Primer

30. Embryonic and adult stem cell therapy

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There are many types of stem cells. All share the characteristics of being able to self-renew and to give rise to differentiated progeny. Over the last decades, great excitement has been generated by the prospect of being able to exploit these properties for the repair, improvement, and/or replacement of damaged organs. However, many hurdles, both scientific and ethical, remain in the path of using human embryonic stem cells for tissue-engineering purposes. In this report we review current strategies for isolating, enriching, and, most recently, inducing the development of human pluripotent stem cells. In so doing, we discuss the scientific and ethical issues associated with this endeavor. Finally, progress in the use of stem cells as therapies for type 1 diabetes mellitus, congestive heart failure, and various neurologic and immunohematologic disorders, and as vehicles for the delivery of gene therapy, is briefly discussed. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: *Stem cells, human embryonic stem cells, induced pluripotent stem cells, regenerative medicine, gene therapy, cell therapy*

Abbreviations used

AHSCT:	Autologous hematopoietic stem cell transplantation
G-CSF:	Granulocyte-colony stimulating factor
GVHD:	Graft-versus-host disease
GVL:	Graft-versus-leukemia
hESC:	Human embryonic stem cell
hESC-CM:	Human embryonic stem cell–derived cardiomyocyte
HSC:	Hematopoietic stem cell
HSCT:	Hematopoietic stem cell transplantation
iPSC:	Induced pluripotent stem cell
LVEF:	Left ventricular ejection fraction
MSC:	Mesenchymal stem cell
NK:	Natural killer
NSC:	Neural stem cell
SCID:	Severe combined immunodeficiency
T1DM:	Type 1 diabetes mellitus
UCB:	Umbilical cord blood

Stem cells are not homogeneous but exist instead as part of a developmental continuum. The most primitive of the cells is the totipotent stem cell. This cell has the potential to develop into a complete embryo (ie, to form any type of cell, including extraembryonic tissues [embryonic membranes, umbilical cord, and placenta]). This unique property is evanescent. It appears with fertilization of the egg and disappears by the time the embryo reaches the 4- to 8-cell stage. With subsequent divisions, embryonic stem cells lose the ability to generate an entire organism. However, they are capable of differentiating into cells present in all 3 embryonic germ layers, namely ectoderm, mesoderm, and endoderm, and on this basis are called pluripotent. With subsequent divisions, cells become more and more restricted in their ability to differentiate into multiple lineages. They are then called multipotent; that is, they are capable of forming a limited number of cell types. This is the property of adult stem cells, also referred to as somatic stem cells or nonembryonic stem cells, which are able to self-renew during the lifetime of the organism and to generate differentiated daughter cells. In the adult, tissues are in a perpetual state of flux under homeostatic conditions. Even in the absence of injury, they are continuously producing new cells to replace those that have worn out. For this reason, adult stem cells can be found in a metabolically quiescent state in most specialized tissues of the body, including the brain, bone marrow, liver, skin, and gastrointestinal tract. These cells are scarce, however, and with the relative exception of hematopoietic stem cells (HSCs), they are difficult to isolate. Typically, preparations of these cells are often contaminated with more differentiated progenitor cells, which decreases the long-term efficiency of the product because progenitor cells are fixed with respect to cell fate and do not self-renew.

One could argue that 3 major technologic achievements have driven the field of stem cell therapeutics. The first occurred in 1961, when the pioneering studies of Till and McCulloch,[1] using a revolutionary *in vivo* bioassay, unequivocally demonstrated the existence of HSCs. The second major enabling technologic leap occurred in 1998, when Thomson et al[2] reported the isolation of human embryonic stem cells (hESCs) from blastocysts and the creation of hESC lines for study. The most recent was reported by Yamanaka's group[3] in 2006, which induced the formation of pluripotent stem cells from murine fibroblasts.

Each of these advances has furthered the ability of researchers to use stem cells for basic research on cell-lineage fate and development, as well as for drug testing, modeling, and treating disease. It is in the latter area, in particular, that exciting progress has been made over the last few years.

SOURCES OF STEM CELLS

Having defined the different types of stem cells, we will now describe currently available sources of stem cells.

