Cell Transplantation as Future Therapy for Cardiovascular Disease?
A Workshop of the National Heart, Lung, and Blood Institute

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Abstract—Despite the development of improved therapies and the significant advances in the understanding of the basis of disease pathogenesis, millions of Americans continue to live with life-threatening cardiovascular diseases. Recent breakthroughs suggest exciting directions that are likely to produce more effective therapies for the treatment of cardiovascular disease. One such area, cell transplantation (grafting of healthy cells into the diseased heart), holds enormous potential as an approach to cardiovascular pathophysiology. Once thought to be a scientific long shot, cell transplantation is becoming recognized as a viable strategy to strengthen weak hearts and limit infarct growth. The technology could also be used for the long-term delivery of beneficial recombinant proteins to the heart, which is a strategy to complement molecular biology advances and provide an alternative strategy for gene therapy. On August 24, 1998, the National Heart, Lung, and Blood Institute convened a workshop to discuss the current status of this fast-moving line of research and to explore its promise for treating cardiovascular disease. The participants included basic and clinical researchers, with representatives from academic and commercial research settings. The workshop was designed to establish the state-of-the-art and to equate current research with practical clinical application. The group recommended short- and long-term goals to assist in realizing, in the most expedient manner, the potential utility of cell transplantation for the treatment of cardiovascular disease. A summary of the meeting discussions and recommendations for future areas of research is presented. (Circulation. 2000;101:e182-e187.)

Key Words: cardiovascular diseases ■ cells ■ grafting ■ myocytes

Early attempts at muscle cell transplantation were encouraging. Proof-of-concept experiments in skeletal muscle beds demonstrated that engrafted myoblasts could differentiate and fuse with the host myotube.1,2 Preliminary experiments with cardiac tissue established that minced adult newt ventricular tissue could reorganize into a contractile mass when affixed to the apex of an injured heart.3 Subsequent studies in rats indicated that minced fetal atrial tissue could form stable, contractile grafts in ectopic skeletal muscle beds.4 A series of breakthroughs in the last few years demonstrated that dispersed preparations of either skeletal myoblasts or cardiac myocytes were stable when engrafted onto donor mouse hearts and, in both cases, donor cells aligned with recipient cells.5,6 Moreover, in the fetal cardiomyocyte grafts, the formation of cell-to-cell contacts, complete with gap junction proteins, was documented.7 More recent work, which is discussed below, has advanced these procedures to larger animal models, and the potential efficacy of cell grafting for myocardial repair in humans has been approached.

In terms of general application, it would seem that fetal cardiac cells constitute the first choice for treatment. On engraftment, they seem to develop into natural components of the host heart and are viable once stably integrated into nonischemic tissue. Other studies show that fetal cardiomyocytes can be engrafted into infarcted or cryoinjured adult hearts and, in some cases, improved cardiac function was noted in engrafted animals compared with nonengrafted controls.8-13 However, fetal cardiomyocytes seem to be highly sensitive to ischemic injury, and their therapeutic use might ultimately require additional interventions (for example, treatment with cardioprotective genes or drugs). Moreover, human fetal cardiomyocytes are difficult to obtain and are limited with respect to their ability to be amplified in culture. The use of fetal cardiomyocytes may also face ethical and political difficulties in human application.

Skeletal myoblasts have many desirable traits as donor cells, including the ability to be amplified in an undifferentiated state in vitro and to remain viable in ischemic tissue. Continued proliferation in vivo (to a point) may be an advantage when engrafting into an injured heart in that the input of a smaller number of cells would ultimately give rise to a large graft. In addition, a gradual increase in graft size in
situation where the engraftment of a large muscle mass would permit concomitant angiogenesis, which in turn would enhance graft viability. Although the uncontrolled growth of donor myoblasts would be detrimental, at least one study indicates myoblast division stops a week after engraftment.

