

28. Embryonic and adult stem cell therapy

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Stem cells are characterized by the ability to remain undifferentiated and to self-renew. Embryonic stem cells derived from blastocysts are pluripotent (able to differentiate into many cell types). Adult stem cells, which were traditionally thought to be monopotent multipotent, or tissue restricted, have recently also been shown to have pluripotent properties. Adult bone marrow stem cells have been shown to be capable of differentiating into skeletal muscle, brain microglia and astroglia, and hepatocytes. Stem cell lines derived from both embryonic stem and embryonic germ cells (from the embryonic gonadal ridge) are pluripotent and capable of self-renewal for long periods. Therefore embryonic stem and germ cells have been widely investigated for their potential to cure diseases by repairing or replacing damaged cells and tissues. Studies in animal models have shown that transplantation of fetal, embryonic stem, or embryonic germ cells may be able to treat some chronic diseases. In this review, we highlight recent developments in the use of stem cells as therapeutic agents for three such diseases: diabetes, Parkinson disease, and congestive heart failure. We also discuss the potential use of stem cells as gene therapy delivery cells and the scientific and ethical issues that arise with the use of human stem cells. (*J Allergy Clin Immunol* 2003;111:S745-53.)

Key words: Adult stem cells, embryonic germ cells, embryonic stem cells, multipotent cells, pluripotent cells, stem cell, telomerase, telomere, teratoma, totipotent

Stem cells, the progenitors of all body tissues, have the remarkable and critical abilities to exist in vivo in a quiescent, undifferentiated state and to self-propagate. They are characterized initially as being totipotent, pluripotent, or multipotent. During normal embryogenesis, the pluripotent (from the Latin word *pluris*, meaning several or many) stem cells are derived from the early human embryo or from fetal tissue that was destined to be a part of the gonads. These cells have the potential to develop into any of the cells that are ultimately derived from the three embryonic germ layers (endoderm, mesoderm, and ectoderm, Fig 1). Multipotent (tissue restricted) stem cells exist within the specialized tissue, and, at least until recently, were thought to give rise only to cell types belonging to that tissue.

Stem cells may be of embryonic or adult origin. Embryonic stem cells (ESCs) are derived from the inner cell mass of the blastocyst, which forms in the early

Abbreviations used

EGC: Embryonic germ cell
EGFP: Enhanced green fluorescent protein
ESC: Embryonic stem cell
HSC: Hematopoietic stem cell
SCID: Severe combined immunodeficiency

stages of embryonic development (4-5 days after fertilization), before implantation in the uterine wall. These cells can self-renew and are pluripotent.¹ Adult stem cells may be found in many specialized tissues of the body, including the brain, bone marrow, liver, skin, gastrointestinal tract, cornea and retina of the eye, and even the dental pulp of the tooth. In the adult, all these tissues are in a perpetual state of flux, and, even in the absence of injury, are continuously giving rise to new cells to replace those that have worn out.² It had been thought that adult stem cells are tissue restricted, but a number of recent investigations have suggested that they may be more plastic than previously thought. For example, adult stem cells derived from the bone marrow, apart from differentiating into blood and immune cells, have been shown to be capable of transdifferentiation, or differentiating into cells of nonhematopoietic lineages, such as skeletal muscle, microglia and astroglia in the brain, and hepatocytes of the liver.¹ These observations have given rise to the notion that adult stem cells may be as programmable as ESCs, suggesting that they might be extremely useful in clinical medicine. However, other researchers have cast doubt on these studies by showing that stem cells are capable of fusing with somatic cells.^{3,4} In addition, others have been unable to reproduce transdifferentiation of bone marrow stem cells into nonhematopoietic tissues.⁵ Thus the question of whether adult stem cells are truly pluripotent, or able to replicate indefinitely in culture, remains to be answered definitively.

EMBRYONIC STEM CELLS

Beginning in 1998, scientists discovered that ESCs could be isolated from early human embryos, grown in culture for long periods while maintaining a normal karyotype, and made to differentiate into a wide array of tissues and organs derived from all three embryonic germ layers.⁶ These long-term cultures of ESCs have active telomerase and maintain long telomeres, which are markers of proliferating cells.⁷ ESCs have also been successfully isolated from the primordial germ cells of the gonadal ridge of the 5- to 10-week fetus, and these are referred to as *embryonic germ cells* (EGCs).⁸ With con-

