Prospects for Gene Therapy of Ischemic Heart Disease

"This is not the end. It is not even

the beginning of the end.

But, perhaps,

it is the end of the beginning."

— **Winston Churchill**, 1942
speaking after the Allied victory in
North Africa at El Alamein.

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Introduction: concepts and definitions

Gene therapy can be defined as the treatment or prevention of disease by introduction of recombinant DNA into tissues of human subjects. This definition is purposefully broad, so as to encompass three general strategies for combating disease by transfer of foreign genes into somatic cells:

1) correction of disorders resulting from a defective gene;
2) alteration of a disease process by imparting a new or augmented function to certain cells;
3) alteration of a disease process by blocking an existing function within certain cells.

Replacement of a missing activity constitutes the most straightforward form of gene therapy - correction of a genetic defect by introduction of a functional copy of the defective gene. This strategy provides the basis for several gene therapy protocols currently entering clinical testing. However, this strategy is limited to well-characterized disorders that are inherited as recessive traits. Such disorders provide important models on which the foundations of gene therapy are based, but they comprise, however, only a small proportion of human diseases that ultimately may be ameliorated by gene transfer techniques.

Even in the absence of a specific genetic defect, a foreign gene may be introduced for the purpose of imparting a new property to a cell or tissue. Genes introduced for this purpose may direct overproduction of a natural protein, production of a natural protein in an ectopic site, or production of a genetically engineered protein that is not be found in nature at all. Likewise, foreign genes may be introduced to inhibit activities of cells that, though natural and customary for that cell, nevertheless contribute to a disease process. Expanding the definition of gene therapy to include these latter two general strategies markedly extends the spectrum of disease processes that ultimately may become amenable to gene therapy.

Our review today will focus on ischemic heart disease, and will present examples to illustrate how these three general strategies eventually may be applied as therapeutic solutions to specific clinical problems arising from this most prevalent of disorders faced by the internist and cardiologist.

Specific targets for gene therapy of ischemic heart disease

A number of pathophysiological features or manifestations of ischemic heart disease present attractive targets for direct gene therapy.

Primary and secondary prevention of coronary atherosclerosis could be improved by gene therapy protocols that eliminate risk factors - hyperlipidemias, hypertension,

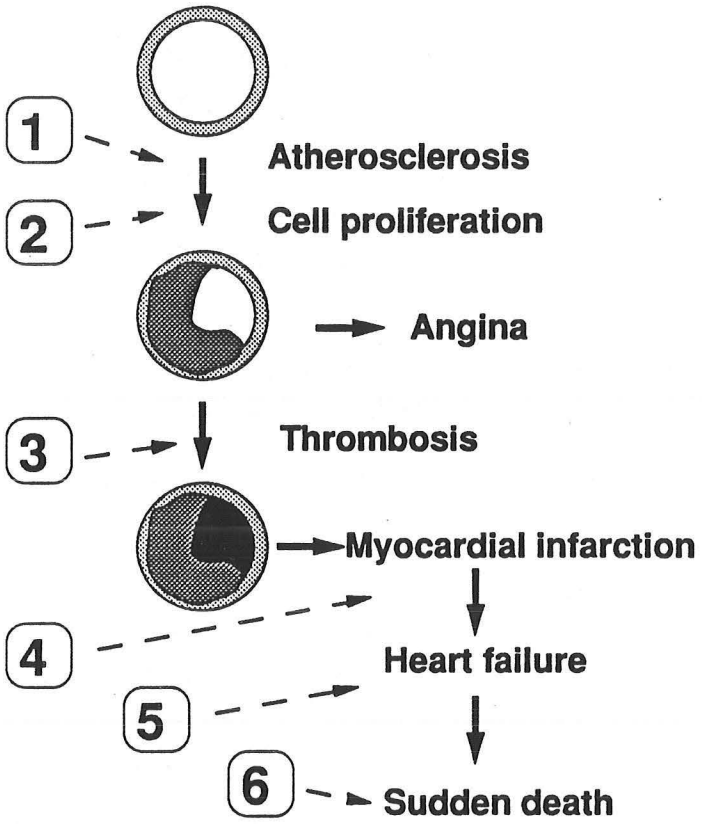

Fig 1. Opportunities to intervene in the natural history of ischemic heart disease by gene therapy. The cartoon illustrates progression of vascular occlusion seen in cross-section of a large epicardial coronary artery, with its resulting clinical sequelae. Gene therapy efforts may plausibly be directed at any one of several steps (1-6) in this process.

diabetes. Gene transfer methods may enhance current approaches to treatment of established atherosclerotic coronary artery disease by reduction of reocclusion following thrombolytic therapy, or by inhibition of acute or late restenosis after angioplasty or vascular surgery. The introduction of foreign genes may be used to enhance development of collateral blood flow to ischemic regions, to redress abnormalities of microvascular function, or to limit cellular damage produced by transient ischemic episodes. Congestive heart failure resulting from ischemic heart disease also may become a target for gene therapy. Foreign genes introduced into vascular endothelial cells or cardiomyocytes could promote improved contractile function. It is even possible now to consider genetic manipulations leading to regeneration of myocytes within the ventricular wall after myocardial infarction.

If we are to utilize gene therapy for these ambitious purposes, several basic problems must be solved: what genes do we use; how do we deliver them to the the appropriate cell target; how do we regulate the activity of the foreign gene in the desired manner; and how do we avoid doing harm in the process?

Let's begin with the question of how we might deliver genes to the cellular targets appropriate for addressing each of the therapeutic goals outlined previously. As shown in

the cartoon, efforts to eliminate risk factors for atherosclerosis can be focused primarily on hepatocytes, while efforts to alter cellular proliferation, thrombosis, or microvascular tone focus on endothelial cells or medial smooth muscle cells of blood vessels themselves. Some of our more fanciful schemes designed to alter susceptibililty to ischemic injury or to enhance contractile function of the heart focus on the cardiomyocyte.