Exciting recent data have shown that skeletal myoblasts engrafted into cryoinjured hearts successfully seeded the lesion and formed nascent myotubes in rats, dogs, and rabbits. Rather than forming scars, the necrosed areas healed with new, fatigue-resistant, slow-twitch muscle. The engrafted regions tolerated cardiac-like duty cycles when examined in vitro. More recently, improved cardiac function (as determined by echocardiographic analysis) was observed in engrafted, cryoinjured rabbit hearts when compared with nonengrafted controls. Given our well-established capacity to amplify primary myoblasts from humans, the potential use of skeletal grafts for treating heart disease has generated considerable interest.

Stem cell–derived myocytes are being pursued as a surrogate source of donor cells. It is well established that totipotent murine embryonic stem (ES) cells can be differentiated into a variety of cell lineages in vitro, including cardiac myocytes. Recent studies detail relatively simple genetic selection protocols to generate pure cultures of cardiomyocytes suitable for transplantation. The isolation of pluripotent human ES cell lines raises the possibility that analogous selection schemes might be used to obtain cells for therapeutic intervention in patients. Recent advances in the field of nuclear transfer raise the possibility that autologous ES cell lines can generate autologous donor cells. The isolation of cardiomyocytes from nontotipotent stem cell lines has also been reported. For example, it is well established that cardiomyocytes differentiate from embryonic carcinoma cell lines. Also, a recent study shows the isolation of a cardiogenic cell line from rat bone marrow. Other studies suggest cardiomyocytes can be differentiated in vitro from a precardiac mesodermal cell line derived from quail. Thus, several surrogate sources of donor cardiomyocytes have already been identified.

In addition to contractile (ie, muscle) cells, other types of cells may impart a benefit when delivered to injured or weakened areas of the heart. For example, it is currently uncertain whether cells transplanted for the purpose of limiting infarct growth need to be myocytes, as opposed to any engraftable cell. Indeed, preliminary findings using smooth muscle cells support this notion. Moreover, implanted cells engineered to secrete proteins encoding cardioprotective activity could be of benefit to a diseased heart. Other cells, such as those comprising the vasculature and peripheral nervous system, may need to be engrafted directly to ensure appropriate cardiovascular function.

Thus, it remains to be established which cell types—cardiac myocytes, skeletal myoblasts, or noncontractile cells—best suit the needs of injured or weakened hearts. Almost assuredly, this choice will be dictated largely by the nature of the injury being treated. Additional work will be required to determine the precise procedures to deliver and maintain appropriate cell populations. Major questions must be addressed before attempting cell transplantation in humans. Of primary concern is the potential need for immune suppression to facilitate the long-term survival of engrafted cells. Most experimental approaches used to date have employed syngeneic or autologous donor cells, which obviates the need for chronic immune suppression. However, it is clear that treatment with cyclosporine and corticosteroid is sufficient to block the short-term (6 weeks) immune rejection of nonautologous fetal cardiomyocytes in a canine model.

Other issues must also be addressed. For example, which other pharmacologic agents are required to develop and maintain the graft? What is the interface of the transplant with cardiac pacemaking? Will the number of implanted cells sufficiently impact function? Would a relatively large area of repair require other procedures, such as revascularization? How can cell grafting halt the progression of heart failure? Can cell transplantation be facilitated by integrating cells with biological, synthetic, or biomimetic scaffolding? These issues require resolution before cell grafting is applied to patients.

Issues and Discussions

The demonstration that donor myocytes survive after engraftment into adult hearts and cardiovascular environments is a milestone on which new advances are building. Current studies are revealing information that will improve the function and endurance of the graft. However, progress is slow because of a lack of understanding of the factors that impact donor cell viability, differentiation, integration, and function. The goal of the workshop was to discuss the issues facing researchers and clinicians interested in the potential clinical application of cardiovascular cell transplantation. The workshop was divided into 3 sessions, as outlined below.

Approaches to Cardiac Grafts

The first session focused on general issues that impact the use of either fetal cardiomyocyte or skeletal myoblast donor cells. As indicated above, the choice of donor cell type is dictated largely by the desired end point. For example, if the goal of the graft is to increase the number of functional myocytes and thereby improve ventricular ejection, cardiac myocytes (of fetal or stem cell origin) would likely be the donor cell of choice. However, for cardiomyocyte engraftment to be efficacious, a complex series of parameters must be met, including appropriate alignment with host cells, terminal differentiation of the donor cells, and proper excitation/contraction coupling with the host myocardium. On the other hand, if limiting the growth of a cardiac infarct is the desired end point, the demands on the donor cell are less stringent and, consequently, the use of other cell types may suffice. Indeed, existing data already indicate that skeletal myoblasts engrafted into damaged hearts differentiate into myotubes and seem to limit infarct growth.