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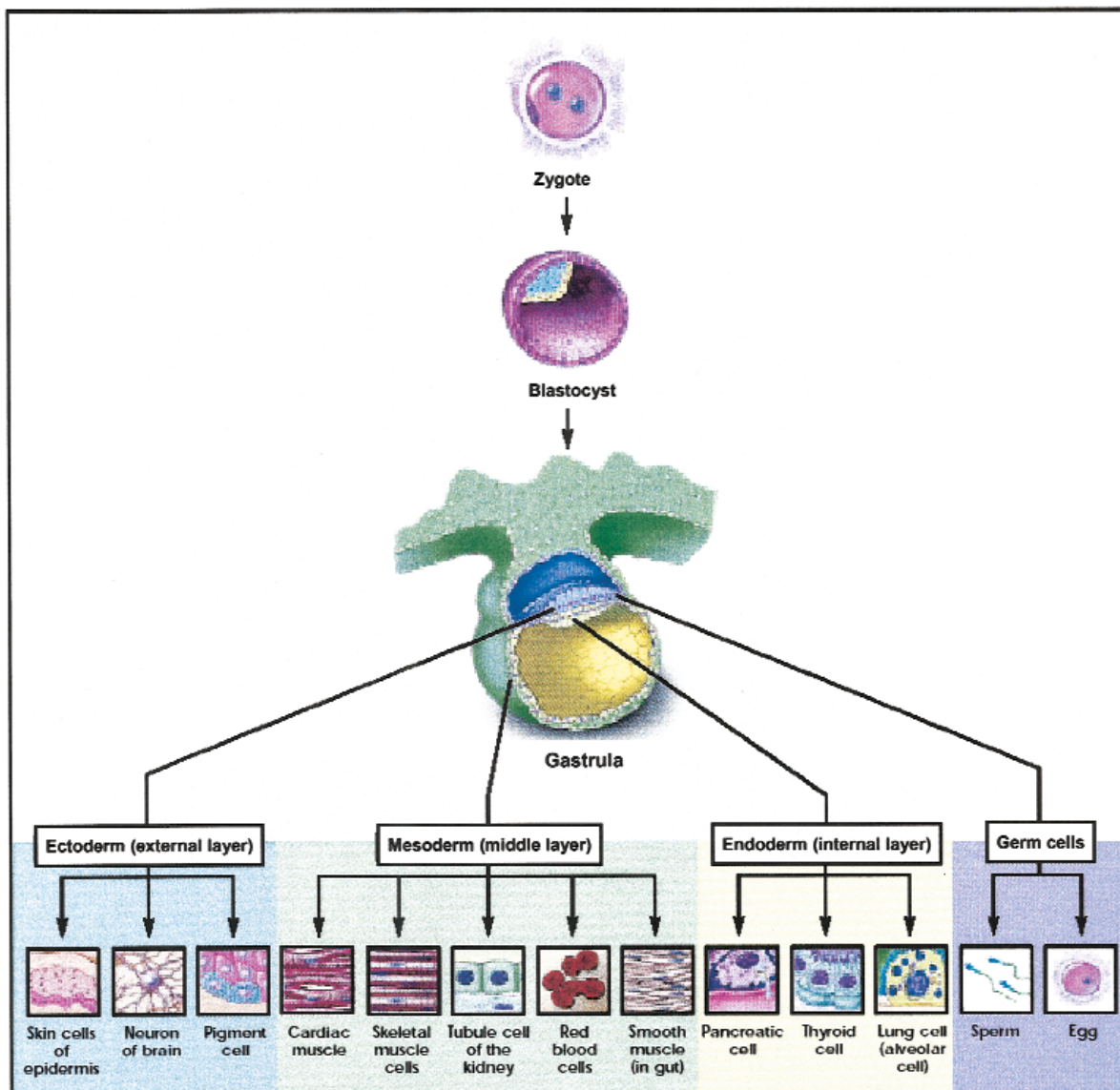


FIG 1. Embryonic origins and developmental potentials of specialized tissues within the human body. Note in particular the gastrula's three main germ cell layers, which develop from the blastocyst (from whose inner cell layer ESCs are derived). Of additional interest, the mesoderm ultimately gives rise to all formed elements of the blood (myeloid and erythroid), as well as the cells of the immune system. Reprinted with permission from Kirschstein R, Skirboll LR. Stem cells: scientific progress and future research directions. National Institutes of Health [online publication] 2001 [cited 2002 Dec 24]. Available from: URL:<http://www.nih.gov/news/stemcell/scireport.htm>.¹

tinued development, the gonadal ridge develops into the testes or ovaries, and the primordial germ cells develop into the eggs or sperm. ESCs and EGCs differ in the conditions required for their isolation, culture, life span in vitro, and differentiation ability. ESCs can proliferate for as long as 300 population doublings and can be passaged for over a year in culture, whereas EGCs can proliferate for only 80 population doublings.^{7,9} Most diploid somatic cells do not express high levels of telomerase and enter replicative senescence after a finite life span of 50 to 80

population doublings in tissue culture.⁹ Thus, properties of the cells of the early embryo, such as normal karyotype and high telomerase activity, are sustained in human ESCs in culture for extended periods.

