Approved: March 15, 2006
Date

MINUTES OF THE HOUSE FEDERAL AND STATE AFFAIRS COMMITTEE

The meeting was called to order by Chairman John Edmonds at 1:30 P.M. on March 7, 2006 in Room 313-S of the Capitol.

All members were present except:
Representative Tom Burroughs- excused
Representative Candy Ruff- excused

Committee staff present:
Athena Andaya, Kansas Legislative Research Department
Dennis Hodgins, Kansas Legislative Research Department
Mary Torrence, Revisor of Statutes Office
Carol Doel, Committee Secretary

Conferees:
David A. Prentice, Ph.D, Senior Fellow for Life Sciences

Others attending:
See attached list

Chairman Edmonds opened the floor for bill introductions and requested a bill regarding mercury emission from power plants. With no objections, that was accepted for introduction.

There were no other requests for bill introduction.

The Chairman introduced Dr. David Prentice from the Family Research Council in Washington, D.C. who gave a briefing on stem cell research. Dr. Prentice stated that within just a few years, the possibility that the human body contains cells that can repair and regenerate damaged and diseased tissue has gone from an unlikely proposition to a virtual certainty. He spoke to the committee on the types of stem cells, stem cell transplant, cloning, and many other aspects of stem cell research. (Attachment 1)

With no further business before the committee, the meeting was adjourned.

FEDERAL AND STATE AFFAIRS
GUEST LIST
Date 3-7-06

NO Guests Signed

Adult Stem Cells—2004 Update

(Submitted October 2004)
For the President's Council on Bioethics
David A. Prentice

Over the last year since submission of the final addendum for the Stem Cell Report in October 2003, literally dozens of additional published articles have added to our knowledge of the substantial abilities of adult stem cells. This brief summary will not attempt to list all of these papers, but will highlight some of the more interesting and significant reports.

Several new reports highlight the pluripotent ability of adult stem cells from new sources including a new isolate from bone marrow[1] and umbilical cord blood.[2] These references also indicate the ability for extensive proliferation of adult stem cells, especially those derived from cord blood. One group reports efficient generation of neural stem cells from bone marrow stromal cells.[3]

The pancreas and potential to treat diabetes has been highlighted in several recent studies. Several published references address the possible existence of a pancreatic stem cell. One reference indicates that regeneration of beta cells in the pancreas is solely due to existing beta cells,[4] while another reference indicates the existence of a multipotent progenitor within pancreas that can form either pancreatic or neural cell lineages.[5] Another group has provided evidence of transdifferentiation of bone marrow-derived stem cells into pancreatic cells.[6] A Harvard group has shown that pancreatic islet progenitors can engraft in mice,[7] and has also shown permanent reversal of diabetes in mice[8] (they are now preparing to move to their first clinical trial.)

Another group has shown effectiveness of endogenous adult stem cells, stimulated by growth factor treatment, in stroke recovery in animals.[9] This again points to the potential of identification of stimuli for endogenous stem cells for use in therapy, obviating culture of stem cells for treatments and relying on the patient's own stem cells to effect tissue repair.

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[1] D'Ippolito G *et al.*, "Marrow-isolated adult multilineage inducible (MIAMI) cells, a unique population of postnatal young and old human cells with extensive expansion and differentiation potential", *J. Cell Science* 117, 2971-2981, 15 July 2004 (published online 1 June 2004)

[2] Kögler G *et al.*, "A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential", *J. Experimental Medicine* 200, 123-135, 19 July 2004

[3] Hermann A *et al.*, "Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells," *J Cell Sci* 117, 4411-4422, 2004

[4] Dor Y *et al.*, "Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation," *Nature* 429, 41-46, 6 May 2004

[5] Seaberg BM *et al.*, "Clonal identification of multipotent precursors from adult mouse pancreas that generate neural and pancreatic lineages", *Nature Biotechnology* 22, 1115-1124, Sept 2004

[6] Oh S-H *et al.*, "Adult bone marrow-derived cells transdifferentiating into insulin-producing cells for the treatment of type I diabetes," *Laboratory Investigation* published online 22 March 2004

[7] Abraham EJ *et al.*, "Human Pancreatic Islet-Derived Progenitor Cell Engraftment in Immunocompetent Mice", *Am J Pathol* 164, 817–830, March 2004

[8] Kodama S *et al.*, "Islet regeneration during the reversal of autoimmune diabetes in NOD mice", *Science* 302, 1223-1227; 14 Nov 2003

[9] Shyu W-C *et al.*, "Functional recovery of stroke rats induced by granulocyte colony-stimulating factor-stimulated stem cells", *Circulation* 110, 1847-1854, 2004

An interesting report on use of a small molecule termed "reversine" indicated the potential of transforming committed adult cells to a stem cell-like state; in the report the researchers induced committed myogenic cells to revert to a multipotent state that could form bone and fat lineages.[10]

Another group reported on the potential ability of olfactory ensheathing cells to promote repair of spinal cord injury, in this case by inducing axonal sprouting.[11]

Building on previous reports of the ability of adult stem cells to repair components of the visual system, a new report shows use of bone marrow-derived stem cells to rescue retinal degeneration in animals.[12]

The potential use of adipose tissue continues to generate papers, including a report on the use of adipose-derived stem cells to heal bone defects in mice.[13]

The potential of adult stem cells for repair of kidney damage was reported by an Italian-UK team.[14]

Reports continue on the use of adult stem cells for repair of heart damage, in this case by another group from Germany.[15]

Construction of new corneas for patients using corneal limbal stem cells has continued to improve, and now a new report indicates that corneas can be constructed and used to treat patients starting with oral mucosa as the cell source.[16]

A very interesting case of patient reconstructive treatment was reported by a German-Australian team. The patient's jaw was reconstructed using a titanium mold seeded with bone marrow stem cells to regrow the jaw and provide blood vessel formation.[17] After transplantation the patient was able to eat solid food for the first time in years.

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[10] Chen S *et al.*, "Dedifferentiation of lineage-committed cells by a small molecule," *Journal of the American Chemical Society* published online December 2003.

[11] Chuah MI *et al.*, "Olfactory ensheathing cells promote collateral axonal branching in the injured adult rat spinal cord," *Experimental Neurology* 185, 15-25, 2004

[12] Otani A *et al.*, "Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells", *J. Clinical Investigation* 114, 765-774, September 2004

[13] Cowan CM *et al.*, "Adipose-derived adult stromal cells heal critical-size mouse calvarial defects," *Nature Biotechnology* 22, 560-567, May 2004

[14] Morigi M *et al.*, "Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure", *J Am Soc Nephrol* 15, 1794-1804, 2004

[15] Wollert KC *et al.*, "Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial", *Lancet* 364, 141-148, 10 July 2004

[16] Nishida K *et al.*, "Corneal reconstruction with tissue-engineered cell sheets composed of autologous mucosal epithelium," *New England Journal of Medicine* 351, 1187-1196, 16 September 2004

[17] Warnke PH *et al.*, "Growth and transplantation of a custom vascularised bone graft in a man," *Lancet* 364, 766-770, 28 August 2004

Appendix K.

Adult Stem Cells

DAVID A. PRENTICE, PH.D.
Professor of Life Sciences at Indiana State University, Terre Haute, Indiana

Within just a few years, the possibility that the human body contains cells that can repair and regenerate damaged and diseased tissue has gone from an unlikely proposition to a virtual certainty. Adult stem cells have been isolated from numerous adult tissues, umbilical cord, and other non-embryonic sources, and have demonstrated a surprising ability for transformation into other tissue and cell types and for repair of damaged tissues. This paper will examine the published literature regarding the identity of adult stem cells and possible mechanisms for their observed differentiation into tissue types other than their tissue of origin. Reported data from both human and animal studies will be presented on the various tissue sources of adult stem cells and the differentiation and repair abilities for each source, especially with regards to current and potential therapeutic treatments.