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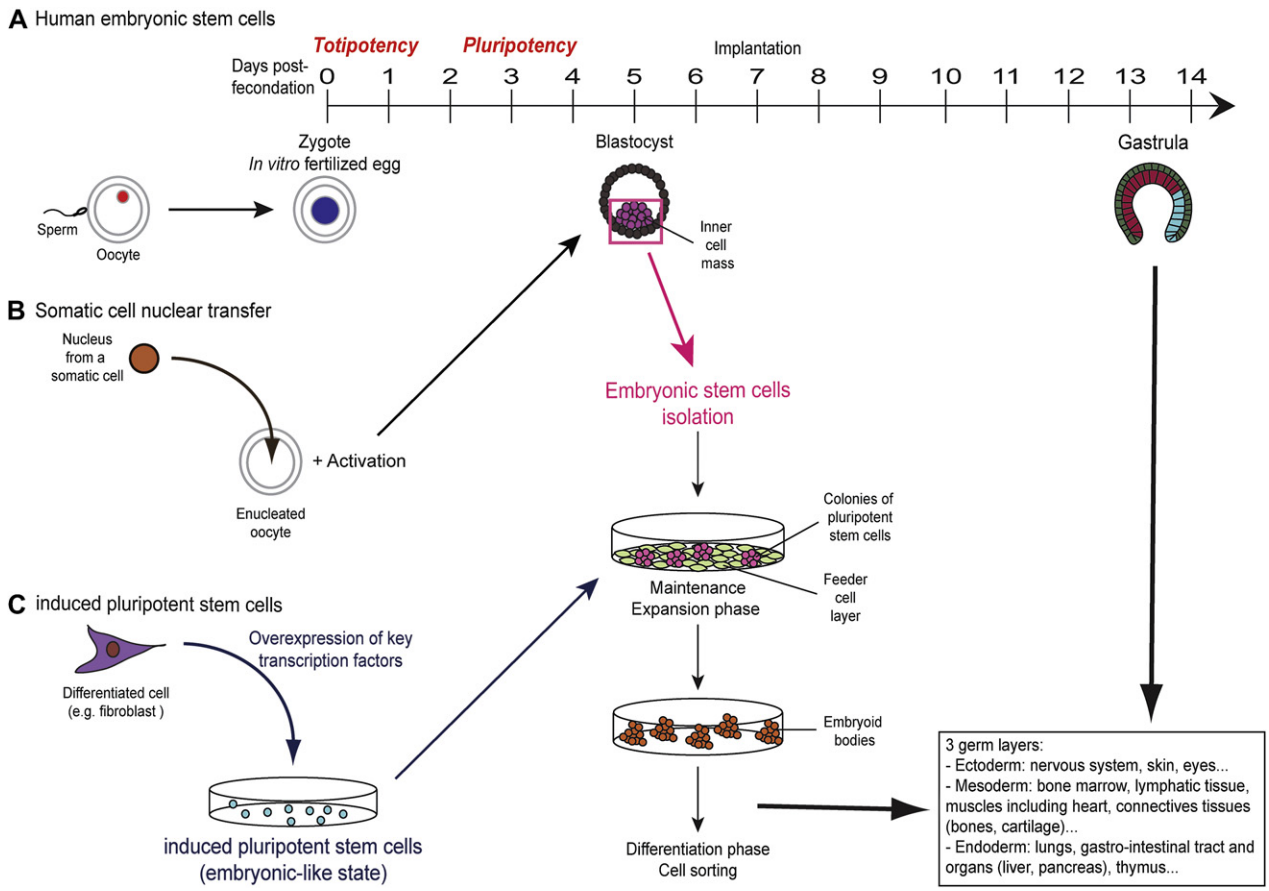

FIG 1. Isolation, generation, and culture of pluripotent stem cells. **A**, After isolation, typically from the inner cell mass of the blastocyst made by means of *in vitro* fertilization, hESCs are expanded in culture. They are classically grown on feeder cell layers, the purpose of which is to expand the cells while maintaining their undifferentiated state (maintenance/expansion phase). Initially those feeder layers were of xenogeneic origin (irradiated murine embryonic fibroblasts), but human feeder layers are being developed and will likely be used with increasing frequency in the future. When removed from feeder layers and transferred to suspension cultures, hESCs begin to form 3-dimensional multicellular aggregates of differentiated and undifferentiated cells termed embryoid bodies. Plated cultures of embryoid bodies spontaneously display a variety of cellular types from the 3 germ lineages at various differentiation stages. Theoretically, cells can be sorted according to differentiation markers, can be differentiated into any desired cells by adding specific growth factors (differentiation phase), or both. On a more practical level, it is difficult to induce hESC differentiation into a specific lineage, and highly definite culture protocols have to be developed for each desired cell type. **B**, Somatic cell nuclear transfer consists of injecting the nucleus from a somatic cell into an enucleated oocyte, followed by activation stimuli. The resulting embryo can be used to generate an hESC line (therapeutic cloning). **C**, iPSCs are generated from differentiated cells that have been reprogrammed to acquire a pluripotent state through overexpression of the key transcription factors *Oct4*, *Sox2*, and either *c-Myc* and *Klf4* or *Nanog* and *Lin28*. Overexpression can be achieved with viral vectors or proteins with or without histone-modifying chemicals. Once they are undifferentiated, they can be grown in culture like hESCs.

hESCs

hESCs are characterized by self-renewal, immortality, and pluripotency. Ongoing attempts to use hESCs in the laboratory finally came to fruition in 1998 with the creation of several human hESC lines,[2,4] although the cloning efficiency of such lines is still very low. Typically, hESCs are derived from the inner cell mass of 5-day-old blastocysts. The blastocysts in turn are made from unused embryos generated by means of *in vitro* fertilization for infertility problems. (It is important to note that the unused embryos can only be used for research purposes with the written informed consent of the parents.) Cells derived from earlier developmental stages can also be used.[5] At the other extreme, pluripotent cells have been isolated from the primordial germ cells of the gonadal ridge of the 5- to 9-week-old embryo. These are cells that normally become either oocytes or spermatozoa. Ironically, spontaneous differentiation in long-term *in vitro* culture of these so-called embryonic germ cells impeded their availability for research.[6] Accordingly, other strategies were and are still being developed with different methods and cell sources. For example, single-cell biopsy of the embryo[5] using a procedure not dissimilar to that used in preimplantation genetic diagnosis and that critically avoids the destruction of the embryo has been used with success, as have parthenogenesis of an unfertilized oocyte[7] and spermatogonial cells from adult human testis.[8] The latter developments are

particularly exciting because they would allow the production of histocompatible cells that could be used in the donor. Technical methods for culture of hESCs are depicted in Fig 1, *A*.