Stem cell lines have been developed from both ESCs and EGCs. They are capable of self-renewal over long periods and can give rise to many differentiated cell types.⁷ This discovery caused ESCs and EGCs to be widely investigated for their potential to cure diseases by repairing or replacing damaged cells and tissues. One of

the current perceived advantages of using ESCs rather than adult stem cells is that ESCs have the ability to proliferate for long periods in vitro and can be directed to differentiate into a broad range of cell types.¹⁰ ESCs, when removed from feeder layers and transferred to suspension cultures, begin to differentiate into 3-dimensional, multicellular aggregates of differentiated and undifferentiated cells termed *embryoid bodies*. Embryoid bodies are composed of a haphazard collection of precursor and more fully differentiated cells from a wide variety of lineages and resemble early post-implantation embryos.¹⁰ Frequently, within 7 to 14 days of transfer, these embryoid bodies progress through a series of stages that begin as a simple, morula-like ball of cells, eventually forming cavitated and cystic embryoid bodies.⁶ In vitro differentiation experiments have shown that plated cultures of embryoid bodies show a variety of different morphologic types, including rhythmically contracting cardiomyocytes, pigmented and nonpigmented epithelial cells, neural cells with outgrowths of axons and dendrites, and mesenchymal cells.⁹ Recent studies have also demonstrated that embryoid bodies derived from the human ESC line H9 express genes specific for each of the three EGC layers, including α -fetoprotein, neurofilament 68-kd subunit, ζ -globin, and α -cardiac actin marking primitive endoderm, neuroectoderm, and mesoderm derivatives.^{6,11}

The full developmental potential of ESCs is unveiled when these cells are studied in an in vivo environment, such as when they are injected into a host blastocyst.⁷ Human ESCs injected into severe combined immunodeficient (SCID) mice form benign teratomas, with advanced differentiated tissues representing all three EGC layers.⁷ These tissues include highly specialized derivatives of ectoderm, such as neural epithelium, of mesoderm, such as bone, cartilage, striated muscle, fetal glomeruli and renal tubules, and of endoderm, such as gut.⁷ The formation of organized tissues during normal embryogenesis is controlled by many inductive events and complex epithelial-mesenchymal interactions. These events and interactions are seen to occur in teratomas but are less clear in the case of in vitro differentiation.⁹ Because we do not yet fully understand the precise inductive elements that regulate embryonic pattern formation so that we can reproduce such formation in vitro, it would be useful to explore methods of extracting cells and tissues of interest from the heterogeneous teratomas or to direct differentiation in vivo toward a particular lineage. Feasible methods of achieving this include (1) adding certain specific combinations of growth factors or chemical morphogens, (2) co-culturing or transplanting ESCs with inducer tissues or cells, (3) implanting ESCs into specific organs or regions, (4) overexpressing transcription factors associated with development of specific tissues, (5) selecting cells that activate a particular lineage-specific program of gene expression, and (6) isolating cells of interest with fluorescence-activated cell sorting.^{9,11-14} Some of these methods have been used to enrich ESC cultures for a particular cell type of interest in vitro; however, much remains to be done in vivo.⁹

POTENTIAL CLINICAL USES OF STEM CELLS

Stem cells have been proposed to have a number of uses, but the most obvious and potentially best is to restore or replace tissue that has been damaged by disease or injury. Studies in animal models have shown that transplantation of fetal stem cell, ESC, or pluripotent stem cell derivatives can successfully treat many chronic diseases, such as Parkinson disease, diabetes, injury of the spinal cord, Purkinje cell degeneration, Duchenne muscular dystrophy, liver or heart failure, and osteogenesis imperfecta.^{12,15-20} In this review, we highlight recent developments for three of these diseases, diabetes, Parkinson disease, and congestive heart failure, which will serve as examples for the use of stem cells as therapeutic agents.

Diabetes

The ability to culture insulin-producing pancreatic islet cells from ESCs holds great promise for the cure of type 1 diabetes. In theory, a line of ESCs that produce islet cells could be generated. These cells could also be genetically engineered to evade immune rejection.⁹ On a more practical level, it is difficult to induce ESCs to differentiate into a specific lineage. Purification of a single cell type from the initial mixed population is also difficult. Percentages of differentiated cells expressing a single phenotype in this mixture are extremely small, ranging typically from 0.1% to 0.5%.²¹ Only rarely have specific culture conditions and growth factors led to the establishment of cultures containing only a single cell type.^{10,11} Even in these cases, the human pluripotent cell lines retain a broad array of multilineage gene expression profiles, despite the addition of specific growth factors.^{10,11} Methods to circumvent the problem of purification of a single cell type include engineering the cells to use a tissue-specific promoter to drive the expression of either a selectable marker, such as an antibiotic resistance gene,¹² or a gene encoding the green fluorescence protein.²² For the isolation of cells containing insulin,¹² ESCs were transfected with a chimeric construct containing the regulatory region of the insulin gene coupled with the gene encoding neomycin resistance. This was followed by isolating cells that were neomycin resistant. These cells, when cloned and cultured under low concentrations of glucose, underwent differentiation and were able to respond to changes in glucose concentration by as much as a 7-fold increase in insulin secretion. When these cells were implanted into the spleens of mice with streptozotocin-induced diabetes, the researchers were able to reverse the symptoms of diabetes and restore blood glucose concentrations to normal. These results showed that diabetes could be among the first applications of stem cell therapy.