Adult stem cells have received intense scrutiny over the past few years due to surprising discoveries regarding heretofore unknown abilities to form multiple cell and tissue types, as well as the discovery of such cells in an increasing number of tissues. The term "adult stem cell" is somewhat of a misnomer, because the cells are present even in infants and similar cells exist in umbilical cord and placenta. More accurate terms have been proposed, such as tissue stem cells, somatic stem cells, or post-natal stem cells. However, because of common usage this review will continue to use the term adult stem cell.

This paper will review the literature related to adult stem cells, including current and potential clinical applications (with apologies to the many who are not cited, due to the exponential increase in papers regarding adult stem cells and the limitations of this review.) The focus will be on human adult stem cells, but will

also include results from animal studies which bear on the potential of adult stem cells to be used therapeutically for patients.

This paper will not attempt to review the literature related to hematopoietic stem cells, *i.e.*, the bone marrow stem cell that is the immediate precursor for blood cells, and the formation of typical blood cells. Nor will this paper review the substantial literature regarding clinical use of bone marrow or bone marrow stem cell transplants for hematopoietic conditions such as various cancers and anemias, nor the striking clinical results seen for conditions such as scleromyxedema, multiple sclerosis, systemic lupus, arthritis, Crohn's disease, etc.[1] In these instances, the stem cells are used primarily to replace the hematopoietic system of the patient, after ablation of the patient's own bone marrow hematopoietic system. Finally, multipotent adult progenitor cells (MAPC's), a bone marrow stem cell that has shown significant abilities at proliferation in culture and differentiation into other body tissues,[2] have been reviewed by Dr. Catherine Verfaillie in a separate paper for the President's Council on Bioethics, and the reader is directed to that review for more information.

Key questions regarding adult stem cells are: (1) their identity, (2) their tissue source of origin, (3) their ability to form other cell or tissue types, and (4) the mechanisms behind such changes in differentiation and effects on tissues and organs. Historically only a few stem cells were recognized in humans, such as the hematopoietic stem cell which produces all of the blood cell types, the gastrointestinal stem cell associated with regeneration of the gastrointestinal lining, the stem cell responsible for the epidermal layer of skin, and germ cell precursors (in the adult human, the spermatogonial stem cell.) These stem cells were considered to have very limited repertoires, related to replenishment of cells within their tissue of origin. These limitations were considered to be a normal part of the developmental paradigm in which cells become more and more restricted in their lineage capabilities, leading to defined and specific differentiated cells in body tissues. Thus, discovery of stem cells in other tissues, or with the ability to cross typical lineage boundaries, is both exciting and confusing because such evidence challenges the canonical developmental paradigm.

STEM CELL MARKERS

Identification of cells typically relies on use of cell surface markers—cellular differentiation (CD) antigens—that denote the expression of particular proteins associated with genomic activity

related to a particular differentiation state of the cell. Identification also has relied on morphological and molecular indications of function, such as expression of specific enzymes. Since stem cells by definition have not yet taken on a specific differentiated function, their identification has relied primarily on use of cell surface markers, and only secondarily on production of differentiated products in various tissues. One stated goal has been to isolate a single putative adult stem cell, characterized fully by specific markers and molecular characteristics, and then to follow the differentiation of this single cell (and/or its progeny) to show that it indeed has multipotent or pluripotent capabilities (clonogenic ability). For bone marrow stem cells, selection of putative adult stem cells has usually excluded typical markers for hematopoietic lineages (lin^-), CD45, CD38, with inclusion or exclusion of the hematopoietic marker CD34 and inclusion of the marker c-kit (CD117). Other proposed markers for adult stem cells are AC133-2 (CD133), which is found on many stem cell populations,[3] and $C1qR_p$, the receptor for complement molecule C1q,[4] found on a subset of $CD34^{+/-}$ human stem cells from bone marrow and umbilical cord blood. When transplanted into immunodeficient mice, $C1qR_p$-positive human stem cells formed not only hematopoietic cells but also human hepatocytes. Other methods of isolation and identification include the ability of putative stem cells to exclude fluorescent dyes (rhodamine 123, Hoechst 33342), allowing isolation by fluorescence-activated cell sorter (FACS) of a "side population" of cells within a tissue that have stem cell characteristics. Expression of the *Bcrp1* gene (ABCG2 gene in humans) is apparently responsible for this dye exclusion, and could provide a common molecular expression marker for stem cells[5]. A study of expressed genes from a single cell-derived colony of human mesenchymal stem cells identified transcripts from numerous cell lineages,[6] and a similar attempt at profiling the gene expression of human neural stem cell in culture with leukemia inhibitory factor (LIF) has been done,[7] perhaps providing an expressed molecular milieu which could identify candidate stem cells. Attempts to determine the complete molecular signature of gene expression common to human and mouse stem cells have shown over 200 common genes between hematopoietic and neural stem cells, with some considerable overlap with mouse embryonic stem cells as well.[8] The function of many of these genes is as yet unknown, but may provide distinctive markers for identification of adult stem cells in different tissues.

However, dependence on particular markers for prospective identification and isolation of adult stem cells seems unreliable. In particular, the use of specific hematopoietic markers such as the

presence or absence of CD34, has yielded mixed results in terms of the identification of putative stem cells. There is evidence that the expression of CD34 and CD133 can actually change over time, and its expression may be part of a cycling phenomenon among human hematopoietic and mesenchymal stem cells in the bone marrow and peripheral blood, and perhaps in other tissues,[9] *i.e.*, an isolated $CD34^+$ cell may become $CD34^-$, and then reacquire CD34 expression. Likewise, a systematic analysis of the cell surface markers and differentiation potential of supposedly distinct isolated populations of human bone marrow stem cells revealed no differences in practice between the cell populations.[10] Moreover, an analysis of genetic and ultrastructural characteristics of human mesenchymal stem cells undergoing differentiation and dedifferentiation has revealed reversibility in the characteristics studied.[11] Thus, any attempt to isolate a single type of adult stem cell for study may not actually capture the intended cell, or may, by using a particular set of isolation or growth conditions, alter its gene expression. This idea has been elaborated by Thiese and Krause,[12] who note that this "uncertainty principle" means any attempt to isolate and characterize a cell necessarily alters its environment, and thereby potentially its gene expression, identity, and potential ability to differentiate along various lineages. Likewise, the stochastic nature of cell differentiation in such dynamic and interacting systems means that attempts to delineate differentiation pathways must include descriptions of each parameter associated with the conditions used, and still may lead only to a probabilistic outcome for differentiation of a stem cell into a particular tissue. Blau *et al.*[13] have raised the question of whether there may be a "universal" adult stem cell, residing in multiple tissues and activated dependent on cellular signals, *e.g.*, tissue injury. When recruited to a tissue, the stem cell would take its cues from the local tissue milieu in which it finds itself (including the soluble growth factors, extracellular matrix, and cell-cell contacts.) Examples of such environmental influences on fate choice have been noted previously.[14] Thus, it may not be surprising to see examples of cells isolated using the same marker set showing disparate differentiative potentials,[15,16,17,18] based on the context of the isolation or experimental conditions, or to see cells with different marker sets showing similar differentiation. In the final analysis, description of a "stem cell", its actual tissue of origin, and even its differentiation ability, may be a moving target describable only within the context of the particular experimental paradigm used, and may require asking the correct questions in context of the cell's identity and abilities not clonally but rather within a population of cells, and within a certain environment.[12,19]

Given the uncertainties involved in isolating and identifying particular adult stem cells, Moore and Quesenberry[20] suggest that we consider an adult stem cell's functional ability to be, at a minimum, taking on the morphology and cell markers of a differentiated tissue, supplemented by any further functional activity and interaction within a tissue. Certainly a physiological response by improvement of function in a damaged organ system is an indication of a functional response.[19,20] As will be discussed later, the function and therapeutic benefit may not necessarily require direct differentiation and integration of an adult stem cell into a desired tissue, but could be accomplished by stimulation of endogenous cells within the tissue.