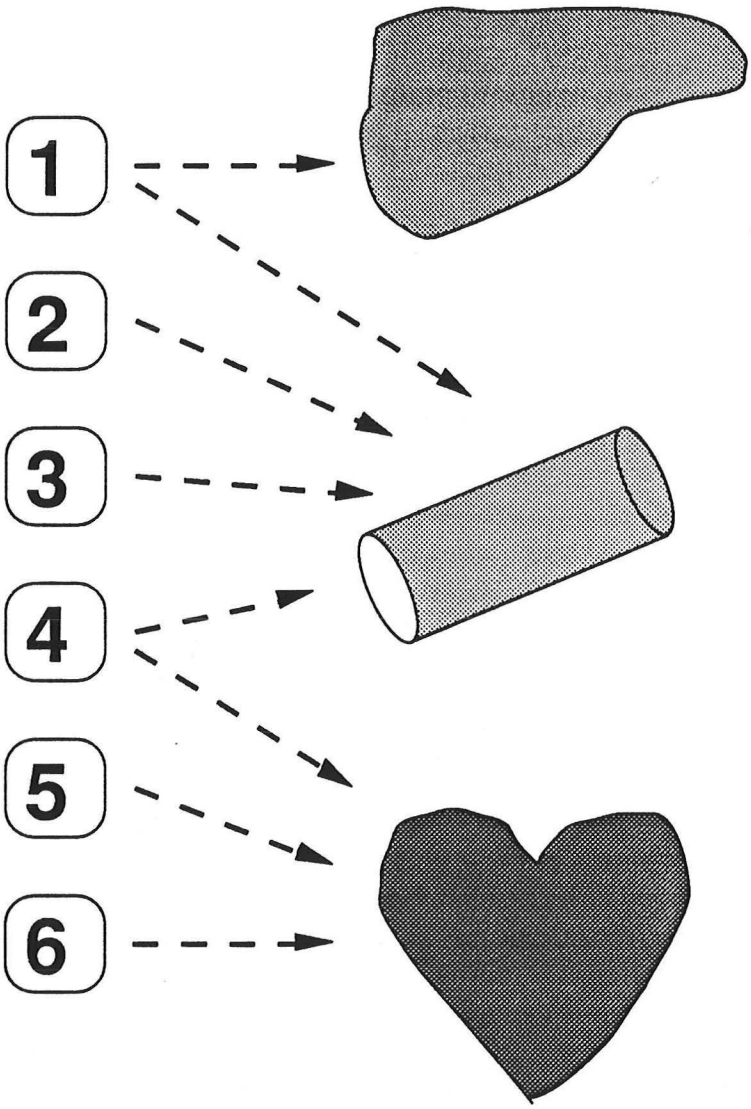

*Fig. 2. **Tissue targets for gene transfer in therapy of ischemic heart disease.** Specific preclinical protocols depend upon efficient gene transfer to liver, blood vessels, or the cardiac wall.*

Gene transfer technologies potentially applicable to humans

Several methods are now available to express foreign genes in tissues of living animals, and thus have potential application to human gene therapy.

Gene transfer methods

- cell transplantation
- viral vectors
  - retrovirus
  - adenovirus
  - adeno-associated virus
  - other
- physical methods
  - native or enhanced endocytosis
    - naked DNA
    - DNA-containing
    - macromolecular complexes
  - microprojectile bombardment (DNA gun)

Cell transplantation: This approach requires three steps: removal of cells from the body, introduction of foreign genes into these cells while they are maintained in tissue culture, and finally, re-implantation of these genetically engineered cells back into the body. This strategy was the first to reach the stage of clinical trials, in an effort to correct immune defects resulting from adenosine deaminase deficiency, using bone marrow as the target tissue . This approach also holds promise for gene therapy of cardiovascular diseases. A clinical trial for reduction of hypercholesterolemia resulting from genetically based defects in the hepatic low density lipoprotein (LDL) receptor has been approved, and relies on transfer of a functional LDL receptor gene into hepatocytes, with subsequent injection of the genetically engineered cells into the portal vein and colonization of the liver (reviewed in Miller, 1992).

Other potential applications of gene therapy for cardiovascular disease include injection of genetically engineered skeletal myoblasts into the myocardial wall or into skeletal muscle for the purpose of secreting a foreign gene product into the circulation or to induce a local effect (Barr, 1991). Likewise, vascular endothelial cells can be harvested, genetically modified in cell culture, and returned to the intact animal on the surface of vascular stents, synthetic grafts, or other bioprostheses (Wilson, 1989; Dichek, 1989).

Gene transfer with viral vectors: A variety of mammalian viruses have been engineered to function as vectors for transfer of foreign genes into somatic cells. This method exploits the highly efficient, receptor-mediated, uptake of virus into target cells, and is aided by other features of the viral life cycle that have been established through evolution. These may include facilitiation of uncoating and nuclear uptake of the viral genome, or integration of the viral genome into chromosomal DNA of the host cell. Viruses used for gene transfer are genetically modified not only to accept insertion of the foreign gene of interest, but to be incapable of sustaining viral replication in the target cells. Homologous recombination and packaging of the viral genome into infectious particles is achieved only in highly specialized cell lines created for this purpose. Because the viruses used for gene transfer have been disabled, the term "transduction" rather than "infection" is used to refer to the process by which the viral genomes enter the target cells.

Retroviruses have been used widely for gene transfer (Dichek, 1989; Zwiebel, 1989; Nabel, 1989), and have features that are attractive for therapeutic applications. Under the appropriate conditions, retroviruses have a very high rate of infectivity (up to 90-100%), and several convenient packaging systems are available. Retroviruses can have a highly specific host range: a specific viral strain may only infect cells of a specific species or a specific cell type (e.g. T lymphocytes). Transduced cells manifest a high frequency of integration of the provirus (including the foreign gene of interest) into the host chromosome.

