabbit carotid artery was infected with a recombinant adenovirus encoding the marker protein β-galactosidase. Staining of the tissue with a chromogenic substrate for β-galactosidase results in intense blue nuclear staining in cells that express the transferred gene. Panels A and B show a segment of artery cut longitudinally and transversely, respectively. Almost all of the endothelial cells lining the vessel lumen express high levels of nuclear β-galactosidase, resulting in a striking blue cobblestone appearance. In addition, numerous cells in the arterial adventitia and smooth muscle cells on the cut surface of the arterial media also express the transferred gene. Panels C and D, histological sections (original magnification × 12 panel C, × 60 panel D) clearly demonstrate discrete blue-stained nuclei on the endothelial surface of the vessel, within the superficial media and in the adventitia.

the vessel wall. Double balloon catheters, which allow the virus solution to dwell between two occlusive balloons, result in predominantly endothelial gene transfer. In contrast, perforated balloons enable high pressure injection of solutions through radial pores, independently of balloon inflation pressure. Some vessel injury occurs, but enhanced transduction of medial smooth muscle cells can be attained. Finally, hydrogel balloons are coated with an absorbent polymer which is soaked in vector solution before application to the vessel wall. This method is only suitable for delivery of small volumes, but results in endothelial and medial delivery without vessel injury and has been particularly useful for gene transfer of plasmid DNA.

Experimental vascular gene therapy
After an initial focus on marker genes, there has been increasing success with in vivo expression of therapeutic transgenes. The first demonstration of gene therapy to prevent angioplasty restenosis used an adenovirus encoding the herpesvirus thymidine kinase (tk) gene to target proliferating smooth muscle cells in porcine coronary arteries. Administration of ganciclovir after gene transfer resulted in selective toxicity to tk-expressing cells and virtually abolished the proliferative response to balloon injury. In the rat carotid injury model, adenoviral gene transfer of a constitutively active retinoblastoma gene (an inhibitor of cellular proliferation) also prevented restenosis. In an alternative approach, modified DNA-liposome complexes
were used to deliver the endothelial nitric oxide synthase (eNOS) gene to injured rat carotid arteries. Expression of functional eNOS restored endothelium-dependent relaxation and reduced neointimal proliferation.

In the peripheral vasculature, transfer of DNA encoding the gene for vascular endothelial growth factor (VEGF), a selective endothelial cell mitogen and mediator of angiogenesis, augmented collateral formation and blood flow in the ischaemic rabbit hind limb. This work forms the basis for the first clinical trial of gene therapy for vascular disease, recently approved by the Food and Drug Administration. Patients with advanced peripheral vascular disease will receive increasing doses of a plasmid vector encoding VEGF, delivered percutaneously by a hydrogel-coated angioplasty balloon, with the aim of promoting collateral vessel growth in the ischaemic limb. End points will include clinical response, capillary flow, and angiographic assessment of collateral vessels.

Although vascular gene therapy remains in its infancy, some important developmental milestones have already been attained. As with any novel and exciting technique, initial over-enthusiasm gave rise to unrealistic expectations. More detailed studies, however, have led to a better understanding of the strengths and limitations of each approach and the hurdles that still need to be overcome to make vascular gene transfer a clinical reality. The first clinical trial of vascular gene therapy signals a very important development in cardiovascular medicine.

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