Hundreds of hESC lines have been generated thus far. The first human stem cell line bank opened in 2004 in the United Kingdom (http://www.ukstemcellbank.org.uk/). The National Institutes of Health registry (http://stemcells.nih.gov/research/registry/) has also archived a number of hESC lines and established criteria for demonstration of the pluripotency of these lines. Specifically, cells should be able to give rise to any cell lineage of the body and thus to form a teratoma (a tumor containing tissues from the 3 primary germ layers) *in vivo* after injection in an immune-compromised animal and should be capable of unlimited self-renewal.

Nuclear reprogramming and induced pluripotency

Nuclear reprogramming is a procedure that causes changes in gene expression that allow a cell of one type to develop into a cell of another type.[9] Recent strategies for generating stem cells are focused on nuclear reprogramming of differentiated cells to force them to become pluripotent. An example is somatic cell nuclear transfer (Fig 1, *B*). This consists of injection of the nucleus of a somatic cell into an enucleated oocyte.[10] The resulting pluripotent cells are genetically matched with the cell donor (this technique is thereby often called "therapeutic cloning"), except for the mitochondrial DNA, which comes from the egg. Another method is accomplished by means of cell fusion with an hESC, which can produce cells with some stem cell characteristics.[11]

A very recent and very exciting advance in reprogramming has been the generation of induced pluripotent stem cells (iPSCs). First reported in 2006 using murine fibroblasts,[3] iPSCs can be made from multiple murine and human somatic cell types,[12,13] and it is now possible to create patient-specific iPSCs.[14] iPSCs can be generated from differentiated cells by using retroviral-mediated expression of core transcription factors known to be required for maintenance of pluripotency and proliferation of embryonic stem cells.[3] These genes are *Oct4*, *Sox2*, and either *c-Myc* and *Klf4* or *Nanog* and *Lin28* (Fig 1, *C*). iPSCs exhibit similar features to embryonic stem cells, including cell morphology, cell-surface markers, growth properties, telomerase activity, expression, and epigenetic marks (ie, methylation or acetylation of histones, which result in changes in gene expression) of pluripotent cell–specific genes[12,13] but not global gene expression signatures.[15] They can give rise to cells derived from all 3 germ layers *in vitro* and *in vivo*, and murine iPSCs injected into murine blastocysts have been shown to contribute to embryonic development.[3]

Using pluripotent stem cells in the clinic: Scientific/medical issues

A number of scientific/medical issues need to be addressed before stem cells can be considered safe for clinical applications. The first hurdle is the tumorigenic potential of pluripotent cells (hESCs and iPSCs). Because pluripotency is evidenced by the ability to form teratomas when transplanted in immunodeficient mice, the concern exists that these cells could form malignant tumors in their new host. One strategy for dealing with this problem is to select pure populations of more committed cells for transfer. Demonstrating genetic and epigenetic stability will therefore be important before these cells are used clinically. In fact, karyotypic abnormalities have been described in several hESC lines, although changes might be at least partially dependent on culture techniques.[16]

In additional to biologic issues directly affecting the stem cell product, it is imperative that controlled, standardized practices and procedures be followed to maintain the integrity, uniformity, and reliability of the human stem cell preparations. Because stem cells are both maintained and expanded *in vitro* before transplantation, culture conditions compatible with human administration must be used. Feeder cells and sera of animal origin have to be reduced and ideally avoided to reduce the potential risk of contamination by xenogeneic protein and pathogens. Finally, transplantation of hESCs into patients is also limited by potential HLA incompatibility. Consequently, life-long immunosuppressive therapy, which can lead to infections and organ-based toxic side effects, such as nephropathy, might be required to prevent graft rejection. In this regard iPSCs hold great promise because they are histocompatible with the patient and because their use avoids one of the major ethical concerns (see below) associated with hESCs.

Although iPSCs solve the tissue-barrier problem, they too have technical drawbacks that are presently limiting their use. First is the issue of the risk of insertional mutagenesis caused by viral integration into the genome. This is of particular concern because patients who have received gene-modified lymphoid cells have had aggressive leukemias as a result of this phenomenon (see below).[17] The possibility that iPSCs might be generated with nonintegrating expression plasmids or adenoviral vectors is being explored in the murine system and appears possible.[18,19]

Another risk is reactivation of a viral oncogene, such as *c-Myc*, used to engineer the cells. Here there are data to suggest that the use of histone-modifying chemicals, such as the histone deacetylase inhibitor valproic acid, improves reprogramming efficiency and avoids the need to use *c-Myc* and that *Oct4* and *Sox2* alone are then sufficient in the generation of human iPSCs.[20] Recently, iPSCs were successfully obtained from murine fibroblasts cultured without any genetic material at all by using only valproic acid and recombinant proteins for the necessary transcription factors.[21] However, even as technical hurdles are overcome, generation of iPSCs still suffers from low efficiency and high cost, although no doubt these problems will be solved in time as well. In particular, the reprogramming efficiency is typically less than 1% but could depend on the differentiation stage of the cells. Indeed, the efficiency of iPSC generation has been recently increased to 28% with the use of hematopoietic stem and progenitor cells.[22]