DIFFERENTIATION MECHANISMS

Several possible mechanisms have been proposed for differentiation of adult stem cells into other tissues. One mechanism that has received attention lately is the possibility of cell fusion, whereby the stem cell fuses with a tissue cell and takes on that tissue's characteristics. *In vitro* experiments using fusion of somatic cells with embryonic stem cells and embryonic germ cells[21] have demonstrated that the cell hybrid can take on characteristics of the more primitively developed cell. However, given that such characteristics of spontaneous cell fusion hybrids *in vitro* have been known for quite some time,[22] and that a cell fusion hybrid does not explain *in vitro* differentiation of adult stem cells unexposed to tissues, the experiments could not verify this as a possible mechanism for adult stem cell differentiation. More recently, *in vivo* experiments have shown that for liver,[23] formation of a cell fusion hybrid is a viable explanation for some of the differentiation as well as repair of liver damage seen in these experiments. In an *in vitro* experiment where human mesenchymal stem cells were co-cultured with heat-shocked small airway epithelial cells, a mixed answer was obtained—some of the stem cells differentiated directly into epithelial cells, while others formed cell fusion hybrids to repair the damage.[24] The ability to form cell hybrids in some tissues may be a useful mechanism for repair of certain types of tissue damage or for delivery of therapeutic genes to a tissue.[25] The reprogramming of cellular gene expression via hybrids is not unlike a novel method reported recently for transdifferentiation of somatic cells. In this method, fibroblasts were soaked in the cytoplasm and nucleoplasm of a lysed, differentiated T lymphocyte cell, taking up factors from the exposed "soup" of the cellular contents of the differentiated cell, and began expressing functional characteristics of a T cell.[26]

In contrast to the results discussed above, other experiments have shown no evidence that cell fusion plays a role in differentiation of adult stem cells into other tissue types. For example, using human subjects it was shown that human bone marrow cells differentiated into buccal epithelial cells *in vivo* without cell fusion,[27] and human cord blood stem cells formed hepatocytes in mouse liver without evidence of cell fusion.[28] In these cases it appears that the adult stem cells underwent changes in gene expression and directly differentiated into the host tissue cell type, integrating into the tissue. It is likely that the mechanism of adult stem cell differentiation may vary depending on the target tissue, or possibly on the state of the adult stem cell used, especially given that normal functioning liver typically shows cell fusion hybrids, with cell fusion functioning as a mechanism for most of the differentiation and repair in tissues such as liver, and direct differentiation (transdifferentiation) into other cell types functioning in other tissues. Much remains to be determined regarding the mechanisms associated with adult stem cell differentiation.

Keeping in mind the uncertainties noted above for identification of a particular adult stem cell and its initial tissue of origin, the majority of this review will focus on some of the evidence for adult stem cell differentiation into other tissues. The cells will be categorized based on general tissue of isolation, with the primary emphasis on human adult stem cells, supplemented with information from animal studies.

BONE MARROW STEM CELLS

Bone marrow contains at least two, and likely more,[2,29] discernable stem cell populations. Besides the hematopoietic stem cell which produces blood cell progeny, a cell type termed mesenchymal or stromal also exists in marrow. This cell provides support for hematopoietic and other cells within the marrow, and has also been a focus for possible tissue repair.[30] Isolation is typically based on some cell surface markers, but also primarily on the ability of these cells to form adherent cell layers in culture. Human mesenchymal stem cells have been shown to differentiate *in vitro* into various cell lineages including neuronal cells,[31,32] as well as cartilage, bone, and fat lineages.[33] *In vivo*, human adult mesenchymal stem cells transferred *in utero* into fetal sheep can integrate into multiple tissues, persisting for over a year. The cells differentiated into cardiac and skeletal muscle, bone marrow stromal cells, fat cells, thymic epithelial cells, and cartilage cells. Analysis of a highly purified preparation of human mesenchymal stem cells[34]

indicated that they could proliferate extensively in culture, constitutively expressing the telomerase enzyme, and even after extensive culture retained the ability to differentiate *in vitro* into bone, fat, and cartilage cells. Isolated colonies of the cells formed bone when injected into immunodeficient mice. Expanding on their previous *in vitro* work with rat and human mesenchymal/stromal stem cells, Woodbury *et al.*[35] performed molecular analyses of rat stromal stem cells and found that the cells express genes associated with all three primary germ layers—mesodermal, ectodermal, and endodermal—as well as a gene associated for germinal cells. The gene expression pattern was also seen in a clonal population of cells, indicating that it was not due to an initial mixed population of cells, but was the typical gene expression pattern of the stromal cells. The results suggested that the stromal stem cells were already multidifferentiated and that switching to a neuronal differentiation pattern involved quantitative regulation of existing gene expression patterns. Koc *et al.*[36] have used infusion of allogeneic donor mesenchymal stem cells in an attempt to correct some of the skeletal and neurological defects associated with Hurler syndrome (mucopolysaccharidosis type-IH) and metachromatic leukodystrophy (MLD). A total of 11 patients received donor mesenchymal stem cells, expanded from bone marrow aspirate. Four patients showed significant improvements in nerve conduction velocities, and all patients showed maintenance or slight improvement in bone mineral density.

Bone marrow-derived cells in general have shown ability to form many tissues in the body. For example, bone marrow-derived stem cells *in vivo* appear able to form neuronal tissues,[18,37] and a single adult bone marrow stem cell can contribute to tissues as diverse as marrow, liver, skin, and digestive tract.[16] One group has now developed a method for large-scale generation of neuronal precursors from whole adult rat bone marrow.[38] In this procedure, treatment of unfractionated bone marrow in culture with epidermal growth factor and basic fibroblast growth factor gave rise to neurospheres with cells expressing neuronal markers.

In vivo studies using fluorescence and genetic tracking of adult stem cells in animals, and tracking of the Y chromosome in humans, has shown that bone marrow stem cells can contribute to numerous adult tissues. Follow-up of patients receiving adult bone marrow stem cell transplants has allowed tracking of adult stem cells within humans, primarily by identification of Y chromosome-bearing cells in female patients who had received bone marrow stem cells from male donors. Biopsy or postmortem samples show that

some of the transplanted bone marrow stem cells could form liver, skin, and digestive tract cells,[39] as well as participate in the generation of new neurons within the human brain.[40] Bone marrow stem cells have also been shown to contribute to Purkinje cells in the brains of adult mice[41] and humans[42]. Generation of this particular type of neural cell is significant in that new Purkinje cells do not normally appear to be generated after birth.

