CARDIAC AND PULMONARY REPLACEMENT

EDITORIAL: INTRACORONARY ADENOVIRUS-MEDIATED TRANSFER OF IMMUNOSUPPRESSIVE CYTOKINE GENES PROLONGS ALLOGRAFT SURVIVAL

R. Morton Bolman III, MD

The recently developed science of gene transfer holds great promise for interventions in cardiovascular disease. Under the theory that cytokines and protein products of recombinant genes introduced into a transplanted heart may contribute to beneficial alterations in alloreactivity, such as reductions in allograft coronary artery disease and acute rejection, gene transfer into the adult heart is being investigated. If, for instance, one could introduce gene coding for cytokines known to modulate the host immune response, the transplanted organ may enjoy better survival.[1] Furthermore, such approaches may allow the avoidance or greatly reduced dosing of conventional systemic immunosuppression.

Gene transfer into the adult heart has been achieved in vivo by direct myocardial injection[2] and by intracoronary infusion.[3] To increase the efficacy of gene transfer and the uptake of recombinant deoxyribonucleic acid by target cells, two vehicles for gene transfer have been successfully used. These vehicles are (1) cationic liposomes and (2) viral vectors.[4] Some studies have used these vehicles in combination. In the adult heart, with nondividing cardiac cells, recombinant adenoviral vectors have emerged as the most feasible vehicle for introduction of genes.[5] Previous studies have focused largely on so-called "reporter" genes for demonstration of transgene expression in the study of various transfer strategies.

Brauner and associates,[1] in the December 1997 issue of the Journal, sought to evaluate the utility of intraccoronary gene transfer for therapeutic benefit in cardiac transplantation in vivo. They constructed recombinant adenoviral vectors including each of two immunosuppressive cytokines, viral interleukin-10 (vIL-10) and human transforming growth factor-β_1 (TGF-β_1). Both these agents have immunosuppressive properties and both have been shown to inhibit host responses in allograft models. IL-10 potently inhibits production of IL-2, TNF-α, and TNF-β, and interferon-γ.[6] These effects have a pronounced impact on antigen presentation in the allograft setting. TGF-β_1 down-regulates both cellular and humoral pathways. At physiologic levels, it suppresses production of IL-2, IL-6, and TNF by alloantigen-specific T lymphocytes. Furthermore, B cell proliferation and immunoglobulin production are reduced by this agent.[7]

The authors have previously tested the abilities of these adenoviral vectors to effect expression of cytokine genes in cardiac allografts. Their studies have led to the development of a model for efficient ex vivo intracoronary transfer of the genes during cold preservation. In the current study, after the slow intracoronary vector delivery method, the authors studied the resulting myocardial distribution and magnitude of expression in the heart via a reporter gene, *Escherichia coli* β-galactosidase. Transcription of the cytokine genes was documented by polymerase chain reaction. The functional effects of the incorporation of these immunosuppressive genes was then examined in an attempt to prolong allograft survival in vivo.

The authors demonstrated consistent high uptake of the vectors by the grafts, averaging 81% for all vectors. Furthermore, the cytokine deoxyribonucleic acid sequences could be detected by polymerase

From the Division of Cardiovascular and Thoracic Surgery, University of Minnesota Hospital, Minneapolis, Minn.

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Address for reprints: R. Morton Bolman, III, MD, Division of Cardiovascular and Thoracic Surgery, University of Minnesota Hospital, Box 207, 420 Delaware St. SE, Minneapolis, MN 55455.

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chain reaction in the grafts, confirming successful gene transfer and expression in myocardial cells. There was some difficulty with distribution of the reporter gene and, by analogy, of the cytokine transgenes. Subepicardial regions took up much more of the genes than the mid-wall and subendocardial regions with the slow intracoronary introduction. The authors modified the administration by intermittent external compression of the left ventricle during the slow inflow of vectors, simulating higher pulsatile pressure. When this method was used, there was more even distribution of the reporter gene, although still with a predilection for the subepicardial regions.