Adult stem cells

The best-known example of the adult stem cell is the HSC, which is located in the bone marrow niche. HSCs and progenitors can be readily harvested from bone marrow and umbilical cord blood (UCB). They can even be collected from peripheral blood after mobilization from the marrow with granulocyte colony-stimulating factor (G-CSF) with or without CXCR4 antagonist.[23] HSCs are characterized by the expression of cell-surface markers, which allows for their isolation. In human subjects the HSC surface phenotype is typically lineage-specific antigen negative (lin^-), $CD34^+CD38^-CD133^+$c-Kit/$CD117^+CD59^+$Thy1/$CD90^+CXCR4^+$. Apart from differentiating into all myeloid and lymphoid lineages, HSCs have been shown to be able to differentiate *in vitro* into cells of nonhematopoietic lineages. However, such plasticity was probably an experimental artifact that

is currently explained by the presence of heterogeneous populations of non-HSCs in hematopoietic organs or by the phenomenon of cell fusion.

Mesenchymal stem cells (MSCs) are another type of adult multipotent cells that are capable of differentiating into various mesodermal cell lineages, including myocytes, osteoblasts, chondroblasts, fibroblasts, adipocytes, and other stromal elements. MSCs are present in almost all organs, but for therapeutic purposes, they are most conveniently isolated from bone marrow and UCB. MSCs can be organ specific. Consequently, populations isolated from various sources, although morphologically similar, might be functionally different. For example, MSCs isolated from the umbilical cord do not have the same abilities to give rise to osteoblasts, chondrocytes, and cardiomyocytes as bone marrow–derived MSCs.[24] MSCs can be readily expanded *ex vivo* and manipulated, if needed, to acquire specific properties. The International Society for Cellular Therapy recommended changing their name to "multipotent mesenchymal stromal cells" because the majority of MSCs lack complete "stemness" property and proposed minimal criteria for standardization of preparations.[25] Human MSCs must be plastic adherent; express CD105, CD73 and CD90; lack hematopoietic markers (CD45, CD34, CD14 or CD11b, CD79α or CD19 m and HLA-DR); and be able to differentiate into osteoblasts, adipocytes, and chondroblasts *in vitro*. MSCs display trophic, anti-inflammatory, and immunomodulatory capacities, both through secretion of soluble factors (indoleamine 2,3-dioxygenase, IL-6, TGF-β1, hepatocyte growth factor, inducible nitric oxide synthase, and prostaglandin) and direct cell-to-cell interaction with immune cells. *In vitro* MSCs suppress effector and cytotoxic T-cell, B-cell, natural killer (NK) cell, and dendritic cell activity and can induce regulatory T cells.[26] However, how MSCs assist in repairing a damaged organ is still unclear. Mounting evidence suggests that direct substitution of injured cells by *in situ* differentiated MSCs is unlikely (although still argued). Establishment of a favorable environment or niche for reconstruction of the tissue by intrinsic stem cells *per se* seems more likely. Regardless, because of their low immunogenicity and claimed beneficial effects on organ regeneration (whatever the mechanism), MSCs are being examined in an increasing number of regenerative medicine applications, as well as in inflammatory and immunologic diseases.

Finally, amniotic fluid, UCB, and the placenta are other sources of nonembryonic stem cells. However, it is not clear yet whether they are pluripotent or multipotent and how clinically useful they will be.

POTENTIAL CLINICAL USES OF STEM CELLS

Stem cells are postulated to have a tremendous number of applications, but tissue engineering seems to generate the greatest excitement. Stem cells can be used in regenerative medicine, immunotherapy, and gene therapy. Animal models and clinical studies have shown that transplantation of stem cells from diverse origins can successfully treat many acute and chronic diseases, such as immunohematologic disorders, type 1 diabetes mellitus (T1DM), Parkinson disease, neuronal destruction, and congestive heart failure.

Hematology-immunology

During the last 50 years, allogeneic hemopoietic stem cell transplantation (HSCT) has progressively become a common procedure for the treatment of a variety of inherited or acquired immunohematologic diseases, including thalassemias, sickle cell disease, Fanconi anemia, inborn errors of metabolism, severe aplastic anemia, severe combined immunodeficiency (SCID), and other primary immune deficiencies. HSCT is also widely used for the treatment of hematologic malignancies, such as acute myeloid and lymphoid leukemias, chronic myeloid leukemia and other myeloproliferative syndromes, myelodysplastic disorders, lymphoma, myeloma, and even solid tumors, such as renal cell cancer, breast cancer, ovarian carcinoma, and neuroblastoma.[27]

The aim of allogeneic HSCT in malignancies is not only a substitution of the malignant bone marrow but also a form of adoptive immunotherapy. In the context of HLA compatibility, donor allogeneic T lymphocytes detect differences in minor histocompatibility antigens in both the host and the tumor and can destroy the residual malignant cells, thereby contributing to the cure of the patient. This is the graft-versus-tumor or graft-versus-leukemia (GVL) effect. Particularly in the case of disease relapse after transplantation, donor lymphocyte infusions might induce or enhance a GVL effect and reinduce the patient into remission.