The versatility of retroviral vectors for gene therapy strategies is limited, however, by several considerations. Only small segments of foreign DNA can be inserted into the retroviral genome and strong transcriptional control signals from the viral promoters (within the long terminal repeat regions) may override cell-specific or inducible promoter elements included with the foreign gene. More importantly, only cells that are rapidly dividing are suitable targets for retroviral infection (Miller, 1990). Thus, retroviral vectors cannot be used to express foreign genes in post-mitotic cells such as cardiomyocytes or neurons, and appear to be quite inefficient for transduction of endothelial cells in situ. Although cultured endothelial cells are readily infected with recombinant retrovirus, the infectivity rates observed when blood vessels are exposed in situ to high titer retroviral suspensions appear to be vanishingly low ($<1 \times 10^{-5}$) (Flugelman, 1992; Meidell, 1992). Retroviruses also present potential risks of reactivation of latent endogenous proviruses or of inducing malignant transformation in target cells due to activation of proto-oncogenes located near to the site of chromosomal integration (insertional mutagenesis).

These limitations of retroviruses as gene transfer vectors have led to the ascendance of recombinant **adenovirus** as a very promising vehicle for gene transfer into animal tissues in situ (Ghosh-Choudhury, 1986; Berkner, 1988). The genomes of adenoviruses currently used for gene transfer will accept inserts up to 7 kbp and additional engineering of the viral genome ultimately may permit transfer of larger and multiple foreign gene inserts. Except in special cell lines used for packaging, the viral genome is transcriptionally inert, thereby limiting transgene expression to that controlled by promoter elements included with the

foreign DNA insert. Construction and packaging systems can produce viral stocks with up to 1011 infectious units per ml. The host range of adenovirus is broad - strains currently in use will transduce most mammalian cells with high efficiency. Finally, adenovirus will transduce non-dividing cells, and transgenes are expressed at high levels without a requirement for cell division or chromosomal integration.

Construction and Packaging of Recombinant Adenovirus

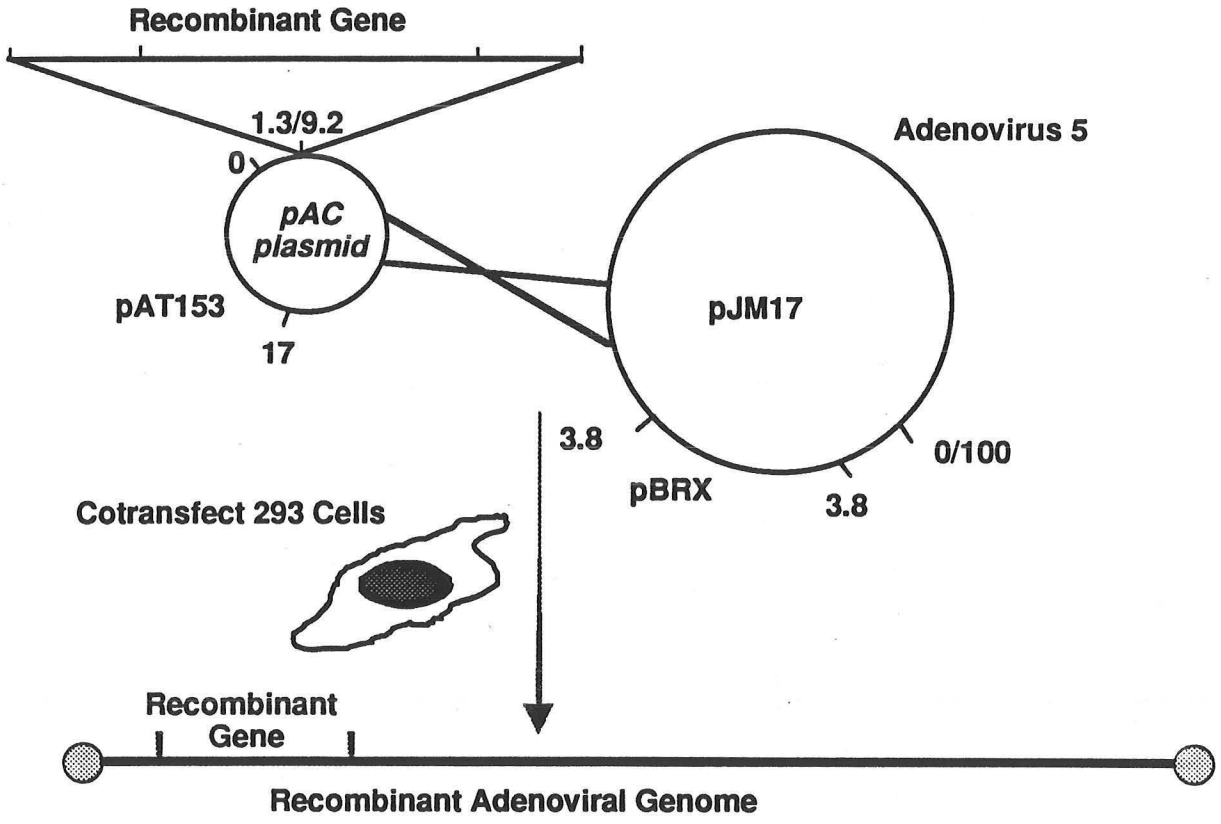

Fig 3. Construction of recombinant adenovirus by homologous recombination in 293 cells. This cell line has been genetically engineered to supply the viral gene product (E1A) required for expression of adenoviral genes.

Recombinant adenovirus has been introduced into laboratory animals in several ways: (a) direct intravascular injection, (b) intramuscular injection, (c) instillation into surgically occluded vascular segments, and (d) instillation into the vessel wall via perforated balloon catheters. All of these approaches show some promise. Direct intravenous or intracardiac injection of high titer viral isolates produces high level expression of selected transgenes in liver and spleen, with lower levels of expression in the heart and other tissues. Intramuscular injection leads to high level expression of foreign genes locally at the site of injection in both cardiac and skeletal muscle. The efficiciency of transduction by

adenovirus in intact tissues may be improved by ancillary measures that delay diffusion of viral particles away from the site of introduction.