In 2000, Schuldiner et al¹¹ reported that they were able to successfully culture human ESCs to form embryoid bodies that spontaneously expressed *PDX-1*, a gene that controls the transcription of insulin. Assady et al²³ reported that these embryoid bodies contained 1% to 3% beta islet insulin-producing cells. They also found that

the embryoid bodies express glucose transporter type 2 and islet-specific glucokinase genes, both of which are important for beta cell function and insulin secretion. When the cells were cultured with glucose, they were found to secrete insulin. The investigators were hopeful that refining the culture conditions could possibly lead to the differentiation of pancreatic islets.²³

Research efforts have also been directed toward culturing islet cells from adult pancreatic tissue. Differentiated beta cells are difficult to proliferate in culture. However, one group has been able to isolate islet cells from human cadavers and introduce DNA encoding genes that stimulate cell proliferation in culture. The cells were also engineered to stimulate the expression of insulin, because these cells in culture lose the ability to produce insulin. When the cells were transplanted into immune-deficient mice, they produced insulin in response to glucose. Investigations on whether these cells will reverse diabetes in mice are currently ongoing.²⁴ The cells that line pancreatic ducts have been shown to be a potential source of islet progenitor cells, and some studies have shown that when these ductal cells are cultured they can be induced to differentiate into clusters containing both ductal and islet endocrine cells, which are found to secrete insulin when given glucose.²⁵

Diseases of the nervous system

Stem cells have tremendous therapeutic potential as a cell-based therapy to rebuild damaged neurons in such diseases of the nervous system as Parkinson disease, amyotrophic lateral sclerosis (Lou Gehrig's disease), Alzheimer disease, and others. Parkinson disease is a neurodegenerative disease that progresses with age and usually strikes after the age of 50 years. It affects neurons in the brain that secrete the neurotransmitter dopamine. As the neurons die, levels of dopamine being produced decrease, which leads to the movement disorders characteristic of Parkinson disease. Levodopa, which is converted to dopamine in the brain, is the mainstay of treatment for this disease, but most patients acquire tachyphylaxis to its effects over time. In contrast to diabetes, where transplantation of islet cells is a therapeutic option, implantation of fully differentiated dopamine-releasing neurons into brains is not currently possible. Dopaminergic neurons do not survive transplantation, and it is extremely difficult to establish the appropriate connections with their normal target neurons in the striatum.²⁶

It was previously thought that nerve cells in the brain do not divide. In recent years, however, many investigators have shown that stem cells that self-renew and differentiate appropriately into the three major neuronal lineages (neurons, astrocytes, and oligodendrocytes) do occur in the adult mammalian brain.²⁷ Human trials with neuronal tissue from electively aborted fetuses have been conducted, and it was initially reported that they were able to achieve a clear reduction in the severity of the symptoms of Parkinson disease.^{28,29} With positron-emission tomographic scanning, scientists were able to measure an increase in dopamine neuron function in the striatum of

the patients.²⁹ However, these trials were done open label, meaning that both the investigator and the patient knew which patients were the recipients of transplanted tissue. In 2001, Freed et al³⁰ reported the results of the first double-blind, placebo-controlled neural transplantation trial for Parkinson disease. These results were not as encouraging as the initial reports. Patients receiving the transplants showed no significant benefit in a subjective assessment of quality of life, which was the primary end point of the study.^{1,30} However, positron-emission tomographic scans revealed that the transplanted dopamine-neurons survived and grew, giving hope that the procedure might be made more effective by some modification in technique. Another double-blind trial is currently being conducted, and the results are anxiously awaited.¹