Major complications of HSCT include organ toxicity from the conditioning regimen used and graft-versus-host disease (GVHD), in which the donor's immune system destroys the recipient's normal tissues, particularly the skin, gastrointestinal tract, and liver. Other important complications of HSCT are graft failure, infertility, growth retardation in children, and secondary cancers thought to arise as a result of chronic immunosuppression and DNA-damaging preparative regimens.

In an effort to decrease these complications, several strategies have been developed. First among these are the reduced-intensity conditioning regimens, or so-called minitransplantations, which have made HSCT available to older and less fit patients.[28] Significant immunosuppression in these patients and GVHD as a result of the conditioning regimen remain serious problems and have suggested to many that the use of the term "mini" is misleading with respect to potential complications.

Second, the cell source has also been examined with respect to complications. Several studies convincingly show that $CD34^+$ cells harvested from peripheral blood engraft faster but are associated with a higher incidence of GVHD.[29]

Finding a histocompatible donor remains a problem for many patients in need of HSCT. Ideally, one would like to use an HLA-matched sibling as a donor. If such a donor is not available, then a matched unrelated donor is an acceptable alternative. This can be a major problem for minority groups underrepresented in registries of volunteer donors, information on which is centralized in the Bone Marrow Donors Worldwide database. In the absence of a compatible donor, HLA-haploidentical mismatched HSCT from a relative can be performed,[30] generally T depleted to avoid GVHD. In that context NK cells could facilitate engraftment and display an antileukemic activity without GVHD.[31]

Finally, UCB transplantation has the potential to significantly enlarge the number of potential HSCT recipients. UCBs are rapidly and easily available from cord blood banks and can be used when only partially HLA matched because they are much less likely to induce acute and chronic GVHD.[32] Remarkably, the GVL effect seems to be preserved, likely as a result of NK cells present in the cord blood preparation.[33] Issues that remain to be solved are delayed engraftment, prolonged T lymphopenia, and defective thymopoiesis.[34] In addition, it is not currently possible to perform donor lymphocyte infusions in the case of relapse, but

an ongoing clinical trial is testing *ex vivo* expansion of UCB T cells. Because the number of HSCs per unit of UCB is small, thereby limiting their ability to be used for adult patients, strategies using combined units or *ex vivo* expanded cells are being developed.[35,36]

Autologous hematopoietic stem cell transplantation (AHSCT) is commonly performed in certain settings as well. Its main role is to lessen the period of aplasia (rescue therapy) after high-dose chemotherapy and thereby lessen the risk of infection and bleeding. When used for the treatment of hematologic malignancies and solid tumors, HSCs to be used for AHSCT are commonly collected after few cycles of chemotherapy to lessen contamination of the graft by tumor cells. Moreover, autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus, might benefit from AHSCT. It is thought that immunoablative therapy resets the immune system by eliminating autoreactive T and B lymphocytes, lessening memory T cells, increasing thymus-derived naive T cells, generating a diverse but distinct T-cell receptor repertoire,[37] and promoting regulatory T cells.[38] Several recent studies have shown, as might be expected, that nonmyeloablative and low-intensity myeloablative regimens have fewer treatment-related complications and mortality than high-intensity myeloablative regimens[39] and that best results might be obtained during the inflammatory phase of autoimmune disease. Allogeneic HSCT has also been used for the treatment of autoimmune disease on the theory of both immune reset and correction of the genetic predisposition, but the risks of GVHD and infection are substantial, and therefore this form of therapy should be reserved only for treatment of very serious and refractory disease.[39]

Finally, the immunomodulatory capability of MSCs is being tested in the treatment of patients with systemic lupus erythematosus, multiple sclerosis, Crohn disease, amyotrophic lateral sclerosis, and T1DM. In solid organ transplantation and HSCT, results from preclinical animal studies, although controversial, suggest that donor-derived MSCs could have a tolerogenic effect and might therefore be used for prevention and/or treatment of graft rejection and GVHD.[40-42]

T1DM

T1DM is an autoimmune disease resulting from the destruction of pancreatic insulin-producing β cells in the islets of Langerhans. Insulin replacement therapy, even when rigorously controlled, is often not efficient enough to prevent long-term complications of the disease. Transplantation of a whole pancreas or isolated mature islets can restore proper glucose regulation, but the former is a morbid high-complication procedure, and the latter appears to be only a transient solution.[43] For these reasons, other sources of β cells suitable for transplantation are being sought. Pancreatic stem cells have been identified in ductal epithelium of injured pancreas on the basis of expression of the transcription factor neurogenin 3.[44] Efforts are now being directed toward methods to expand these cells *ex vivo* or to stimulate their proliferation *in vivo*.

Generation of insulin-secreting cells from hESCs and iPSCs holds great promise for the cure of T1DM. hESC-derived pancreatic endoderm can be differentiated *in vivo* into glucose-responsive insulin-secreting cells in immunodeficient mice with streptozotocin-induced diabetes (a β cell–selective destruction).[45] Unfortunately, teratoma formation was found in approximately 15% of the recipient mice and is a safety concern. The immunologic incompatibility of hESCs could be resolved by using bioengineered porous capsules, which are designed to protect the graft from immune cells but remain permissive to the passage of small molecules. In regard to iPSCs, a particularly exciting study from Zhou et al[46] reported the reprogramming of differentiated murine pancreatic exocrine cells into β-like cells *in vivo*. These investigators found that transient expression of 3 key developmental transcription factors, neurogenin 3, MafA, and Pancreatic duodenal homeobox-1 (Pdx1), by adenoviral vectors injected directly into the pancreas was sufficient to reprogram exocrine cells into insulin-secreting cells responding to hyperglycemia.