In the attempt to determine the functional implications of these incorporated transgenes, allograft survival was investigated in animals receiving the vector only (controls) and in animals receiving the vector with attached cytokines. Heterotopic allografts transfected with the vector alone (controls) maintained mechanical activity for 6.9 ± 0.9 days after transplantation. Mean survival, on the other hand, for the TGF-β–infected grafts was 11.1 ± 1.7 days ($p < 0.0004$ compared with controls). Similarly, IL-10–infected allografts survived for 11.2 ± 3 days ($p < 0.0004$ compared with controls). Furthermore, average rejection scores for the cytokine-infected grafts were significantly lower than for controls ($p < 0.001$).

This and other studies have demonstrated the feasibility of myocardial gene transfer by intracoronary administration. Replication-defective adenoviral vectors are apparently the most efficient vehicle for in vivo gene transfer in nondividing cells such as myocytes, demonstrating several-fold greater transduction than liposomal compounds. The use of viral vectors in vivo raises some safety concerns. However, the type 5 adenoviruses used as vectors have not been associated with any known malignant diseases. Furthermore, this entity has been used for routine vaccination in human beings with no apparent ill-effects. There are two problems limiting the potential usefulness of adenoviral vectors for gene delivery, specifically with respect to transplant applications. First, the newly introduced gene remains episomal and is not incorporated in the permanent genetic makeup of the cell. This results in transient expression of the gene product and short-lived duration of the immunosuppressive effects. The second problem relates to the use of transgenes in the transplant setting relates to initial cellular immune activation and antigen-presenting cell function. These processes occur immediately after the establishment of reperfusion and therefore are outside the time frame of transgene expression. Rather, they occur during the initial transcription and translation of the inserted genes. It will thus be necessary to supplement gene therapy with either conventional immunosuppression or soluble cytokines until the transgene expression of cytokines can occur.

The present study presents a first report of a functioning gene therapy strategy in the setting of heart transplantation targeted to modulation of the allograft response. Acute allograft rejection is shown to be significantly inhibited by the introduction of exogenous immunosuppressive cytokine genes delivered by ex vivo intracoronary gene transfer. Viral IL-10 and human TGF-β^1 genes, attached to adenovirus vectors, were able to be introduced during cold preservation of the heart and resulted in internalization, transcription, and expression of both cytokines within the graft. Allograft survival was prolonged significantly, a result that is intriguing and suggests that future approaches may be applicable to clinical transplantation. Many steps remain before human application can be entertained. However, other authors are investigating systems of gene transfer in settings related to thoracic surgery. Takeda and associates[8] have used a liposome-mediated method to introduce the endothelin-1 gene into rodents and have demonstrated the development of a lesion very similar to bronchiolitis obliterans in these animals. This represents an advance in our understanding of this disease process. Work in our own laboratory by Al-Dossari and coworkers[9] produced the first large-animal model of bronchiolitis obliterans. The use of gene transfer strategies promises to further elucidate the mechanisms of this disease and, very likely, many other diseases. Boasquevisque and colleagues[10] have demonstrated gene transfer into lung allografts using adenoviral vectors. There are many attempts ongoing to explore gene transfer strategies in transplantation, neoangiogenesis, and enhancement of myocardial contractility. The current problems preventing effective application of these therapies to human disease have already been alluded to. Gene transfer is a short-lived therapy, as currently described. There may very likely be immune responses in the host to the vectors that are introduced, further limiting the potential of this strategy. The viruses used as vectors need to be demonstrably safe for use in human beings. Despite these and other concerns, gene transfer holds tremendous promise both as a tool to

investigate disease mechanisms and as a potential novel and very specifically targeted therapy. Another realm of potential utility for gene transfer approaches is the field of xenograft transplantation. In attempts to make pig organs more acceptable to human beings, some investigators have bred transgenic pigs to express human complement inhibitors on their endothelial surfaces. This prevents the activation of complement by immunoglobulin M natural antibodies and thereby abrogates hyperacute rejection.[11] The creation of transgenic animals is time and labor intensive and very expensive. Gene transfer strategies would potentially possess a great appeal for modulation of the xenograft response. The local introduction of complement inhibitors and cytokines could significantly improve xenograft organ survival and help approach the ideal of organ tolerance with reduced requirement for systemic immunosuppression.

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