Even if the trials noted here prove successful, the use of cell implantation has a number of drawbacks. These include the lack of sufficient amounts of well-characterized and standardized cell material. Further, widespread application will likely be limited as long as access to embryonic donor tissue is required. For these reasons, development of alternative sources of cells for therapeutic purposes is the object of intense investigation. Candidate cells would form a renewable, unlimited source of cells capable of differentiating into functional dopamine neurons. Recently, reports from Zhang et al³¹ and Reubinoff et al³² discussed a potential alternative to the use of fetal tissue transplantation. They developed methods of inducing human ESCs to differentiate into neural stem cells that differentiated in vitro into the three neural lineages. When these neural stem cells were transplanted into the brains of neonatal rodents, they incorporated into the host brain, demonstrated widespread distribution, and differentiated into all three neural lineages. The transplanted cells migrated along established tracks and differentiated in a region-specific manner, which showed their capacity to respond to local cues and participate in the processes of host brain development. Both groups also reported that they observed no evidence of teratoma or non-neural tissue formation in the brains of transplant recipients. Further studies are required to show the functionality of the newly generated neurons. Recent studies reported by Bjorklund et al³³ showed that undifferentiated mouse ESCs transplanted into the rat striatum spontaneously differentiated into dopaminergic neurons that restored cerebral function in the animal. Kawasaki et al³⁴ identified a stromal cell-derived inducing activity that promoted the in vitro differentiation of neural primate ESCs. When these dopamine-producing cells were transplanted into the brains of SCID mice, they were observed to give rise to dopaminergic neurite formation in the target tissue within 2 weeks. Kim et al²⁰ also reported successful results after infecting murine ESCs with a Nurr1 expression construct that allowed increased survival and neuronal differentiation of the transplanted ESCs.

It should be noted that there is much debate regarding how many populations of central nervous system stem cells exist, how they are related, and how they function in vivo. There are currently no identifiable markers that

exist for identifying the neuronal stem cells *in vivo*, and the only way to test if a certain population of central nervous system cells contains stem cells is to manipulate them *in vitro*.³⁵ Many of the transplantation studies that used nonselected ESCs as transplants resulted in spheroid aggregates containing differentiated neurons.³⁶ A selection procedure has been established that allows for the enrichment of nestin-expressing neural precursor cells, which have been shown to differentiate and participate in normal rat brain. Transplantation of selected neural precursor cells has shown better integration into the host tissue.³⁷ Recently, Andressen et al³⁷ genetically engineered murine ESCs to express the enhanced green fluorescent protein (EGFP) under the control of a thymidine kinase promoter and nestin second intron and were able to specifically detect EGFP in nestin immunoreactive neural precursor cells. On transplantation into rat brains, these cells were observed to integrate into the host tissue and served as a pool for successive neuronal and glial differentiation. Thus, a combination of a specific selection protocol for neural precursor cells, together with the specific expression of EGFP in these cells, may offer a valuable opportunity to gain a pure neural precursor cell population for *in vitro* maintenance and expansion, followed by *in vivo* transplantation. Also, fluorescence-activated cell sorting of EGFP-labeled neural precursor cells will be useful for the standardization of a donor cell population for cell replacement therapies.³⁷

Another promising area in stem cell therapy of the nervous system involves the repair mechanism. It has been found that on brain injury stem cells in two specific areas of the brain, the subventricular zone and the dentate gyrus of the hippocampus, proliferate and migrate towards the site of damage.³⁸ Fallon et al³⁹ have shown that transforming growth factor- α , a peptide involved in tissue repair and present in the earliest embryos, induces a wave of migration of stem cells when injected into damaged areas of brain. Of equal interest, these stem cells subsequently differentiate into dopaminergic neurons. Also, it is important to note that treated rats do not show any of the behavioral abnormalities associated with the loss of the neurons. Further investigations on the beneficial effects on Parkinson-like symptoms are ongoing.

The study of stem cell therapeutics for curing Parkinson disease is a good model for nervous system disorders, because it is a relatively easy target involving replacement of only one particular cell type in a single area of the brain. Therapies for other disorders, such as an injury of the spinal cord, in which many cell types are destroyed, face much larger hurdles. Clearly more research needs to be done, but the use of stem cells for treating nervous system disorders is rapidly advancing and appears to hold great promise for the future.

Repairing a damaged heart

Congestive heart failure afflicts millions of people around the world, with 400,000 new cases being reported in the United States every year. Congestive heart failure may result from many causes, including hyperten-