The immunomodulatory effects of MSCs are also being explored in the setting of T1DM. At the time of diagnosis, β-cell destruction is often not yet complete. In theory, amelioration of the immune attack might allow the survival of the residual islet cells. In diabetic immunodeficient mice human MSCs decreased hyperglycemia and increased endogen insulin levels and β-cell numbers.[47] Clinical studies testing allogeneic MSCs in patients with recently diagnosed diabetes are ongoing.

Finally, nonmyeloablative AHSCT was performed in 23 patients with early-onset T1DM.[48] Twenty enjoyed a variable insulin-free period, and 12 of these patients remained insulin free after a mean follow-up of 31 months. Interestingly, benefit was demonstrated in those patients with transient responses as well. In this small group (8 patients) daily insulin doses were significantly diminished, and C-peptide levels (reflective of endogenous insulin synthesis) were increased.

Diseases of the nervous system

It was thought for a long time that nerve cells do not divide in the adult mammalian brain, but this has now been shown to be incorrect. Neurogenesis not only occurs during prenatal and postnatal development but also in adults. Neurogenic niches have been identified in the subventricular zone of the lateral ventricles and in the subgranular zone of the dentate gyrus of the hippocampus. In brain-injury models neural stem cells (NSCs) proliferate in those neurogenic regions and are even able to migrate toward the site of damage.[49] NSCs are multipotent and capable of self-renewing. *In vitro* they cluster in "neurospheres," which are able to differentiate into the 3 major neuroectodermal lineages (neurons, astrocytes, and oligodendrocytes).

Neural stem/progenitor cells can be isolated from embryonic, fetal, or adult brain tissue by sorting cells on the basis of nestin expression primarily, as well as other markers, and can then be placed into culture for expansion. Clearly, for autologous stem cell therapy, more accessible NSCs are required. Curiously, dental pulp and peridontium have been shown to be sources of NSC, as well as olfactory mucosa, which is readily harvested by means of nasal biopsy.[50] Investigations for NSC-based therapy are ongoing for various neurologic diseases. The neural repair probably results from a replacement of defective cells but also from neuroprotective, trophic, and immunomodulatory effects. To date, the ideal NSC source, schedule, and route of transplantation have not been established and are likely to be disease specific. The use of NSCs to treat Parkinson disease might be instructive in this regard.

Parkinson disease is an incurable, progressive, neurodegenerative disease that affects dopaminergic neurons. Levodopa, which is converted to dopamine in the brain, is the mainstay of

treatment, but most patients acquire tachyphylaxis to its effects over time. In contrast to patients with diabetes, in whom transplantation of islet cells is a therapeutic option, implantation of fully differentiated dopamine-releasing neurons into the brain is not presently feasible because such cells do not survive. Transplantation of embryonic/fetal nigral dopaminergic neurons was tested in 2 double-blind, placebo-controlled trials, but results were not as encouraging as those from previous open-trial reports.[51,52] However, modest clinical improvement was noted in some patients, and striatal fluorodopa uptake was significantly enhanced. Unfortunately, several patients subsequently had dyskinesias. Postmortem examination of the brains of some patients provided evidence that transplanted dopamine neurons can differentiate and survive for many years without immunosuppression. However, it appeared that at least some of the grafted tissue was involved by disease over a period lasting from 9 to 16 years.[53] Widespread application of this therapy will likely be limited as long as access to fetal donor tissue is required. Moreover, the safety of those cells is not completely assessed because they have not been tested in a large number of patients and because development of cerebral mass lesions of donor origin was reported in a patient less than 6 years after fetal transplantation for Huntington disease.[54] Accordingly, finding alternative sources of NSCs for therapeutic purposes is the object of intense investigation.

In one case autologous NSCs were harvested by means of cerebral biopsy, expanded, and differentiated into dopaminergic neurons *ex vivo* and then injected back to the patient's putamen 9 months later.[55] Clinical evaluation and fluorodopa uptake were improved after transplantation, but all benefits had disappeared by 5 years. In animal studies other stem cell types partially alleviated Parkinson symptoms, including MSCs,[56] olfactory NSCs,[57] hESC-derived neurons,[58] and iPSCs.[59] Human clinical trials with cells of these types can therefore be anticipated.

Unlike Parkinson disease, which theoretically requires replacement of only 1 cell type, therapies for other neurologic diseases, such as stroke and spinal cord injury, in which large numbers of cells of many types (neurons, glia, and endothelial cells) are destroyed, face much larger hurdles. In recent years, human NSCs from diverse origins, including hESCs, HSCs, and MSCs, have been tested in preclinical models of ischemic stroke. These experiments have enabled the development of treatment strategies and have demonstrated the critical importance of transplantation timing for clinical success. Only a few clinical studies have been performed for the treatment of stroke. Stereotactic injection of neuronal cells derived from embryonal carcinoma cell line (NT2/D1) did not display significant benefits compared with control results, but some patients experienced improvement.[60] In other studies an investigation into the utility of fetal porcine cells was stopped because of adverse events (temporary worsening of deficits and seizures) in 2 of 5 patients,[61] whereas intravenous infusion of MSCs was shown to be safe without significant benefit. HSCs are currently being evaluated in phase I/II protocols. The injection of specific growth factors to stimulate proliferation of intrinsic neuroprogenitors in the brain is a novel approach to this problem.[62]