Regeneration or replacement of dead or damaged cells is the primary goal of regenerative medicine and one of the prime motivations for study of stem cells. It is thus of significant interest that bone marrow stem cells have shown the ability to produce therapeutic benefit in animal models of stroke. In mice, fluorescence-tracked bone marrow derived stem cells expressed neuronal antigens and also incorporated as endothelial cells, possibly producing therapeutic benefit by allowing increased blood flow to damaged areas of the brain.[43] In rats, intravenous (IV) administration of rat[44] or human[45] bone marrow stromal cells resulted in significant behavioral recovery after stroke. Interestingly, only a small percentage of the stromal stem cells appeared to incorporate into the damaged brain as neuronal cells (1-5% in the case of the human marrow stromal cells), but the levels of neurotrophin growth factors within the brains increased and were possibly the signal for repair of damaged brain tissue, perhaps by stimulation of endogenous neuronal precursors. It is also of interest that the marrow stromal cells were injected IV and not intracerebrally, indicating that the stem cells somehow "homed" to the site of tissue damage. Most studies showing adult stem cell differentiation into other tissues show an increased incorporation of cells, or even an absolute requirement for differentiation, relying on tissue damage to initiate the differentiation. This may indicate that without a "need" for replacement and repair, there is little or no activation of adult stem cells. The recruitment and homing of adult stem cells to damaged tissues are fascinating but relatively unexplained phenomena. One report[46] indicates that recruitment of quiescent stem cells from bone marrow to the circulation requires release of soluble c-kit ligand (stem cell factor), but the range of factors necessary for recruitment and homing to organs other than bone marrow is unknown at this time and warrants increased investigation.

Bone marrow stem cells have also shown the ability to participate in repair of damaged retinal tissues. When bone marrow stem cells were injected into the eyes of mice, they associated with retinal astrocytes and extensively incorporated into the vascular (blood vessel) network of the eye.[47] The cells could also rescue and

maintain normal vasculature in the eyes of mice with a degenerative vascular disease. In another animal study, bone marrow derived stem cells were observed to integrate into injured retina and differentiated into retinal neuronal cells.[48] Stromal stem cells have also shown capability in mice to repair spinal cord which was demyelinated.[49] One of the problems related to spinal cord injury is loss of the protective myelin sheath from spinal cord after injury. A mixed bone marrow stem cell fraction was injected into the area of damage in the spinal cord, and remyelination of the area was seen. In another mouse study, marrow stromal cells injected into injured spinal cord formed guiding strands within the cord;[50] interestingly, the effect was more pronounced when the stromal cells were injected 1 week after injury rather than immediately after injury.

Because bone marrow stem cells are of mesodermal lineage, it is not surprising that they show capabilities at forming other tissues of mesodermal origin. Human marrow stromal cells, which have been shown to form cartilage cells, have been used in an *in vitro* system to define many of the molecular events associated with formation of cartilage tissue.[51] Bone marrow derived stem cells have also been shown capable of regenerating damaged muscle tissue.[52] In an elegant study following genetically marked bone marrow stem cells in mice, LaBarge and Blau were able to document multiple steps in the progression of the stem cells to form muscle fibers and repair muscle damage.[53] The ability of human bone marrow derived stem cells to form muscle cells and persist in the muscle was recently documented. In this case, a patient had received a bone marrow transplant at age 1, and developed Duchenne muscular dystrophy at age 12. Biopsies at age 14 showed donor nuclei integrated within 0.5-0.9% of the muscle fibers of the patient, indicating the ability of donated marrow cells to persist in tissue over long periods of time.[54]

Bone marrow stem cells have also shown capability at forming kidney cells. Studies following genetically marked bone marrow stem cells in rats[55] and mice[56] showed that the stem cells could form mesangial cells to repopulate the glomerulus of the kidney. In the mouse study, formation of cell fusion products was ruled out as a mechanism for differentiation of the bone marrow stem cells. Other animal studies have shown contribution of bone marrow stem cells to repair of damaged renal tubules in the kidney;[57,58] taken together, animal studies indicate that bone marrow stem cells can participate in restoring damaged kidney tissue.[59]

Liver was one of the earliest tissues recognized as showing potential contribution to differentiated cells by bone marrow stem

cells. Bone marrow stem cells have been induced to form hepatocytes in culture[60] and liver-specific gene expression has been induced *in vitro* in human bone marrow stem cells.[61] *In vivo*, bone marrow stem cells were able to incorporate into liver as hepatocytes and rescue mice from a liver enzyme deficiency, restoring normal liver function.[62] Bone marrow stem cells also repopulated liver after irradiation of mice to destroy their bone marrow.[63] Examination of livers of female patients who had received male bone marrow transplants, and male patients who had received female liver transplants, showed that similar repopulation of liver from bone marrow stem cells could take place in humans.[64] Examination of the kinetics of liver repopulation by bone marrow stem cells in a mouse model indicated that the replacement was slow, with only small numbers of cells replaced by the bone marrow stem cells.[65] As noted previously, two recent studies have found that replenishment of liver by bone marrow stem cells occurs primarily via cell fusion hybrid formation, even in repair of liver damage.[23] A side-population of stem cells has been identified in mouse liver, similar to that seen in bone marrow. This hepatic side-population, which contributes to liver regeneration, can be replenished by side-population bone marrow stem cells.[66]

Pancreas and liver arise from adjacent endoderm during embryological development, and show relatedness in some gene expression and interconversion in some instances. Bone marrow derived cells have shown the ability to form pancreatic cells in animal studies. Mouse bone marrow stem cells containing a genetic fluorescent marker that is only expressed if insulin is expressed were transplanted into irradiated female mice.[67] Within 6 weeks of transplant, fluorescent donor cells were observed in pancreatic islets; donor cells identified in bone marrow and peripheral blood did not show fluorescence. *In vitro*, the bone marrow derived cells showed glucose-dependent insulin secretion as well. Bone marrow derived stem cells have also demonstrated the ability to induce regeneration of damaged pancreas in the mouse.[68] Mice with experimentally induced hyperglycemia from pancreatic damage were treated with bone marrow derived stem cells expressing the c-kit marker. Interestingly, only a low percentage of donor cells were identified as integrating into the regenerating pancreas, with most of the regeneration due to induced proliferation and differentiation of endogenous pancreatic cell precursors, suggesting that the bone marrow stem cells provided growth signals for the tissue regeneration.

Heart, as a mesodermally-derived organ, is a likely candidate for regeneration with bone marrow derived stem cells. Numerous references now document the ability of these adult stem cells to contribute to regeneration of cardiac tissue and improve performance of damaged hearts. In animal studies, for example, rat[69], mouse[70,71,72] and human[73,74] stem cells have been identified as integrating into cardiac tissue, forming cardiomyocytes and/or cardiac blood vessels, regenerating infarcted heart tissue, and improving cardiac function. In mice, bone marrow derived stem cells injected into old animals seems capable of restoring cardiac function,[75] apparently through increased activity for cardiac blood vessel formation. One fascinating study using xenogeneic (cross-species) transplants suggests that stromal cells may show immune tolerance by the host.[76] Mouse marrow stromal cells were transplanted into fully immunocompetent rats, and contributed formation of cardiomyocytes and cardiac vessels. Even after 13 weeks, the mouse cells were not rejected by the rat hosts. Evidence has accumulated from postmortem studies that bone marrow stem cells can contribute to cardiomyocytes after damage to the human heart as well.[77,78] The evidence has led numerous groups to use bone marrow derived stem cells in treatment of patients with damaged cardiac tissue.[79,80,81,82] Results from these clinical trials indicate that bone marrow derived stem cells, including cells from the patients themselves, can regenerate damaged cardiac tissue and improve cardiac performance in humans. In terms of restoring angiogenesis and improving blood circulation, results in patients are not limited to the heart. Tateishi-Yuyama *et al.*[83] have shown that bone marrow derived stem cells from the patients themselves can improve blood circulation in gangrenous limbs, in many cases obviating the need for amputation.