sion, infection, and coronary artery disease. Although many medical and surgical treatments for congestive heart failure are available, the long-term prognosis of these patients remains guarded, with an average life expectancy of approximately 5 years after diagnosis.¹ Transplanting autologous stem cells would have clear advantages to transplanting a heart, because it would obviate the need for a donor and for the requisite immunosuppression needed to suppress rejection of the transplanted heart. Bone marrow stem cells can be driven toward differentiating into cardiomyocytes, vascular endothelial cells that form the inner lining of new blood vessels, and smooth muscle cells that form the walls of blood vessels by using highly specific culture conditions.⁴⁰ Recently, Orlic et al⁴¹ showed that injection of adult bone marrow-derived hematopoietic stem cells (HSCs) into the heart of a mouse that had been induced to have a myocardial infarction led to formation of new cardiomyocytes, vascular endothelium, and smooth muscle cells. Within 9 days after injection, the newly generated myocardium, including coronary arteries and capillaries, occupied as much as 68% of the damaged portion of the ventricle. Mice that received the transplanted cells survived in much greater numbers than did the control mice. These results suggest that the HSCs were able to respond to internal signals within the heart, migrate toward the injured ventricle, and give rise to specialized, differentiated heart cells. Jackson et al⁴² performed a similar study with adult stem cells derived from mouse bone marrow, delivering these cells into the model mice by bone marrow transplantation. They found that after 2 to 4 weeks the survival rate was 26%, with the damaged heart tissue showing the presence of donor-derived cardiomyocytes and endothelial cells. This study provided yet another alternative for the delivery and therapeutic strategy of stem cells for the repair and growth of cardiac tissue. Kocher et al⁴³ took this one step further, demonstrating that human bone marrow-derived adult stem cells, when injected into rats, were able to give rise to vascular endothelial cells, forming new blood vessels.

Human ESCs are also showing much promise for cell-based cardiac therapy. In 2000, Itskovitz-Eldor et al⁶ showed that human ESCs could reproducibly differentiate in culture into cells that exhibited cellular markers and a physical appearance of heart cells. Kehat et al⁴⁰ have also shown that human ESCs can differentiate into myocytes that portray cardiomyocytic structural and functional properties. The next step in this research is to see whether these ESCs can repair and replace damaged heart cells in an animal model.

There is no doubt that adult and ESCs are proving useful in stem cell therapy for replacing damaged heart tissue. However, many questions still remain to be answered. For instance, how many cells would be required to repair a single human heart? How long will the replacement cells continue to function? Do the results from the animal models accurately reflect human heart conditions and transplantation responses? Would it be feasible for at-risk patients to donate stem cells in

advance, so that no time is lost between getting a heart attack and undergoing stem cell transplantation? The answers to these questions are being pursued by many investigators, and when received, they will give a much better idea of how useful stem cell therapy of damaged myocardium will turn out to be.

Clinical applications of HSCs

It is probably fair to say that stem cell research began with the hematology community and the many scientists who were engaged in studies of hematopoiesis, the process of blood formation. The pioneering work of Till and McCullough⁴⁴ in the 1960s on hematopoietic cells helped define the two hallmark characteristics of all stem cells, namely their abilities to self-renew and to differentiate into all the formed elements of the blood. Much research has been done to identify and characterize HSCs. This task has proved difficult, however, because the cells are rare and morphologically indistinguishable from other primitive cells. Thus, one has to rely on cell surface proteins as markers for the isolation of HSCs. Much of the initial research on establishing such markers was done with mice, which laid the groundwork for human analyses.⁴⁵ For human HSCs, some of these markers are CD34⁺, lineage (lin)⁻, Thy1⁺, c-kit^{-low} and CD38^{-low}.⁴⁶

After destruction of the patients' own hematopoietic cells with radiation or chemotherapy, HSCs collected from the blood or bone marrow of the donor are transfused into the recipient. An adult typically requires 7 to 10 million stem cells/kg body weight for transplantation, and these are enriched in the CD34⁺ population of cells. CD34⁺ cells obtained from peripheral blood have been reported to engraft more quickly, but probably at the expense of causing more graft-versus-host disease than bone marrow-derived cells.⁴⁷ HSCs present in umbilical cord blood units are the least likely to incite graft-versus-host disease but are difficult to use for adult transplantation because only infrequently can enough HSCs be derived from a single cord.⁴⁸ There are many complications of HSC transplantation, in addition to graft-versus-host disease; these include prolonged immunosuppression and secondary malignancies.

Adult HSC transplants are by now a common procedure for the treatment of a variety of hematologic diseases. Non-malignant diseases include aplastic anemia, β -thalassemia, globoid cell leukodystrophy, and inborn errors of metabolism, such as Hunter syndrome and Lesch-Nyhan syndrome.¹ Transplants are also widely employed for treatment of malignant hematologic diseases, such as leukemia, lymphoma, and myeloma, and more recently for autoimmune disease, such as systemic lupus erythematosus.⁴⁹ Finally, the graft-versus-host disease engendered by the transplantation of competent donor immune cells is also finding utility in the treatment of other solid tumor malignancies.⁵⁰

Stem cells and gene therapy

A final area in which stem cells may influence clinical medicine is gene therapy. Gene therapy uses genetic engi-

neering to alter, supplement, or replace the activity of an abnormal gene by introducing a normal copy of the gene or to provide a gene that adds new functionality. The initial clinical trials with gene therapy focused on replacing a defective gene with a normal copy of that gene in patients with single-gene disorders, such as hemophilia⁵¹ and X-linked SCID.⁵² The early studies revealed problems that need to be addressed, such as difficulties controlling protein levels without endogenous gene regulatory regions, difficulties maintaining gene expression through long periods, and, most recently, the development of a leukemia-like process by insertional mutagenesis of the retrovirus transgene vector.⁵³ Nevertheless, as efforts are directed toward overcoming these problems, they are also being directed toward using gene therapy to treat complex, chronic diseases that involve more than one gene, including heart disease, arthritis, and Alzheimer disease.