Spinal cord injury often results in permanent motor deficiency, sensory deficiency, or both, thereby rendering treatment particularly challenging. In a small number of paraplegic and quadriplegic patients, olfactory NSCs have been injected into intralesional and perilesional areas.[50] Feasibility and safety were acceptable, but unfortunately, clinical improvement remained slight after 3 years of follow-up. Logically, early-phase treatment might yield the best results. In fact, a phase I/II clinical trial tested infusion of HSCs into the spinal cord associated with G-CSF injections: the Association Impairment Scale grade improved in 30.4% of patients treated quite early (<8 weeks) after the initial lesion; however, no enhancement was noted when the treatment was performed later.[63]

Cardiac repair

Congestive heart failure afflicts millions of persons around the world, with 400,000 new cases being reported each year in the United States alone. The most common cause is coronary artery disease. After myocardial necrosis has occurred, the cell loss is irreversible, and although many medical and surgical treatments are available for the subsequent congestive heart failure, the long-term prognosis of these patients remains guarded, with a 5-year mortality of 50%. Transplanting stem cells would have clear advantages to transplanting a heart because it would obviate the constraint of a donor and, in case of autologous cells, for the requisite immunosuppression.

hESC-derived cardiomyocytes (hESC-CMs) have been successfully generated. Intramyocardial injection of hESC-CMs a few days after infarction in immunodeficient rodents seemed to enhance left ventricular ejection fraction (LVEF) compared with that seen in a control group when evaluated at 4 weeks.[64] Unfortunately, this enhancement was not sustained after 12 weeks of follow-up. Another study suggested that a coinfusion of hESC-CMs and MSCs in mice was of benefit because, according to the authors, a "synergistic trophic effect that enhanced repair of injured host tissue" was brought about.[65] Importantly, no teratoma was found in animals receiving hESC-CMs.[64,65]

Despite a controversial plasticity *in vitro*, a considerable amount of data from actual preclinical studies suggest that it is unlikely that transdifferentiation of HSCs and MSCs into functional cardiomyocytes happens to any significant degree *in vivo*. In very specific culture conditions, MSCs might be driven toward differentiating into cardiomyocyte-like cells at a very low frequency (approximately 0.07%) that would not be enough for cardiac repair.[24] It is now generally agreed that the transplanted cells exert their beneficial role through paracrine effects and by creating a favorable trophic environment for intrinsic cell recovery, enhancing angiogenesis, and limiting ventricular remodeling.[66] A study comparing the efficacy of transplanted bone marrow mononuclear cells, MSCs, skeletal myoblasts, and fibroblasts in mice with experimental myocardial infarcts was carried out and showed that HSCs had the most beneficial effect on left ventricular function in that model.[67] Recently, infusion of endogenous cardiac stem cells isolated from endomyocardial biopsy specimens and expanded *ex vivo* appeared to enhance myocardial viability and LVEF in a murine infarction model.[68]

Intracoronary infusions of HSCs[69] or MSCs[70] have been performed a few days after percutaneous coronary intervention for acute myocardial infarction. Despite contradictory results of clinical trials, meta-analyses reported moderate but significant benefits of such therapy compared with the condition of control patients, with improvement in LVEF, infarct size, and end-systolic volume.[71] In patients with chronic ischemic disease, intracoronary and intramyocardial injections of HSCs are associated with modest enhancements as well.[71] Other cell types are also

being tested, such as skeletal myoblasts (although lack of electrical synchronization with cardiomyocytes could potentially be arrhythmogenic) or endothelial progenitor cells.

Stem cells and gene therapy

The goal of gene therapy is to cure diseases caused by malfunctioning genes. It does so by substituting the function of a normal gene for the one that causes disease. Until now, the most commonly used procedure in human gene therapy clinical trials is the insertion of a normal copy of the target gene in a nonspecific location into the host genomic DNA. The therapeutic transgene is packaged into a delivery vehicle, which is typically a replication-deficient virus. Nonintegrating virus (adenovirus or adeno-associated virus) can be used in nondividing cells, such as neurons and cardiomyocytes. In dividing cells, such as stem cells, vectors that integrate into host DNA, such as γ-retrovirus or lentivirus, are required to have a transmission to daughter cells.

Stem cells are of great benefit to cell-based gene therapy because they are self-renewing and thus might reduce or eliminate the necessity for repeated administrations of the therapeutic cells. Single-gene inherited diseases are particularly good candidates for gene therapy. In theory the host's own stem cells can be repaired through genetic engineering and then used in an autologous transplantation. This avoids all the risks of transplanted allogeneic cells, including the risks associated with long-term immunosuppression, as well as GVHD, in patients receiving HSCT. The first clinical trials with engineered HSCs involved patients with genetic immunodeficiency diseases, such as adenosine deaminase–deficient SCID.[72] Trials have also been carried out in patients with X-linked SCID (γ-common [γ-c] cytokine receptor deficient or SCID-X1)[17] and chronic granulomatous disease.[73] The clinical results have been quite promising but have been marred by the development of leukemia, which has been shown to be caused by insertional mutagenesis in a number of these patients (see below). Gene therapy for hemoglobinopathies, such as β-thalassemia and sickle cell disease, are ongoing. Easily accessible mucosal and skin stem cells are also being used, for example in treatment of diseases such as junctional epidermolysis bullosa.[74]