Several reoccurring issues were noted, regardless of the donor cell employed. For example, the acute donor cell death that occurs immediately after engraftment is thought to have a major negative impact on the ensuing graft size. It will be imperative to identify the basis for this cell death (ie, necrosis or apoptosis) and to develop strategies aimed at limiting the process. It is also not clear what constitutes the optimal...
context of donor cells (for example, disaggregated cells, tissue “chunks,” tissue slices, or cells seeded on inert matrices). The context of the engrafted cells will impact the method of delivery, cell survival, and composition of the graft. Clearly, the initial size of the graft will determine the viability of the cells. Thicker tissue grafts, for example, will likely require greater vascularization compared with smaller grafts comprised of dispersed cells. Although several studies indicate that angiogenesis, including the appearance of large blood vessels, occurs spontaneously within or near the grafts, this was not universally observed.

Another important issue is to develop methodologies to control donor cell growth and differentiation. Further understanding in this area could allow for regulated growth of the grafts. In the case of fetal cardiomyocyte grafts, donor cells that couple to the host myocardium unexpectedly seemed to withdraw from the cell cycle to a greater degree than those that did not. By extending the proliferative capacity of the donor cells, it follows that larger grafts would be obtained. Indeed this seems to be the case with skeletal myoblast grafts. Primary skeletal myoblasts initially proliferate after engraftment, but then seem to terminally differentiate after about a week. Engraftment with established myogenic cell lines (such as C2C12 cells) typically give rise to larger grafts; indeed, these cells seem to replace entire scars when engrafted into cryoinjured rat hearts. This undoubtedly reflects the enhanced growth propensity of the established cell lines compared with primary cells. However, it should also be noted that established cell lines are oncogenic in some instances, which precludes their clinical application.

Nonetheless, it is clear from these observations that understanding the molecular events that coordinate cell growth and death in donor cells will impact the ultimate size (and, consequently, function) of the graft. The expression of a number of factors that regulate cell cycle and apoptosis is coordinately regulated during both cardiac and skeletal myocyte differentiation, with significant consequences on proliferation and survival. In the case of skeletal myoblasts, the roles of myogenin, p21, and Gax (which seems to be a key regulator of pathways to exit the cell cycle) are under active investigation. The potential to exploit such cell growth and death regulatory factors to enhance proliferation or confer apoptosis resistance to donor cells will likely provide new tools to improve cell transplant efficiency.

Sources of Donor Cells
The second session focused on identifying suitable sources of donor cells. A major obstacle for the clinical implementation of any cell transplantation protocol is to generate sufficiently large quantities of cells suitable for human application. In the case of generating grafts comprised of cardiomyocytes, this may prove to be problematic. Cardiomyocytes have little growth potential in vitro and, as such, the amplification of suitable numbers is not possible with current technologies. Their use may be further limited by ethical and political considerations. A potential solution to this problem entails the development of non-totipotent stem cell lines with cardiogenic potential. Ideally, these cells could be amplified indefinitely in culture and would undergo cardiogenic differentia-

tion when transplanted into a diseased heart. Alternatively, they could be stimulated to differentiate in vitro and then engrafted using approaches analogous to those used for fetal cardiomyocytes. The cultures would also lend themselves to extensive characterization and gene engineering to promote desirable characteristics, such as improved contractile function. Understanding how to control cardiomyocyte differentiation may be a critical step in the progression of cell transplant technology.