Two major strategies are used for delivering therapeutic transgenes to patients. The first is to use a direct delivery system, wherein the therapeutic transgene is packaged into a delivery vehicle such as a disabled virus and injected into the patient's target organ. This delivery method is limited to specific types of human cells that the viral vehicle can infect, composed largely of dividing cells. This limits the method's utility in treating diseases of organs such as the heart or brain, which are primarily composed of nondividing cells. The second strategy is to use cell-based delivery, whereby the therapeutic transgene is packaged into a delivery vehicle and introduced into a delivery cell, such as a stem cell that is derived from the patient. These genetically modified cells are then multiplied in the laboratory and then infused back into the patient. This method is advantageous relative to direct gene transfer because (1) it allows researchers more control over selection of genetically modified cells when they are manipulated outside the body, and (2) investigators can control the level and rate of production of the therapeutic agent in the cells.⁵⁴

Stem cells are of great benefit to cell-based therapy or gene therapy, because they are self-renewing and thus may reduce or eliminate the need for repeated administrations of the gene therapy. Furthermore, they can be readily isolated from the circulating blood or bone marrow of adults or the cord blood of neonates and redelivered easily into the patient through injection. Of the approximately 450 gene therapy clinical trials conducted in the United States so far, 40% have been cell based, and 30% have used HSCs as the means for delivering transgenes into patients.

HSCs differentiate into a number of lineages in which the therapeutic transgene resides. Apart from "homing" to the bone marrow, these stem cells are also capable of migrating to many different areas in the body, including the bone marrow, liver, spleen, and lymph nodes, which would be useful in the treatment of diseases other than those of the blood, such as liver metabolic disorders and Gaucher disease.¹ So far, HSCs have been the only kind of adult stem cell used in human trials. However, several other adult stem cells are being studied as vehicle candi-

dates for gene therapy, including muscle-forming stem cells, or myoblasts,⁵⁵ bone-forming stem cells, or osteoblasts,⁵⁶ and neural stem cells.⁵⁷

ESCs possess qualities such as pluripotency and unlimited proliferative capacity, making them extremely attractive for use in cell-based gene therapy. At this stage, however, such ESC therapy is still highly hypothetical and experimental, because research is limited to only a few laboratories that have access to human ESCs. As time progresses and more advances are made, it will be necessary to identify optimal stages of differentiation for transplantation and to prove that the transplanted ESCs are able to integrate, function, and survive in the recipient.

In addition, there are some potential drawbacks and concerns with the use of ESCs in therapy. Because human ESCs can form malignant teratomas when transplanted in mice, and human ESCs can form "benign" teratomas in SCID mice, there is the concern that human ESCs could form teratomas if transplanted into human beings.^{58,59} Although there is little evidence to demonstrate teratoma formation by ESCs injected into mice with normal immune systems, the theoretic concern exists. In the SCID mouse experiments, it is thought that the undifferentiated ESCs induce teratomas. Thus, the potential for teratoma formation might be eliminated if methods were devised to remove the undifferentiated cells before transplantation. Researchers are now considering the possibility of genetically modifying ESCs, differentiating them in culture, and then isolating large, pure populations of differentiated cells for transplantation. They could also be used as laboratory models for differentiation, allowing the evaluation of vectors for gene delivery and translation within the patient.¹

The immunologic status of human ESCs has not been studied in great detail, and it still remains to be determined whether these cells would trigger immune rejection in the recipient. It is also important to understand the mechanisms by which ESCs can proliferate and yet remain undifferentiated in culture. The choice between self-renewal and differentiation is highly regulated by intrinsic signals and the external microenvironment.⁶⁰ To realize the true therapeutic potential of ESCs and EGCs, it is important first to understand the molecular and genetic bases by which these cells continue to replicate for prolonged periods. Second, to perform successful transplants in patients, it is important to have a sufficient number of cells that can be manipulated to differentiate into the desired cell type. Achieving clinical success with ESCs looks promising and may provide solutions for many of the technical hurdles faced by therapeutic gene transfer today.