Bone marrow derived adult stem cells have also been found to contribute to various other adult tissues. Animal studies indicate evidence that bone marrow stem cells can contribute as progenitors of lung epithelial tissue[84], and mesenchymal stem cells can home to damaged lung tissue, engraft, and take on an epithelial morphology, participating in repair and reduction of inflammation.[85] Bone marrow derived stem cells also have been shown to contribute to regeneration of gastrointestinal epithelia in human patients.[86] A recent study in mice has indicated that bone marrow stem cells can also participate in cutaneous healing, contributing to repair of skin after wounding.[87]

PERIPHERAL BLOOD STEM CELLS

There is abundant evidence that bone marrow stem cells can leave the marrow and enter the circulation, and specific mobilization of bone marrow stem cells is used to harvest stem cells more easily for various bone marrow stem cell treatments.[88] Therefore, it is not surprising that adult stem cells have been isolated from peripheral blood. Mobilized stem cells in peripheral blood have been administered intravenously in a rat model of stroke, ameliorating some of the behavioral deficits associated with the damaged neural tissue[89], leading to a proposal that stem cell mobilization in patients might be used as a treatment for stroke in humans.[90] Mobilized stem cells have also been used in cardiac regeneration in mice[72]. Two recent studies have found that human peripheral blood stem cells exhibiting pluripotent properties can be isolated from unmobilized human blood. One study showed that the isolated cells were adherent, similar to marrow mesenchymal cells, and could be induced to differentiate into cells from all three primary germ layers, including macrophages, T lymphocytes, epithelial cells, neuronal cells, and liver cells.[91] The other study showed induction of the peripheral blood stem cells could produce hematopoietic, neuronal, or cardiac cells in culture.[92] In the latter study, undifferentiated stem cells were negative for both major histocompatability antigens (MHC) I and II, expressed high levels of the Oct-4 gene (usually associated with pluripotent capacity in other stem cells), and could form embryoid body structures in culture.

NEURONAL STEM CELLS

One extremely interesting finding of the past few years has been the discovery of neuronal stem cells, indicating that cell replenishment was possible within the brain (something previously considered impossible.) Neuronal stem cells have been isolated from various regions of the brain including the more-accessible olfactory bulb[93] as well as the spinal cord[94], and can even be recovered from cadavers soon after death.[95] Evidence now exists that neuronal stem cells can produce not only neuronal cells but also other tissues, including blood and muscle.[96,97,98,99,100,101] Animal studies have shown that adult neural stem cells can participate in repair of damage after stroke, either via endogenous neuronal precursors[102] or transplanted neural stem cells.[103] Evidence indicates that endogenous neurons and astrocytes may also secrete growth factors to induce differentiation of endogenous precursors.[104] In

addition, two studies now provide suggestive evidence that neural stem cells/neural progenitor cells may show low immunogenicity, being immunoprivileged on transplant,[105] and raising the possibility for use of donor neural stem cells to treat degenerative brain conditions.

Pluchino *et al.*[106] recently used adult neural stem cells to test potential treatment of multiple sclerosis lesions in the brain. Using a mouse model of chronic multiple sclerosis—experimental immune encephalitis—they injected neural stem cells either intravenously or intracerebrally into affected mice. Donor cells entered damaged, demyelinated regions of the brain and differentiated into neuronal cells. Remyelination of brain lesions and recovery from functional impairment were seen in the mice. Neural stem cells have also been used to investigate potential treatments for Parkinson's disease. Using experimentally-lesioned animals as models for Parkinson's disease, human neural stem cells have been observed to integrate and survive for extended periods of time.[107] Dopaminergic cells (the cells degenerated in Parkinson's disease) can be induced in these systems,[108] and neural stem cells are capable of rescuing and preventing the degeneration of endogenous dopaminergic neurons,[109,110] also producing improved behavioral performance in the animals. In these studies, the data suggest that the transplanted neural stem cells did not participate to a large extent in direct formation of dopaminergic neurons, but rather secreted neuroprotective factors and growth factors that stimulated the endogenous neural cells. In this respect, infusion of transforming growth factor into the brains of Parkinson's mice induced proliferation and differentiation of endogenous neuronal precursors in mouse brain.[111] Following this potential for stimulation of endogenous neuronal cells, Gill *et al.* recently reported on a Phase I trial in which glial derived neurotrophic factor (GDNF) was infused into the brains of five Parkinson's patients.[112] After one year there was a 61% increase in the activities of daily living score, and an increase in dopamine storage observed in the brain. In a tantalizing clinical application with direct injection of neural stem cells, a Parkinson's patient was implanted with his own neural stem cells, resulting in an 80% reduction in symptoms at one year after treatment.[113] Further clinical trials are underway.

The olfactory ensheathing glial (OEG) cell from olfactory bulb has been used extensively in studies regarding spinal cord injury and axon regrowth. Human OEG cells can be expanded in number in culture and induced to produce all three main neural cell types.[93] Transplant of the cells into animal models of spinal cord injury has

shown that the cells can effect remyelination of demyelinated spinal cord axons,[114] and provide functional recovery in paraplegic rats,[115] including in transected spinal cords.[116] Another study has found that infusion of growth factors such as GDNF can stimulate functional regeneration of sensory axons in adult rat spinal cord.[117] Interestingly, one group has made use of the similarities between enteric glial cells and OEG cells, and shown that transplanted enteric glial cells can also promote regeneration of axons in the spinal cord of adult rats.[118] Clinical trials are underway to test the abilities of OEG cells in spinal cord injury patients. Finally, a significant impediment to recovery from spinal cord injury is the formation of a glial/astrocyte scar at the site of injury, which can prevent growth of axons no matter what the source of the cells. Menet *et al.* have shown, using a mutant mouse model, that much of the scar can be prevented by inhibition of glial fibrillary acidic protein and vimentin.[119] In mutant mice that lacked these genes, there was increased sprouting of axons and functional recovery after spinal cord injury. Thus, endogenous neural cell growth and reconnection might suffice for repair of damage if inhibitory mechanisms can be removed from neural systems.

hNT CELLS

Embryonal carcinoma (EC) cells can be derived from teratocarcinomas of adult patients, and show multipotent differentiation abilities in culture. From one such isolation, a "tamed" (non-tumorigenic) line of cells with neuronal generating capacity has been developed, termed hNT (NT-2) cells. Because of their capacity to generate neuronal cells, these cells have been studied for possible application in regeneration of neuronal tissues. The hNT neurons show the ability to generate dopaminergic neurons,[120] and have shown some benefit of transplantation in animal models of amyotrophic lateral sclerosis (ALS, Lou Gehrig's disease).[121] Early clinical trials using hNT neurons transplanted into stroke patients have shown initial positive results.[122]

MUSCLE STEM CELLS

Muscle contains satellite cells that normally participate in replacement of myoblasts and myofibers. There are also indications that muscle additionally may harbor other stem cells, either as hematopoietic migrants from bone marrow and peripheral blood, or as intrinsic stem cells of muscle tissue. Muscle appears to contain a side population of stem cells, as seen in bone marrow and liver, with the ability to regenerate muscle tissue.[123] Muscle derived stem

cells have been clonally isolated and used to enhance muscle and bone regeneration in animals.[124] An isolated population of muscle-derived stem cells has also been shown to participate in muscle regeneration in a mouse model of muscular dystrophy.[125] Stimulation of muscle regeneration from muscle-derived stem cells, as observed in other tissues, is greatly increased after injury of the tissue.[126,127] An interesting use of muscle-derived stem cells has been the regeneration and strengthening of bladder in a rat model of incontinence.[128] Because of the similar nature of muscle cells between skeletal muscle and heart muscle, muscle-derived stem cells have also been proposed for use in repairing cardiac damage,[129] with evidence that mechanical beating is necessary for full differentiation of skeletal muscle stem cells into cardiomyocytes.[130] At least one group has used skeletal muscle cells for clinical application to repair cardiac damage in a patient, with positive results.[131]