The complexity of target organs, such as the heart, indicates a need for varied cell types if the goal is to supplement dead or dysfunctional cells. It may be necessary to engraft cardiomyocytes together with Purkinje cells to ensure electrical impulse propagation. In that regard, it is important to note that the molecular factors regulating cardiomyocyte sublineage differentiation are currently being unraveled. Transcription factors and growth factors that help specify the differentiation of atrial, ventricular, and conduction system cells have recently been identified. These factors could be exploited to generate specific cardiomyocyte sublineages that are tailor-made for a particular application. It should also be noted that the fetal cardiomyocyte grafting studies reported to date used total heart–dispersed cell preparations either directly or after brief in vitro culture. As such, a variety of cell types was present, and it was difficult to know with certainty the distribution of cell types besides the cardiomyocytes required for successful engraftment. It is of interest to note that genetically selected cardiomyocytes from differentiating ES cultures (>99% pure) were competent to form stable grafts. It remains to be determined if the addition of other cell types will enhance grafting efficiency.

Cell sourcing for grafts designed to limit scar expansion may be less problematic. Skeletal myoblasts may provide numerous advantages, one of which is the availability of autologous cells (from the patient’s own leg or back). Autologous transplants would be free of the concerns of rejection, which generally dictate a need for immunosuppressive drugs. Although skeletal myoblasts do not differentiate into cardiac muscle, their use for cellular transplantation may provide a practical resolution, at least for limiting infarct growth. In addition to skeletal myoblasts, preliminary results suggest that the engraftment of other cells, such as smooth muscle cells, may also be satisfactory to arrest infarct expansion. Sourcing these cell types for clinical use is readily accomplished, and the generation of autologous donor cells is possible.

The transplantation of cells across species (xenotransplantation) is also a major effort in progress. Although most current research focuses on whole organ transplantation, the potential usefulness of cellular xenotransplantation should not be overlooked. Recent advances demonstrate that major issues presented in the past are being overcome. Immunogenicity may be resolved by genetically engineering a donor animal, such as the pig, to suppress the expression of cell surface antigens. Xenotransplanted cells offer the potential to be banked, cryopreserved, or combined with other cell types. Although a number of hurdles still exist, such as the possi-
bility that xenotransplanted cells would not survive over practical periods for human use, rapid progress is being made, and the xenotransplantation of porcine pancreatic islets and blood vessels in humans has been successful in a small number of procedures.38

Identification and Development of Interfacing Technologies

The third session focused on interventions that could enhance graft formation and/or function. To perform successful transplants, the donor cells will need to survive for extensive periods of time while simultaneously carrying out specialized functions. It is quite likely that advances in other areas of tissue engineering and bioengineering can be applied directly to the myocardium.

One example is the use of clever biomaterials and biometrics to serve as scaffolds on which to seed and grow desirable cells. Scaffolds can be made from animal small intestine, biopolymers, collagen, and bioactive glass. These substances can be produced in specialized shapes and are being tested for a variety of clinical uses, such as the formation of artificial blood vessels and pulmonary valves. These substances present a substantial array of advantages for cell transplantation. For example, treated bioscaffolds from the intestine seem to be minimally rejected by the host because the scaffold is resorbed over a short period. Preliminary data suggest that sputtering a “mesh” of various cells onto the recipient heart was effective in promoting survival and beating. In other studies, totally synthetic meshes, such as polyglycan polyglycolic acid, were nontrombogenic and seemed to be useful after cell ingrowth to produce cardiac valve leaflets and artery segments. Further work is required to determine how best to achieve the specialized functions of the replaced tissue (for example, how to produce proper stiffness in valve leaflets). However, the recent advances in this area are highly promising.

Attention to vascular support of the engrafted cells is also likely to be of critical importance. Angiogenic growth factors may be required to ensure appropriate perfusion of the new tissue. It is possible that “priming” the damaged area of the heart with such factors may be needed before grafting. Alternatively, the donor cells could be genetically modified to express, constitutively or inducibly, the requisite growth factors. It is of interest to note that the bioscaffolds described above may contain (or synthetic ones can be endowed with) beneficial physiological substances, such as angiogenic factors, neurotrophins, and pharmaceutical agents that enhance cardiomyocyte function.