STEM CELL ISSUES: SCIENTIFIC AND ETHICAL

Stem cells hold great promise to cure many diseases that were earlier thought to be incurable. Their potential use in clinical therapy has raised many concerns, however; both scientific and ethical. From the purely scientific perspective, many questions still remain to be answered before

stem cells are considered safe for clinical applications, as discussed previously. Furthermore, there is sufficient concern about origin and identification of cells to warrant the existence of a safety net composed of a set of safeguards for human stem cells to be used in clinical applications. These include the following: (1) Donor sources of stem cells should be carefully screened for pedigree evaluation, genetic testing, and testing for infectious diseases. (2) Because most stem cells are maintained and expanded in culture before transplantation, it is imperative that controlled, standardized practices and procedures be followed to maintain the integrity, uniformity and reliability of the human stem cell preparations. (3) Stem cells, especially those of embryonic origin, are highly pluripotent and have the capacity to differentiate into all cell types. It is therefore imperative to gauge the purity of a cellular preparation by rigorous and quantitative identification of cell types within a heterogeneous population of differentiating human cells. (4) Before transplantation, human stem cell preparations must be shown to possess relevant biologic activity. For example, pancreatic islet-like cells must release insulin, cells intended for liver tissue regeneration must be capable of storing glycogen, and cardiomyocytes intended for heart transplantation must be shown to contract synchronously. (5) Proof of concept must be clearly established in an animal model to demonstrate the validity, efficacy, and safety of the intended therapy.¹

Of equal importance, however, are logistic and ethical issues associated with the use of material of fetal origin. The use of human ESCs in medical research has drawn much attention from the public, and many ethical issues have been raised that concern their use solely for the purpose of medical benefit. Many consider this research to be immoral, illegal, and unnecessary.⁶¹ The fact that experiments with ESCs, as performed currently, result in the destruction of an embryo cannot be ignored. Stem cell research must not lead to an underground black market in "spare" embryos.⁶² In principle, because pluripotent cells proliferate indefinitely, it should be possible for medical research to be conducted on the directed differentiation of stem cells with the relatively few human pluripotent stem cell lines that have been derived so far. This is the basis for current federal policy, which has limited funding of human ESC research to the 64 human cell lines already established before August 9, 2001. This ruling is causing unrest among the scientific community, which is of the opinion that not all 64 lines can be used for research and is concerned that distribution of the cell lines is restricted by patenting, commercial secrecy, and restrictive national and international material transfer agreements.⁶³ These ethical issues are not relevant to adult stem cell research, in which cells are isolated from the adult, for example, the harvesting of HSCs for bone marrow transplantation. However, this approach is somewhat problematic, because adult stem cells are thought not to be as plastic as ESCs. Contrary to this view, the most recent experimentation to address this issue suggests that a population of multipotent mesenchymal stem cells with all the developmental potential of ESCs may

TABLE I. Stem cell biology: Key concepts

1. Stem cells are the source of all specialized cells of the body.
2. Stem cells are defined by two essential characteristics: ability to self-renew and ability to differentiate into multiple different tissue types
3. There are two main types, depending on origin, ESCs and adult stem cells.
 - a. ESCs are derived from inner layer of blastocyst; they have the greatest ability to proliferate and differentiate into virtually any specialized tissue.
 - b. Adult stem cells are derived from differentiated tissues of developed organs; relative to ESCs, they appear to have more limited ability to proliferate and differentiate.
4. The potential for treating disease resulting from damaged tissue is great, but its utility has not yet been proved.
5. Ethical, religious, and political issues associated with derivation and use of ESCs have not been fully resolved and remain stumbling blocks that impede scientific investigation in this area.

reside in human marrow.⁶⁴ If these observations are confirmed, and the cells can be prospectively identified and isolated in sufficient numbers from marrow, one very difficult issue for stem cell therapy will have been solved.

SUMMARY AND CONCLUSIONS

As discussed in detail, and summarized in Table I, stem cells can be found in both embryonic and adult tissues, and their potential for treating a wide variety of common, and uncommon, diseases is almost as limitless as their ability to undergo cell division. Nonetheless, this therapeutic potential will only be fulfilled if stem cells can be isolated and purified in numbers large enough to truly affect organ function. This would mean not only that the cells are present in sufficient number but also that they function as efficiently as the cell population that they are designed to replace. If the current pace of research keeps on unabated, however, we will soon learn more about the differences and similarities between ESCs and adult stem cells and about their ability to survive, proliferate, and function after transplantation. Accordingly, although it is difficult to predict the ultimate utility of stem cell-based therapy at this time, it is not difficult to conclude that this is an enormously important area of scientific research, one that has great potential monetary rewards as well. For these reasons, attempts to regulate stem cell research on a global basis will probably prove difficult. In countries such as the United States, one must hope for thoughtful legislative action on this front, or else the whole endeavor will be driven underground and potentially into the hands of less ethical and less regulated scientists. Open discussions between political bodies and the various interest groups in the scientific, medical, and religious communities need to take place to address the concerns of each and to provide an ultimate solution that is clearly in the interest of humanity.

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