LIVER STEM CELLS

As noted before, there are similarities between liver and pancreas which could facilitate interconversion of cells between the two tissues. This concept has been demonstrated using genetic engineering to add a pancreatic development gene to liver cells, converting liver to pancreas.[132] Rat liver stem cells have been converted *in vitro* into insulin-secreting pancreatic cells.[133] When transplanted into immundeficient mice which are a model for diabetes, the converted liver stem cells were able to reverse hyperglycemia in the mice. One other interesting observation regarding liver stem cells has been the possible formation of myocytes in the heart by liver stem cells. A clonal cell line derived from adult male rat liver and genetically tagged was injected into female rats, and marked, Y-chromosome bearing myocytes were identified in the host hearts after six weeks.[134]

PANCREATIC STEM CELLS

Interconversion between pancreas and liver has also been demonstrated starting with pancreatic stem cells, in which mouse pancreatic cells repopulated the liver and corrected metabolic liver disease.[135] For pancreas, however, the possibility of solutions to the scourge of diabetes has been a driving force in efforts to define a stem cell that could regulate insulin in a normative, glucose-dependent fashion. The success of the Edmonton protocol,[136] where cadaveric pancreatic islets are transplanted into patients, has provided a glimmer of hope, but more readily-available sources of

insulin-secreting cells are needed. Fortunately, there seems to be no shortage of potential candidates that can form insulin-secreting cells. The pancreas itself appears to contain stem/progenitor cells that can regenerate islets *in vitro* and *in vivo*. Studies indicate that these pancreatic stem cells can functionally reverse insulin-dependent diabetes in mice.[137] Similar pancreatic stem cells have been isolated from humans and shown to form insulin-secreting cells *in vitro*,[138] the hormone glucagon-like peptide-1 appears to be an important inducing factor of pancreatic stem cell differentiation. Interestingly, the same hormone could induce mouse intestinal epithelial cells to convert into insulin-producing cells *in vitro*, and the cells could reverse insulin-dependent diabetes when implanted into diabetic mice.[139] Besides pancreatic and intestinal stem cells, other adult stem cell types showing the ability to secrete insulin and regenerate damaged pancreas include bone marrow[57,58] and liver.[133] Genetic engineering of rat liver cells to contain the pancreatic gene PDX-1 has also been used to generate insulin-secreting cells *in vitro*; the cells could also restore normal blood glucose levels when injected into mice with experimentally-induced diabetes.[140]

CORNEAL LIMBAL STEM CELLS

Corneal limbal stem cells have become commonly used for replacement of corneas, especially in cases where cadaveric donor corneas are insufficient. Limbal cells can be maintained and cell number expanded in culture,[141] grown on amniotic membranes to form new corneas, and transplanted to patients with good success.[142] A recent report indicates that human corneal stem cells can also display properties of functional neuronal cells in culture.[143] Another report found that limbal epithelial cells or retinal cells transplanted into retina of rats could incorporate and integrate into damaged retina, but did not incorporate into normal retina.[144]

MAMMARY STEM CELLS

Reports have indicated that mammary stem cells also exist. Isolated cells from mouse could be propagated *in vitro* and differentiated into all three mammary epithelial lineages.[145] Clonally-propagated cells were induced in culture to generate complex three-dimensional structures similar to that seen *in vivo*. Transcriptional profiling indicated that the mammary stem cells showed similar gene expression profiles to those of bone marrow stem cells. In that respect, there is a report that human and mouse mammary stem cells exist as a side population, as seen for bone marrow, liver, and muscle stem cells.[146] When propagated in culture, the isolated

mammary side population stem cells could form epithelial ductal structures.

SALIVARY GLAND

A recent report indicates that stem cells can be isolated by limiting dilution from regenerating rat salivary gland and propagated *in vitro*.[147] Under differing culture conditions, the cells express genes typical of liver or pancreas, and when injected into rats can integrate into liver tissue.

SKIN

Multipotent adult stem cells have been isolated from the dermis and hair follicle of rodents.[148] The cells play a role in maintenance of epidermal and hair follicle structures, can be propagated *in vitro,* and clonally isolated stem cells can be induced to form neurons, glia, smooth muscle, and adipocytes in culture. Dermal hair follicle stem cells have also shown the ability to reform the hematopoietic system of myeloablated mice.[149]

TENDON

A recent report notes the isolation of established stem cell-like lines from mouse tendon. The cells exhibited a mesenchymal morphology, and expressed genes related to osteogenic, chondrogenic, and adipogenic potential, similar to that seen in bone marrow mesenchymal stem cells.[150]

SYNOVIAL MEMBRANE

Stem cells from human synovial membrane (knee joint) have been isolated which show multipotent abilities for differentiation, including evidence of myogenic potential.[151] These stem cells were used in a mouse model of Duchenne muscular dystrophy to test their ability to repair damaged muscle. Stem cells injected into the bloodstream could engraft and incorporate into muscle, taking on a muscle phenotype, and with evidence of muscle repair.[152]

HEART

Beltrami *et al.* analyzed the hearts of post-mortem patients who succumbed 4-12 days after heart attack, and found evidence of dividing myocytes in the human heart. While it is unclear from the study whether the cells were originally cardiomyocytes or were other

stem cells which had homed to damaged heart tissue, such as bone marrow stem cells, the evidence indicated dividing cells within the heart.[153]

CARTILAGE

Human cartilage biopsies placed into culture show apparent dedifferentiation into primitive chondrocytes with mesenchymal stem cell appearance.[154] These chondrocytes have been used for transplants to repair articular cartilage damage, and in treatment of children with osteogenesis imperfecta.[155,156,157]

THYMIC PROGENITORS

Bennett *et al.* have reported the isolation of thymic epithelial progenitor cells.[158] Ectopic grafting (under the kidney capsule) of the cells into mice allowed production of all thymic epithelial cell types, as well as attraction of homing T lymphocytes. In separate experiments, Gill *et al.* also isolated a putative thymic progenitor cell from mice and were able to use these cells to reform miniature thymuses when the cells were transplanted under mouse kidney capsule.[159]

DENTAL PULP STEM CELLS

Stem cells have been isolated from human adult dental pulp that could be clonally propagated and proliferated rapidly.[160] Though there were some similarities with bone marrow mesenchymal stem cells, when injected into immunodeficient mice the adult dental pulp stem cells formed primarily dentin-like structures surrounded by pulpy interstitial tissue. Human baby teeth have also been identified as a source of stem cells, designated SHED cells (Stem cells from Human Exfoliated Deciduous teeth).[161] *In vitro*, SHED cells could generate neuronal cells, adipocytes, and odontoblasts, and after injection into immunodeficient mice, the cells were indicated in formation of bone, dentin, and neural cells.