Finally, the potential to develop engraftment protocols designed to replicate or enhance specialized functions of the heart and blood vessels was discussed. One such example with particular complexity is cardiac pacing. Many advances have been made in achieving pacing using the inorganic technology of device implantation. However, cell transplantation may play a role in replacing existing pacing therapies or treating specific arrhythmias. Cell transplantation could also complement pacemakers or other devices in the treatment of heart failure by supplementing the sinoatrial or atrial-ventricular nodes or improving refractoriness in condi-

Recommendations

These results and discussions illustrate the need for additional work. It is of critical importance to adopt standard assays and reagents to permit the direct comparison of results from different laboratories and in different species. Issues relevant for postgraft monitoring include estimates of the viability of engrafted cells and the surrounding tissue. It is also necessary to determine whether grafted cells function in a predictable way. Finally, we have a great need for molecular markers that would determine the nature of the engrafted cells and their progeny. The discussions yielded the following recommendations.

Short-Term Goals

1. Standardize the assessment of graft success to permit the direct comparison of results of experiments performed in different laboratories and in different species. This could be accomplished by developing a practical protocol to monitor several experimental end points. Thus, it would be useful to establish standardized criteria to (1) quantitate graft cell survival, (2) ascertain the differentiated status of successfully engrafted cells, (3) assess donor/host electromechanical coupling, and (4) determine if grafts have a beneficial impact on heart function.

2. Optimize the methods and postinjury timing for graft cell delivery (intramyocardial injection, intracoronary delivery, and use of biologic/artificial scaffolding). We must determine how the cell dosage and cell proliferation in the graft contribute to ensuing graft size.

Long-Term Goals

1. Determine to what extent cellular engraftment exerts an active effect (ie, contributes to contractile activity) versus a passive effect (ie, prevention of infarct expansion and/or remodeling) on cardiac function.

2. Determine the spectrum of cardiovascular pathologies for which cellular engraftment is beneficial (eg, infarct, cardiomyopathy, pacing applications, and recombinant protein delivery).

3. Determine the advantages and disadvantages of different donor cells (fetal cardiac myocytes, fast and/or slow skeletal myoblasts, and stem cell–derived myocytes) and the utility, if any, for noncontractile (fibroblasts) or vascular smooth muscle cells (ie, in controlling infarct expansion and/or remodeling).

4. Develop strategies to enhance graft cell survival/viability (eg, blocking apoptosis, promoting angiogenesis, and blocking matrix proteases), and determine to what extent the nature of host myocardial injury impacts donor cell survival.

Future Perspectives

The concept of cell transplantation provides a unique opportunity to address the pathophysiology of cardiovascular dis-
ease. Whether cell transplantation will become an alternative to organ transplantation is not resolved. However, the research is producing novel, imaginative concepts, and the indications are that a variety of other uses will emerge from this technology. In concert with other modern technologies, cell transplantation presents some exciting possibilities for disease therapy and research.

For example, cell transplantation could take advantage of advances in gene discovery and developmental biology. Donor cells could be genetically manipulated to improve autologous survival and function and to improve the local environment. Recent studies clearly indicate that the inactivation of the sarcoplasmic reticulum protein, phospholamban, enhances cardiomyocyte contractility, independent of adrenergic stimulation. Donor cells modified in this manner might more efficiently supplement the function of a weakened heart compared with genetically naive donor cells. The transplantation of cells engineered to deliver recombinant proteins could also impact cardiac function (for example, cells could express a growth hormone to promote the thickening of the cardiac wall in idiopathic dilated cardiomyopathy). In addition, selected proteins may be desirable to promote the survival and function of the graft itself. Transplanted cells could also be genetically engineered to serve as an in situ means of monitoring local environments and the course of changes during disease progression. For example, donor cells could contain calcium probes or voltage-sensitive reporter genes to monitor graft and/or heart function. Reporter genes sensitive to oxygen free radicals could be used to monitor the amounts of these potentially harmful molecules in intact tissues.

Despite the exciting advances described, many gaps in current knowledge remain. For example, does angiogenesis occur spontaneously to an extent sufficient to support the graft? Can angiogenesis be augmented? What is the optimal cell source? What will be the role of human ES cells be in this technology? Is the transdifferentiation of embryonic or adult cells possible? At present, the spectrum of cardiovascular diseases amenable to therapeutic intervention via cell engraftment also remains to be established. Future work will determine the sequence of events and the importance of these myriad factors in cell transplantation.

Appendix

**Meeting Participants**

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