Routes of administration of gene transfer vectors

Intravenous injection
- Specificity of transgene expression acquired through:
  - cell-specific delivery
  - cell-specific promoter
  - function of transgene product

Local administration
- regional perfusion
- needle injection
- microprojectile bombardment

Despite this progress, many questions concerning the efficacy and safety of this approach to gene transfer remain to be answered through pre-clinical testing. Over what duration will transgenes continue to be expressed at levels sufficient to induce biologically relevant effects? What is the frequency of chromosomal integration of foreign gene sequences as compared to stable maintenance of episomal DNA or degradation and loss of the viral DNA? How significant is the theoretical risk for activation of latent endogenous viruses? What are the immunological responses of the host following virus injection? Can we generate new strains of adenoviral vectors with superior features?

This last question is of special interest to investigators within our Molecular Cardiology group at this institution. Bob Gerard and colleagues have purified and cloned the adenoviral protein (knob) responsible for binding of the adenoviral particle to a receptor on the surface of mammalian cells. They have crystallized this protein for precise determination of its molecular structure. Further work on knob may expand the versatility of recombinant adenovirus as a gene transfer vector in several ways. Purified knob provides an invaluable reagent for identification of the adenovirus receptor, a more detailed understanding of which may provide opportunities to enhance the efficiency of adenoviral gene transfer. Moreover, strategic or random mutagenesis of knob may permit construction of adenoviral vectors that specifically transduce certain cellular targets while sparing other cells.

Physical methods for gene transfer: A variety of methods for gene transfer into animal tissues rely on physical perturbations of the cell membrane to permit entry of for-

eign DNA into the cell, which is followed by transport or diffusion of DNA to the nucleus and expression of the foreign gene. Such methods range from the shockingly simple - direct injection of plasmid DNA requiring only a tuberculin syringe - to more elaborate schemes that require specialized instrumentation.

Striated myocytes, including both skeletal muscle fibers and cardiomyocytes, have the unique ability to take up DNA from the extracellular space after simple intramuscular injection of concentrated DNA solutions (Acsadi, 1991; Lin 1990). The basis for this effect is unclear, though it may require mechanical stretch of the sarcolemmal membrane. Expression of transgenes can be sustained - up to several months. We have used this method in our laboratories, and generally confirm the published findings. At this time, however, enthusiasm for this method is tempered by several issues. Despite scrupulous attention to experimental technique, the results of direct injection of plasmid DNA can be excessively variable. Injection of the same DNA solution by the same operator into two muscles of the same animal has yielded levels of transgene expression that differ by 1000-fold. In addition, this method may produce cell injury or inflammation, and can induce cardiac arrhythmias. These considerations, and greater success with alternative methods, lead us to favor other approaches at this time.

Incorporating DNA into lipid vesicles increases the range of cell types that will take up foreign genes from the extracellular space, and other variations on the theme of including DNA within macromolecular complexes to facilitate uptake continue to be developed (Felgner, 1987; Wagner, 1990). Lipid-DNA complexes have been introduced into vascular segments to deliver transgenes into endothelial cells (Lim, 1991), but virally mediated gene transfer techniques appear to be superior for gene transfer into muscle, endothelial cells, or liver.

Another physical method for gene transfer relies on propulsion of DNA-coated microprojectiles across the cell membrane. This approach was utilized initially for transgene expression in plants and lower eukaryotes, including genetic transformation of mitochondria and chloroplasts (Johnston, 1988). More recently this technique has been applied to mammalian cells, including tissues of living animals (Williams, 1991). This method requires special instrumentation but otherwise is as simple as the direct injection of plasmid DNA. Plasmid DNA bearing the gene of interest is precipitated onto the surface of gold microparticles (approx. 1 uM in diameter) and spread on the surface of a rigid plastic disc. The disc is placed within the apparatus and accelerated with a shock wave of expanding helium gas. The plastic disc travels toward the exit port of the apparatus where a wire grid halts its progress but permits the DNA-coated microprojectiles to exit and strike the target tissue. The plastic disc also serves to seal the exit port so that the helium gas does not escape from the apparatus, thus minimizing trauma to the tissue.

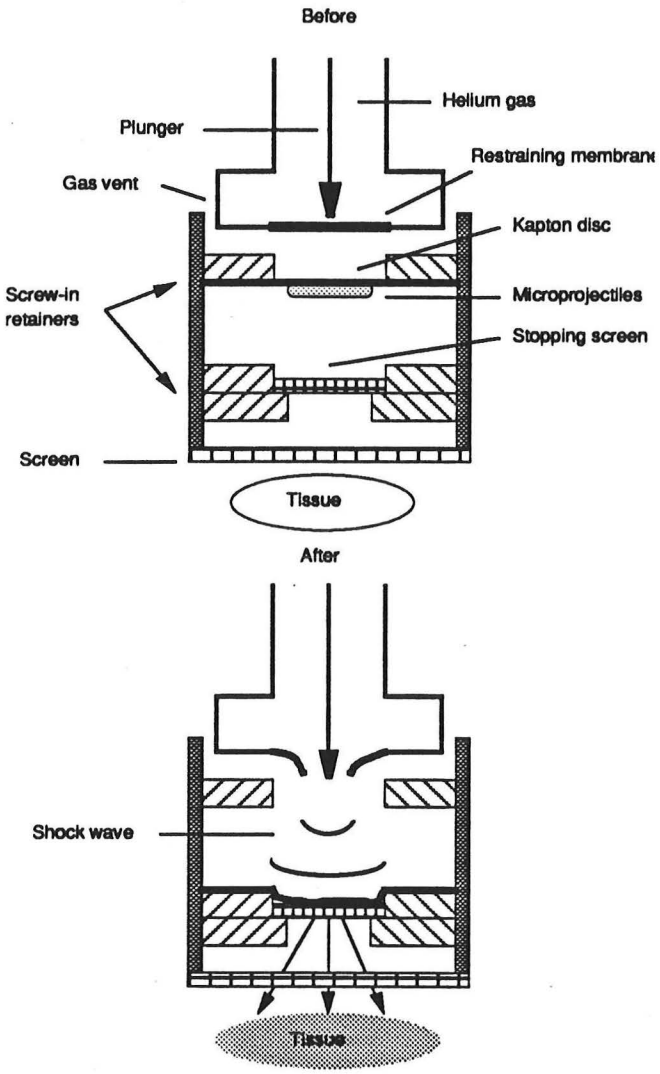

Fig. 4. Principles of operation of the DNA gun.