ADIPOSE (FAT) DERIVED STEM CELLS

One of the more interesting sources identified for human stem cells has been adipose (fat) tissue, in particular liposuctioned fat. While there is some debate as to whether the cells originate in the fat tissue or are perhaps mesenchymal or peripheral blood stem cells passing through the fat tissue, they represent a readily-available source for isolation of potentially useful stem cells. The cells can be

maintained for extended periods of time in culture, have a mesenchymal-like morphology, and can be induced *in vitro* to form adipose, cartilage, muscle, and bone tissue.[162] The cells have also shown the capability of differentiation into neuronal cells.[163]

UMBILICAL CORD BLOOD

Use of umbilical cord stem cells has seen increasing interest, as the cells have been recognized as a useful source for hematopoietic transplants similar to bone marrow stem cell transplants, including for treatment of sickle cell anemia.[164] Cord blood shows decreased graft-versus-host reaction compared to bone marrow,[165] perhaps due to high interleukin-10 levels produced by the cells.[166] Another possibility for the decreased rejection seen with cord blood stem cell transplants is decreased expression of the beta-2-microglobulin on human cord blood stem cells.[167] Cord blood can be cryopreserved for over 15 years and retain significant functional potency.[168] Cord blood stem cells also show similarities with bone marrow stem cells in terms of their potential to differentiate into other tissue types. Human cord blood stem cells have shown expression of neural markers *in vitro*,[169] and intravenous administration of cord blood to animal models of stroke has produced functional recovery in the animals.[89,170] Infusion of human cord blood stem cells has also produced therapeutic benefit in rats with spinal cord injury,[171] and in a mouse model of ALS.[172] A recent report noted establishment of a neural stem/progenitor cell line derived from human cord blood that has been maintained in culture over two years without loss of differentiation ability.[173] Several reports also note the production of functional liver cells from human cord blood stem cells.[174] Additional differentiative properties of human umbilical cord blood stem cells are likely to be discovered as more investigation proceeds on this source of stem cells.

UMBILICAL CORD MESENCHYME (WHARTON'S JELLY)

While most of the focus regarding umbilical cord stem cells has focused on the cord blood, there are also reports that the matrix cells from umbilical cord contain potentially useful stem cells. Using pigs, this matrix from umbilical cord, termed Wharton's jelly, has been a source for isolation of mesenchymal stem cells. The cells express typical stem cell markers such as c-kit and high telomerase activity, have been propagated in culture for over 80 population doublings, and can be induced to form neurons *in vitro*.[175] When transplanted into rats, the cells expressed neuronal markers and

integrated into the rat brain, additionally without any evidence of rejection.[176]

AMNIOTIC STEM CELLS

Amniotic fluid has also been found to contain stem cells that can take on neuronal properties when injected into brain.[177] These stem cells were recently isolated from human amniotic fluid,[178] and were found to express Oct-4, a gene typically associated with expression in pluripotent stem cells.

MESANGIOBLASTS

Mesangioblasts are a multipotent stem cell that has been isolated from large blood vessels such as dorsal aorta.[179] The cells show long term proliferative capacity in culture as well as the capability of differentiation into most mesodermally derived types of tissue. In a recent report, the cells were injected into the bloodstream of mice that are a model for muscular dystrophy,[180] and participated in repair of the muscle tissue.

Adult stem cells in other tissues very likely exist, but this survey of many of the known adult stem cells and their capacities for differentiation and tissue repair can serve as a beginning point for discussion regarding the progress as well as potential of adult stem cells. Some final thoughts on current and potential utilization of adult stem cells follow.

ADULT STEM CELL MOBILIZATION FOR TISSUE REPAIR

An important point to consider as we look ahead regarding utilization of adult stem cells for tissue repair is that it may be unnecessary first to isolate and culture stem cells before injecting them back into a patient to initiate tissue repair. Rather, it may be easier and preferable to mobilize endogenous stem cells for repair of damaged tissue. Initial results regarding this possibility have already been seen in some animal experiments, in which bone marrow and peripheral blood stem cells were mobilized with injections of growth factors and participated in repair of heart and stroke damage.[72,89,90] The ability to mobilize endogenous stem cells, coupled with natural or perhaps induced targeted homing of the cells to damaged tissue, could greatly facilitate use of adult stem cells in simplified tissue regeneration schemes.[181]

GENE THERAPY APPLICATIONS WITH ADULT STEM CELLS

Adult stem cells can provide an efficient vehicle for gene therapy applications, and engineered adult stem cells may allow increased functionality, proliferative capacity, or stimulatory capability to these cells. The feasibility of genetically engineering adult stem cells has been shown, for example, in the use of bone marrow stem cells containing stably inserted genes. The engineered stem cells when injected into mice could still participate in formation and repair of differentiated tissue, such as in lung.[182] As another example, engineered stem cells containing an autoantigen, to induce immune tolerance of T cells to insulin-secreting cells, were shown to prevent onset of diabetes in a mouse model of diabetes,[183] a strategy that may be useful for various human autoimmune diseases. Introduction of the PDX-1 gene into liver stem cells stimulated differentiation into insulin-producing cells which could normalize glucose levels when transplanted into mice with induced diabetes.[140] Simply engineering cells to increase their proliferative capacity can have a significant effect on their utility for tissue engineering and repair. For example, McKee *et al.*[184] engineered human smooth muscle cells by introducing human telomerase, which greatly increased their proliferative capacity beyond the normal lifespan of smooth muscle cells in culture, while allowing retention of their normal smooth muscle characteristics. These engineered smooth muscle cells were seeded onto biopolymer scaffolds and allowed to grow into smooth muscle layers, then seeded with human umbilical vein endothelial cells. The resulting engineered arterial vessels could be useful for transplants and bypass surgery. Similarly, human marrow stromal cells that were engineered with telomerase increased their proliferative capacity significantly, but also showed enhanced ability at stimulating bone formation in experimental animals.[185] Genetically-engineered human adult stem cells have already been used in successful treatment of patients with genetic disease. Bone marrow stem cells, from infants with forms of severe combined immunodeficiency syndrome (SCID), were removed from the patients, a functional gene inserted, and the engineered cells reintroduced to the same patients. The stem cells homed to the bone marrow, engrafted, and corrected the defect.[186,187,188]

Adult stem cells could also be used to deliver stimulatory or protective factors to tissues and endogenous stem cells. This would utilize the innate homing ability of adult stem cells, but would not necessarily rely on differentiation of the stem cells to participate in tissue replenishment. For example, Benedetti *et al.* utilized the

homing capacity of neural stem cells in brain by engineering mouse neural stem cells with the gene for interleukin-4. Transfer into brain glioblastomas in mice led to the survival of most of the mice, and imaging analysis documented the progressive disappearance of large tumors.[189] Likewise, engineered mesenchymal stem cells were transplanted into the brains of mice that are a model of Niemann-Pick disease; the enzyme acid sphingomyelinase is lost in the disease, resulting in neurological damage and early death. The mesenchymal stem cells were engineered to overexpress the missing enzyme. When injected into brains of the mouse model, the mice showed a delay in onset of neurological abnormalities and an extension of lifespan, suggesting that the stem cells delivered and secreted the necessary enzyme to the brain tissue.[190] Muscle-derived stem cells that were engineered to express the growth factor bone morphogenetic protein-2 were used to stimulate bone healing in mice with skull bone defects. While the muscle-derived stem cells did show differentiation as bone cells, the results indicated that the critical factor was delivery of the secreted growth factor by the stem cells to the areas of bone damage, allowing much more rapid healing than in control animals.[191] As noted previously, neural stem cells show an ability to rescue degenerating neurons, including the dopaminergic neurons whose loss is associated with Parkinson's disease. The delivery of neuroprotective substances is postulated as the most likely explanation for this phenomenon, rather than substantial differentiation by the injected neural stem cells.[109] In support of this hypothesis, when neural stem cells were specifically engineered to overexpress a neurotrophic factor similar to glial derived neurotrophic factor, degeneration of dopaminergic neurons was prevented.[110]

STIMULATING ENDOGENOUS CELLS

The indications from the previous examples suggest that direct stimulation of endogenous stem cells within a tissue may be the easiest, safest, and most efficient way to stimulate tissue regeneration. Such stimulation need not rely on any added stem cells. This approach would circumvent the need to isolate or grow stem cells in culture, or inject any stem cells into the body, whether the cells were derived from the patient or another source. Moreover, direct stimulation of endogenous tissue stem cells with specific growth factors might even preclude any need to mobilize stem cells to a site of tissue damage. A few experimental results suggest that this approach might be possible. One group has reported that use of glial derived neurotrophic factor and neurotrophin-3 can stimulate regeneration of sensory axons in adult rat spinal cord.[117]