These early studies revealed problems that need to be addressed, such as difficulties controlling protein levels without endogenous gene regulatory regions, maintenance of gene expression through long periods, low protein production, and insertional mutagenesis of the retroviral transgene vector. Indeed, the major side effect was thus far the occurrence of T cell–acute lymphoblastic leukemia in 5 of 19 patients successfully treated for SCID-X1 in 2 distinct French and British trials.[17,75] In all cases the retroviral vector was found in the leukemic clone, integrated near a proto-oncogene, and particularly before the LIM domain–only 2 in 4 cases and was associated with acquired somatic mutations. γ-Retroviral vectors were subsequently shown to integrate preferentially in the 5′ ends of genes[76] near transcription start sites in a nonrandom manner near genes that provide selective advantage to the clone. Interestingly, when the same retroviral vector, the murine leukemia virus, was used to deliver other transgenes, no case of leukemia was observed, suggesting that γ-c receptor overexpression might be involved in the oncogenesis.

New techniques are being developed to enhance efficiency and to avoid the risk of insertional oncogenesis. First, safer delivery systems are being developed. For example, HIV-derived lentivirus is able to transduce nondividing cells, is easier to use, and can induce less mutagenesis than γ-retrovirus, as assessed in murine models.[77] Other modifications under development include the use of inducible and tissue-specific promoters, a weaker viral promoter/enhancer, and self-inactivating retroviral vectors and introduction of suicide genes. At last, the improvement of direct gene correction with homologous recombination (a normal copy of the gene is switched with the defective allele) is promising in murine models.[78] That last technology could be particularly significant in the treatment of dominant genetic diseases.

The potential utility of hESCs and iPSCs was discussed earlier, but the use of such cells is under active investigation for human gene therapy.

Finally, new attempts are focusing on cell-based delivery vehicles for tumor-specific therapy. MSCs appear to be good agents for this purpose because, in addition to their properties described above, they have been shown to migrate toward tumors. For example, MSCs engineered to express the TNF-related apoptosis-inducing ligand induced apoptosis in tumor cells, reduced tumor growth *in vivo*, and prolonged survival in murine models of human glioma.[79]

ETHICAL ISSUES

The use of hESCs in medical research has drawn much attention from many sectors of the public. Religious, historical, cultural, medical, and other points of view have contributed to a very vigorous and wide-ranging discourse over the use of these materials.[80] Some consider research with hESCs to be inherently immoral because these individual's believe that life begins with fertilization of the ovum, and the destruction of an embryo with the potential to develop into a viable human being is thought tantamount to infanticide. For this reason, the American federal government severely restricted access and use of hESCs in 2001. These restrictions have now been largely overturned by the Obama administration. In contrast, proponents of this line of research insist that the potential benefits to humankind from this research mitigate such concerns. They also argue that hESCs are made from unwanted fertilized ovum that would likely be destroyed in any event.

Stem cells created by means of nuclear transfer share the same ethical concerns. Furthermore, because these cells have the potential to generate a complete embryo, they also raise the even more highly charged possibility of cloning human beings, so-called reproductive cloning. Many organizations and countries have already banned reproductive cloning of human beings. Because this procedure can be used to generate stem cells for therapeutic purposes, in countries where this type of cloning is legal, such as Australia and the United Kingdom, the created embryos must be destroyed within 14 days. Federal laws in the United States are not clear on the legality of therapeutic cloning, but the Obama administration has pledged establishment of strict guidelines to ensure that cloning research will not be used for human reproduction.

Because of the shortage of human oocytes, generation of human-animal chimeras was legalized in 2008 in the United Kingdom for research purposes only. A human nucleus is transferred into an animal's oocyte, creating a hybrid embryo that must be destroyed within 2 weeks and cannot be implanted. Clearly, creation of such tissues raises even more complex issues.

Finally, the issue of financial compensation for embryo and gamete donors is also controversial, with guidelines for this problem being proposed by the International Society of Stem Cell Research (http://www.isscr.org/guidelines/index.htm). All parties involved in the debate want very much to avoid the development of an underground black market in spare embryos.

CONCLUSIONS AND PERSPECTIVES

The promise of stem cell therapeutics powers the field of regenerative medicine and has generated a huge amount of excitement, anticipation, and hope. Accordingly, research with hESCs is increasing exponentially worldwide, particularly in the United States, where important limitations on research with such cells were overturned in 2009. Furthermore, the US Food and Drug Administration recently approved the world's first phase I clinical trial using hESC-based therapy in patients with spinal cord injury.

Nonetheless, a number of substantive scientific and ethical issues remain to be resolved before hESCs can enter the therapeutic mainstream. In the meantime, recent breakthroughs in generating iPSCs would obviate the need to solve the most vexing of these problems. In fact, it seems reasonable to hope that in the next few years many of the enabling issues relevant to iPSCs will be solved, allowing the field of regenerative medicine to deliver on its vast potential promise.

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 extremely important area of scientific research. Surrounded by controversy and a good many ethical concerns, thoughtful legislative action could both foster the field and ensure continued progress. This would clearly be more desirable than having the whole endeavor 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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