This approach permits delivery of foreign genes either to the skin or internal organs of anesthetized mice. At the current stage of development, expression of transgenes is present at high levels for several days and detectable for several weeks. With attention to particle size and velocity appropriate for a given tissue, tissue damage or inflammation is minimal or absent. We regard this technology as promising for gene therapy applications in which transient expression of a foreign gene within a spatially localized region of tissue is adequate to produce the desired effect. For example, Stephen Johnston and colleagues have employed microprojectile bombardment to produce "genetic immunization" of mice against foreign antigens (Tang, 1992). Introduction of a foreign gene into skin of the mouse ear leads to detectable levels of antibodies directed against the foreign gene product that appear with a time course resembling that produced by injection of protein antigens. Delivery of the antigen as the product of a transgene on a second occasion acts similarly to a conventional booster injection, rapidly inducing higher titers of antibody.

Gene therapy of ischemic heart disease: current pre-clinical protocols at this institution

The preceeding section has illustrated the increasingly varied repertoire of techniques available for delivering foreign genes to cells and tissues that are pertinent to the pathophysiology of ischemic heart disease. These technologies remain suboptimal but are sufficiently developed to permit pre-clinical testing of specific transgenes that may be useful for the therapeutic goals that were previously described. A variety of projects are underway at this institution, and the principal investigators involved in these studies have been kind enough to let me include unpublished findings in this review.

The efforts I will describe have emanated from our Molecular Cardiology group, but are expanding to include collaborators from several other departments. The multidepartmental nature of this effort has led recently to the organization of a Gene Therapy Working Group, an institutional effort led by Stephen Johnston, Chris Newgard, and myself, with the strong support of Dr. Foster.

Forced expression of the LDL receptor (LDL-R) in hepatocytes of normal and hypercholesterolemic mice: Joachim Herz and Bob Gerard have forced expression of the human LDL-R in hepatocytes of normal mice, and of mice with severe hypercholesterolemia resulting from a homozygous null mutation of the mouse LDL-R gene. The transgene was delivered by intravenous injection of recombinant adenovirus. In normal mice, increasing expression of the LDL-R leads to a marked (>4-fold) increase in the rate of LDL clearance from plasma (Herz and Gerard, 1993). Hypercholesterolemic mice treated similarly have died within days, apparently from acute hepatic failure due to fatty infiltration. It appears that massive and rapid overexpression of the LDL-R in these animals presents the hepatocyte with an overwhelming burden of LDL that the cell cannot metabolize. Nevertheless, the dramatic biological effect of this intervention suggests that this approach has potential clinical relevance. Current efforts focus on: (1) titrating expression of the LDL-R to normalize plasma LDL without hepatotoxicity, (2) assessing the duration of transgene expression, and (3) scaling up the gene delivery system to larger animals.

Forced expression of apolipoprotein AI (ApoAI) in hepatocytes and endothelial cells of laboratory animals: Bob Meidell and colleagues also have employed an adenoviral vector to deliver the human apoAI gene to hepatocytes of normal mice. Forced expression of this transgene results in secretion of human apoAI into plasma to levels roughly equal to that of the endogenous mouse protein, and effects a 25% increment in plama levels of high density lipoprotein cholesterol. Current efforts focus on: (1) assessing the consequences of this degree of elevation in plasma HDL-C on atherosclerotic lesions in mouse models; (2) assessing the effects of local delivery of an ApoAI transgene into the vessel wall within atherosclerotic lesions.

Inhibition of the fibroproliferative response induced by vascular injury: Restenosis occurs in 25-30% of coronary atherosclerotic lesions treated by percutaneous intervention, irrespective of the device employed (balloon angioplasty, atherectomy, or laser angioplasty). Extensive efforts to identify pharmacologic strategies to retard the aggressive proliferation of smooth muscle cells that accounts for this process have been largely unrewarding (McBride, 1988; Klein, 1990). Thus, prevention of restenosis following percutaneous intervention presents an attractive target for gene therapy proprosals, particularly if a catheter-based gene transfer system could be amended to the primary procedure. A variety of dominant negative mutational schemes have been proposed and are being tested for the purpose of inhibiting smooth muscle proliferation. Many of these rely on generation of anti-sense RNA to inhibit synthesis of peptide growth factors, receptors for peptide growth factors, and proteins (often proto-oncogene products) that are components of intracellular pathways of signal transduction that are triggered by peptide growth factors.

John Willard and colleagues are expressing antisense RNA complementary to basic fibroblast growth factor (bFGF) mRNA within the vessel wall as a potential countermeasure to combat the cellular proliferation that occurs as a consequence of vascular injury, such as that induced by balloon angioplasty. Recent work by investigators at other centers suggests other genes that may be appropriate targets for dominant negative strategies to block occlusive cellular proliferation.

Enhanced local fibrinolysis at specific vascular sites: Acute coronary occlusion usually is the result of thrombus formation at a site of atherosclerotic disease, thereby precipitating myocardial infarction (DeWood, 1980). Blood flow can be restored, and injury to the myocardium reduced, by adminstration of agents that promote fibrinolysis (Guerci, 1987; ISAM Study Group, 1986; White, 1987). Tissue plasminogen activator (tPA, the activator of endogenous fibrinolysis) is effective for this purpose when delivered in high concentrations as a pharmacologic agent. Increased local expression of tPA theoretically could augment endogenous fiibrinolytic activity at vascular sites with increased risk for occlusive thrombus formation, without increasing the risk for bleeding at other sites. Such enhanced local thrombolytic activity is an attractive goal for gene therapy protocols based on increasing the secretion and/or activity of tPA following gene transfer into endothelial cells.

While it may prove possible to achieve this goal simply by supplying additional copies of the tPA gene to the endothelial cells, this strategy becomes more robust when the tPA molecule itself has been modified to acquire novel properties. Elegant structure-function studies of the tPA molecule by Ed Madison, Joe Sambrook and others led to the creation of a mutant tPA that is resistant to the serpin PAI-1 that is the major endogenous inhibitor of tPA (Madison, 1989). Conditioned medium from cultured endothelial cells engineered to secrete this inhibitor-resistant form of tPA exhibits more than 500-fold greater thrombolytic activity than medium from control cells. Bob Meidell and colleagues

are introducing transgenes encoding wild-type or mutant forms of tissue plasminogen activator (tPA) in endothelial cells of laboratory animals. Experiments to demonstrate clot lysis within intact vessels are underway.

Limitation of ischemic injury by stimulation of collateral vascular growth: Charles Landau and colleagues at this institution have engineered the bFGF gene into an adenoviral vector and are assessing the effects of this more potent delivery system on vascular growth within cardiac and skeletal muscles. The rationale for this line of investigation has been enhanced further by the recent observation that pharmacologic administration of bFGF reduces infarct size resulting from coronary occlusion in experimental animals (Yanigasawa-Miwa, 1992).

Limitation of ischemic injury by forced expression of heat shock (stress) proteins: Ivor Benjamin is leading efforts to determine the effects of forced expression of selected heat shock (stress) proteins (Benjamin et al. 1992) on the susceptibility of myocardial cells to lethal ischemic injury. The scientific rationale of this project rests on recent studies from our group to indicate that forced expression of hsp70 prolongs the period of time after the onset of simulated ischemia before irreverstible injury occurs in cultured mouse cells. This field of research was reviewed recently at Medical Grand Rounds by Dr. Benjamin.

Regeneration of functional myocardium after myocardial infarction: Perhaps the most futuristic idea within our current portfolio of studies, this goal has been elevated from science fiction to a serious research endeavor by several recent discoveries. First, studies by Field (Field, 1988) and others have demonstrated that it is possible to overcome mitotic growth arrest within cardiomyocytes in the intact animal. Forced expression of large T antigen, a transforming protein from SV40 virus, within atrial or ventricular cardiomyocytes during embryonic development of transgenic mice produces cardiac tumors. Second, a family of genes known as myogenic basic helix-loop-helix (bHLH) proteins have been shown to have the remarkable properties of converting non-muscle cells into skeletal myocytes. This class of genes was discovered independently by several labs, including that of Woody Wright at this institution (wright, 1989).

Without going into complex details of these important discoveries and a vast body of additional research that addresses mitotic growth control and myogenic differentiation, it is sufficient to say that these findings raise expectations that it may become possible to improve contractile function of the failing heart following myocardial infarction by increasing the number of functional myocytes within the left ventricular wall.

Research directed at this ultimate goal is being carried out in the Molecular Cardiology group by Rhonda Bassel-Duby, myself, and our collaborators. The current point of attack is the identification of genes that direct differentiation of cardiomyocytes. We have identified transcriptional control elements within a defined region of the myoglobin gene

that are required for its activation during embryonic development of the heart (Parsons, 1993), and we have begun to identify transcription factors (Bassel-Duby, 1992) that modulate this response.

Current problems

The growing power of biotechnologies available for gene transfer has fostered overt pre-clinical protocols for gene therapy of cardiovascular diseases. Major problems remain to be solved, however, before the promising beginning of the gene therapy era can be followed by significant improvements in clinical care of patients with ischemic heart disease. The technology of gene transfer must continue to improve. Many of the methods described above continue to be hindered by inconsistencies or low efficiencies that are incompatible with the stringent requirements necessary for clinical use. For applications that require long-term or permanent expression of transgenes, countermeasures to eliminate silencing or inactivation of transgenes must be developed. The potential toxicities of each gene transfer system and of specific transgenes proposed for clinical use must be evaluated systematically in pre-clinical testing. The ideal gene transfer system would permit targeting to specific cell types and modulation of expression of the foreign gene, while minimizing risks for oncogenic transformation or cytotoxicity.

Evaluating gene transfer methods in pre-clinical protocols

Duration of transgene expression
- chromosomal integration
- stability of episomes
- promoter activity
- immunological responses

Biological effects of transgene product

Toxic and unwanted consequences
- unexpected effects of transgene product
- activation of latent endogenous viruses
- carcinogenesis
- autoimmune disease

Given the creative effort going into this field, we can reasonably assume that technical capabilities will continue to advance. A reasonable prediction, however, is that the rate at which gene therapy protocols begin to alter clinical management of common cardiovascular diseases is more likely to be limited by the biological complexity of the important pathophysiological events than by deficiencies in the available biotechnologies.

In organizing our program in cardiovascular gene therapy at this institution, we have explicity sought to draw from the respective talents of molecular biologists, virologists, cardiovascular physiologists, and clinicians. It is our premise that the institutions that can most effectively integrate the efforts of such individuals will assume leadership roles as we enter the era of gene therapy. The following diagram illustrates the basic organizational premise on which our program has been constructed.

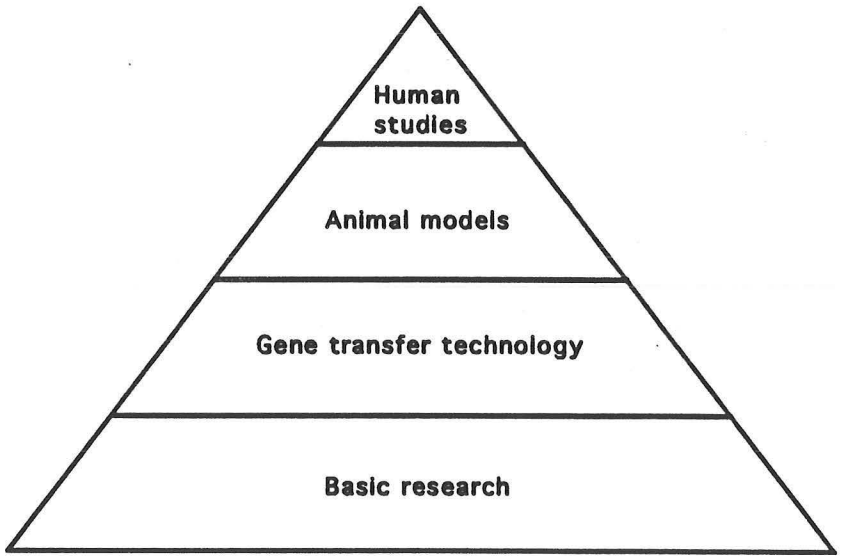

Fig. 5. Basic organization of gene therapy program.

Ethical and regulatory aspects of cardiovascular gene therapy

The approaches to gene therapy proposed in this review present no major departure, in either a conceptual or ethical sense, from traditional precepts of medical practice and human experimentation. When gene transfer methods are limited to somatic cells, as in all of the protocols envisaged here, gene therapy can be construed as conceptually and ethically identical to pharmacologic therapy in which an inactive precursor is administered and the active form of the drug is produced by metabolism within the body. In either case, a foreign substance is introduced and distributed to target cells and tissues. In the case of gene therapy, that foreign substance happens to be DNA, which serves as a precursor for the generation of RNA and protein products designed to have a therapeutic impact, in much the same way as if a foreign protein, peptide, or other chemical were delivered by more conventional means.

All of the approaches to gene therapy we have touched on have potential risks, including the induction of malignancies or autoimmune diseases. However, these same risks are attendent to the use of drugs, blood products, and procedures currently in clinical use. Assessments of relative benefits and risks of gene therapy protocols should be weighed on the basis of empirical data from pre-clinical trials in animal models and from randomized controlled trials in selected patient populations, which should not be hindered by categorical restrictions based on artificial distinctions between gene therapy and other forms of medical treatment.

Although somatic cell gene therapy appears to present no new ethical or moral dilemmas, the propects that gene transfer methods may be used for trivial cosmetic purposes, for military or terrorist activities, or for manipulation of the germline in humans are real and disturbing, and suggest a need for reasonable safeguards against abuse. Safeguards against unethical applications of gene transfer methods ideally would be determined in open political debate among a scientifically literate citizenry. The medical community has a special obligation to educate both itself and the public at large so that such restriction will not hinder legitimate research efforts.

Glossary

A minimal essential lexicon: terminology for gene therapy

construct - a recombinant DNA molecule that includes the protein coding region of a gene of interest, along with other regions of DNA that direct efficient transcription, stability of mRNA, and protein translation within the target cell. Constructs are engineered ("constructed") into specially designed bacterial plasmids so that large quantities of DNA (up to several mg) can be produced and purified conveniently from bacteria in the laboratory. Specific recombinant DNA sequences carried in plasmid constructs can be introduced into the genomes of retroviruses or adenoviruses and packaged into infections viral particles by procedures that require cultured mammalian cells genetically engineered for this purpose.

episome - a recombinant DNA molecule present within the nucleus without integration into the chromosomes of the host cell. Foreign genes located within episomes can be transcriptionally active for extended periods.

germ cell - sperm and egg cells, or their precursors. Transfer of foreign genes into germ cells poses ethical and moral questions that do not apply to gene transfer into somatic cells.

integration - insertion of foreign DNA sequences into chromosomes of the target cell. Such chromosomal insertion enables the foreign DNA to be transferred to daughter cells if the target cell divides, but is not essential for the function of the foreign gene. Some gene therapy protocols require chromosomal integration for success, while others may be successful without integration.

promoter - a region of DNA included within a construct that constitutes a genetic off-on switch by controlling transcription of the gene of interest. Promoters can be "constitutive" (active in all cell types), "cell-specific" (active only is a specific type of cell), or "inducible" (capable of being switched on by specific stimuli, such as hormones). Some promoters are induced by metabolic stresses encountered during myocardial ischemia (Benjamin, 1992).

somatic cell - cells other than germ cells. Most current gene therapy protocols are directed explicitly at somatic cells, meaning that the consequences of gene therapy would affect only the patient, and foreign genes would not subsequently be passed to offspring of the individual undergoing therapy.

transfection - the process of introducing foreign DNA into a cell. When the foreign DNA is introduced after packaging within a replication-defective viral particle, the process may be called "transduction".

transgene - genetic information carried within a DNA molecule that is foreign to the host cell. Transgenes can be active transiently to produce a foreign gene product (RNA and/or protein) and then be lost from the cell or inactivated by a variety of mechanisms, or they can become active on a permament basis.

References

Ascadi, G., Jiao, S., Jani, A., Duke, D., Williams, P., Chong, W., and Wolff, J.A. (1991) Direct gene transfer and expression into rat heart in vivo. New Biologist 3: 71-81.

Barr, E. and Leiden, J.M. (1991) Systemic delivery of recombinant proteins by genetically modified myoblasts. Science 254: 1507-1509.

Bassel-Duby, R., Hernandez, M.D., Gonzalez, M.A., Krueger, J.K., and Williams, R.S. (1992) A 40 kD protein binds specifically to an upstream sequence element essential for muscle-specific transcription of the human myoglobin promoter. Mol Cell Biol 12: 5024-5032.

Benjamin, I.J,. Horie, S., Greenberg, M.L., Alpern, R.J., and Williams, R.S. (1992) Induction of stress proteins in cultured myogenic cells: molecular signals for the activation of heat shock transcription factor during ischemia. J Clin Invest 89: 1685-1689.

Berkner, K.L. (1988). Development of adenovirus vectors for the expression of heterologous genes. Bio Techniques 6:616-629.

DeWood, M.A., Spores, J., Notske, R., Mouser, L.T., Burroughs, R., Golden, M.S., and Lang, H.T. (1980). Prevalence of coronary occlusion during the early hours of transmural myocardial infaction. N Engl J Med 303:897-902.

Dichek, D.A., Neville R.F., Zwiebel, J.A., Freeman, S.M., Leon, M.B., and Anderson, F. (1989). Seeding of intravascular stents with genetically engineered endothelial cells. Circulation 80:1347-1353.

Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M., Northrop, J.P., Ringold, G.M., and Danielsen, M. (1987). Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc. Natl. Acad. Sci. USA 84:7413-7417.

Field, L.J. (1988). Atrial natriuretic factor-SV40 T antigen transgenes produce tumors and cardiac arrhythmias in mice. Science 239: 1029-1033.

Flugelman, M.Y., Jaklitsch, M.T., Newman, K.D., Casscells, W., Bratthauer, G.L., and Dichek, D.A. (1992). Low level in vivo gene transfer into the arterial wall through a perforated baloon catheter. Circulation 85:1110-1117.

Ghosh-Choudhury, G., Haj-Ahmad, Y., Brinkley, P., Rudy, J., and Graham, F.L. (1986). Human adenovirus cloning vectors based on infectious bacterial plasmids. Gene 50:161-71.

Guerci, A.D., Gerstenblith, G., Brinker, J.A., Chandra, N.C., Gottlieb, S.O., Bahr, R.D., Weiss, J.L., Shapiro, E.P., Flaherty, J.T., Bush, D.E., Chew, P.H., Gottlieb, S.H., Halperin, H.R., Ouyang. P., Walford, G.D., Bell W.R., Fatterpaker, A.K., Llewellyn, M., Topol, E.J., Healy, B., Siu, C.O., Becker, L.C., and Weisfeldt, M.L. (1987). A randomized trial of intravenous tissue plasminogen activator for acute myocardial infarction with subsequent randomization to elective coronary angioplasty. N Engl J Med 317:1613-1618.

Herz, J. and Gerard, R.D. (1993) Adenovirus-mediated low density lipoprotein receptor gene transfer accelerates cholesterol clearance in normal mice. Proc Nat Acad Sci USA : (in press).

Johnston, S.A., P.Q. Anziano, K Shark, J.C. Sanford, and R.A. Butow. (1988). Mitochondrial transformation in yeast by bombardment with microprojectiles. Science 240: 1538-1541.

Klein, L.W., and Rosenblum, J. (1990). Restenosis after successful percutaneous transulminal coronary angioplasty. Prog Cardio Dis 32:365-382.

Lim, C.S., Chapman, G.D., Gammon, R.S., Mühlstein, J.B., Bauman, R.P., Stack, R.S., and Swain, J.L. (1991) In vivo gene transfer into canine coronary and peripheral arteries. Circulation 83: 2007-2011.

Lin, H., Parmacek, M.S., Morle, G., Bolling, S., and Leiden, J.M. (1990) Expression of recombinant genes in myocardium after direct injection of DNA. Circulation 82: 2217-2221.

Madison, E.L., Goldsmith, E.J., Gerard, R.D., Gething M-J., and Sambrook, J.F. (1989). Serpinresistant mutants of human tissue-type plasminogen activator. Nature 339:721-724.

McBride, W., Lange, R.A., and Hillis, L.D. (1988). Restenosis after successful coronary angioplasty. Pathophysiology and prevention. N Engl J Med 318:1734-1737.

Meidell, R.S., Gerard, R.D., and Williams, R.S. (1992). The end of the beginning: gene transfer into the vessel wall. Circulation 85:1219.

Miller, A.D. (1992) Human gene therapy comes of age. Nature 357: 455-460.

Miller, D.G., Adam, M.A., and Miller, A.D. (1990). Gene transfer by retrovirus vectors occurs only in cells that are actively replicating at the time of infection. Mol Cell Biol 10:4239-42.

Nabel, E.G., Plautz, G., Boyce, F.M., Stanley, J.C., and Nabel, G.J. (1989). Recombinant gene expression in-vivo within endothelial cells of the arterial wall. Science 244:1342-1344.

Parsons, W.J., Richardson, J.A., Graves, K.H., Williams, R.S., and Moreadith, R.W. (1993) Gradients of transgene expression directed by the human myoglobin promoter in the developing mouse heart. Proc Nat Acad Sci USA 90:1726-1730.

Tang, D., DeVit, M., and Johnston, S.A. (1992) Genetic immunization is a simple method for elliciting an immune response. Nature 356: 152-154.

Wagner, E., Zenke, M., Cotten, M., Beug, H., and Birnstiel, M.L. (1990) Transferrin-polycation conjugates as carriers for DNA uptake. Proc. Natl. Acad. Sci USA 87: 3410-3414.

Wagner, E., Zatloukal, K., Cotten, M., Kirlappos, H., Mechtler, K., Curiel, D.T., and Birnstiel, M.L. (1992) Coupling of adenovirus to transferrin-polylysine/DNA complexes greatly enhnaces receptor-mediated gene delivery and expression of transfected genes. Proc. Natl. Acad. Sci USA 89: 6099-6103

White, H.D., Norris, R.M., Brown, M.A., Takayama, M., Maslowski, A., Bass, N.M., Ormiston, J.A., and Whitlock, T. (1987). Effect of intravenous streptokinase on left ventricular function and early survival after acute myocardial infarction. N Engl J Med 317:850-855.

Williams, R.S., Johnston, S.A., Riedy, M., DeVit, M.J., McElligott, S.G., and Sanford, J.C. (1991). Introduction of foreign genes into tissues of living mice by DNA-coated microprojectiles. Proc Natl Acad Sci USA 88:2726-2730.

Williams, R.S., Johnston, S.A., Meidell, R.S., Willard, J.E., and Gerard, R.D. (1993) Current prospects for gene therapy of cardiovascular disease. In "Genes in medical diagnosis and therapy." Kurstak E (ed.), New York: Marcel Dekker, Inc (in press).

Wilson, J.M., Birinyi, L.K., Salomon, R.N., Libby P., Callow A.D., and Mulligan, R.C. (1989). Implantation of vascular grafts lined with genetically modified endothelial cells. Science 244:1344-1346.

Wright, W. E., D. A. Sassoon, and V. K. Lin. (1989). Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. Cell 56: 607-617.

Yanigasawa-Miwa, A., Uchida, Y., Nakamura, F., Tomaru, T., Kido, H., et al. (1992). Salvage of infarcted myocardium by angiogenic action of basic fibroblast growth factor. Science 257: 1401-1403.

Zwiebel, J.A., Freeman, S.M., Kantoff, P.W., Cornetta, K., Ryan, U.S., and Anderson, W.F. (1989). High-level recombinant gene expression in rabbit endothelial cells transduced by retroviral vectors. Science 243:220-222.