Administration of transforming growth factor to the brains of Parkinson's mice stimulated proliferation and differentiation of endogenous neuronal stem cells and produced therapeutic results in the mice,[111] and infusion of glial derived neurotrophic factor into the brains of Parkinson's patients resulted in increased dopamine production within the brain and therapeutic benefit to the patients.[112] And, Zeisberg *et al.* have found that bone morphogenetic protein-7 (BMP-7) can counteract deleterious cell changes associated with tissue damage. In this latter study, a mouse model of chronic kidney damage was used. Damage to the tissue causes a transition from epithelial to mesenchymal cell types in the kidney, leading to fibrosis. The transition appears to be initiated by the action of transforming growth factor beta-1 on the tissues, and BMP-7 was shown to counteract this signaling *in vitro*. Systemic administration of BMP-7 in the mouse model reversed the transition *in vivo* and led to repair of severely damaged renal tubule epithelial cells.[192] These experiments indicate that direct stimulation of tissues by the correct growth factors could be sufficient to prevent or repair tissue damage. The key to such treatments would be identification of the correct stimuli specific to a tissue or cell type.

In summary, our current knowledge regarding adult stem cells has expanded greatly over what was known just a few short years ago. Results from both animal studies and early human clinical trials indicate that they have significant capabilities for growth, repair, and regeneration of damaged cells and tissues in the body, akin to a built-in repair kit or maintenance crew that only needs activation and stimulation to accomplish repair of damage. The potential of adult stem cells to impact medicine in this respect is enormous.

Adult Stem Cells—Addendum (October 2003)
For the President's Council on Bioethics
David A. Prentice

Since initial submission of the commissioned paper, numerous additional published references have documented the abilities of adult stem cells to stimulate regeneration of damaged tissues. Just a few of the most significant are mentioned here. Mesenchymal stem cells engineered to express the *Akt1* gene, when transplanted into mice, demonstrated the ability to repair and restore performance of infarcted heart, essentially to a normal state.[a] Another clinical trial in addition to those mentioned in the paper has shown significant improvement in patients with heart damage, with reduction in the area of damage and improved heart function after adult stem cell treatment.[b] Three more published articles support the existence of a stem cell in the heart and its participation in cardiac regeneration.[c] Stroke damage in rats was repaired using human neural stem cells[d] and prostate was regenerated *in vivo* in mice using adult stem cells.[e] Another report indicates that human mixed bone marrow stem cells can contribute significant amounts of lung tissue in patients[f] and pluripotent stem cells were discovered in the mouse inner ear[g], which can form all 3 primary germ layers and might lead to potential therapies for hearing loss. Finally, bone marrow stem cells were discovered to have a protective as well as regenerative role in diabetes.[h]

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Current Science of Regenerative Medicine with Stem Cells

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ABSTRACT

Regenerative medicine with stem cells holds great hope for the treatment of degenerative disease. The medical potential of embryonic stem cells remains relatively untapped at this point, and significant scientific hurdles remain to be overcome before these cells might be considered safe and effective for uses in patients. Meanwhile, adult stem cells have begun to show significant capabilities of their own in repair of damaged tissues, in both animal models and early patient trials.

Key Words: regenerative medicine, stem cells, stem cell transplant

Regenerative medicine holds great hope for millions of patients with degenerative diseases and injuries. Repair of damaged organs and tissues using stem cells could potentially address the needs of these patients, encompassing most of the top 15 leading causes of death in the United States. However, the emotional appeal of stem cells and the political debate in which the science is embroiled have clouded much of the actual results in this area. It is imperative that a complete review of the scientific results and potential promises be a part of any fully informed debate.

A stem cell has two chief characteristics: (1) it continues to proliferate so that a pool of cells is always available and (2) it responds to appropriate signals by differentiating into one or more specialized cell types (Figure 1A). Numerous sources of human stem cells exist, including those from early (5–7 day postconception) embryos, fetal tissues, umbilical cord blood and matrix, placental tissues, and most or all body tissues; postnatal sources are often grouped together under the term "adult stem cells" (Figure 1B). The "plasticity" of a stem cell, that is, its ability to form differentiated cell types, ranges from unipotent (able to form only one differentiated type), to multipotent (able to form multiple cell types), to pluripotent (able to form most or all tissues of the adult body), to totipotent (able to form all postnatal and extraembryonic tissues, potentially able to regenerate a complete new embryo).

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DOI = 10.2310/6650.2005.05043

EMBRYONIC STEM CELLS

Mouse embryonic stem (ES) cells were first grown in culture in 1981,[1,2] but human ES cells were not successfully cultured until 1998.[3] Isolation of ES cells requires the disaggregation of the early embryo—hence the ethical debate regarding these cells. At about the same time, another team successfully cultured stem cells, termed embryonic germ cells, with similar properties from fetal primordial germ cells.[4] ES cells are considered the archetypal pluripotent stem cell; they proliferate extensively in culture and, based on their normal function during development or results from reinsertion into another embryo, have the potential to form any tissue. Although this potential is attractive for treatment of degenerative disease, the results to this point have been modest, and there are still many scientific hurdles to overcome before ES cells might be used clinically, including generation of functional differentiated cells, tumor formation, and immune rejection.[5] The best examples of potential success to date are in animal models of spinal cord injury and Parkinson's disease. Keirstead and colleagues showed some success at ameliorating acute (although not chronic) spinal cord injury in rats, including improvement in locomotor activity,[6] and

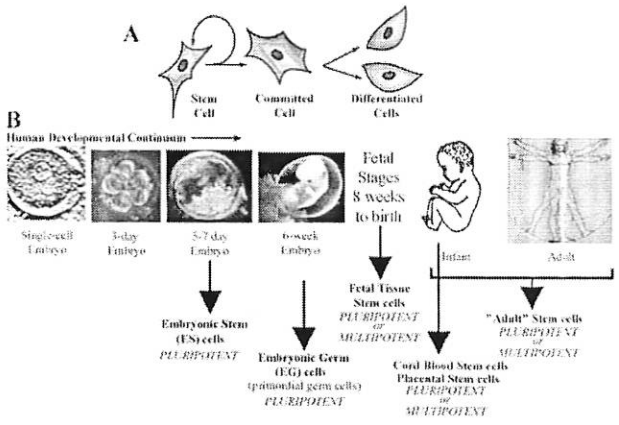

FIGURE 1 Characteristics and sources of stem cells. *A*), Stem cells maintain proliferation (***circular arrow***) and respond to differentiation signals (*arrow to right*). *B*), Sources include embryos, primordial germ cells, differentiated fetal tissue, and "adult" stem cells, including umbilical cord matrix and blood, placenta, and postnatal body tissues.

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Nistor and colleagues showed remyelinating activity of human ES cells in a rat model.[7] In animal models of Parkinson's disease, ES cells have been successfully transplanted and achieved dopamine secretion, alleviating some of the behavioral symptoms in monkeys[8] and rats,[9] although in the latter example, the ES cells stopped growth after 12 weeks. However, some experiments, although showing partial behavioral improvement, have also shown tumorigenesis of the injected ES cells.[10,11] Tumor formation continues to be a problem for the potential clinical use of ES cells; the uncontrolled growth of native or even ES-derived progenitor cells is one factor that has so far precluded their use in humans.[12,13] A few animal studies also show some ability of ES cells for cardiac repair,[14,15] although in vitro studies have indicated potential problems with arrhythmia induced by ES-derived cardiac cells.[16] Whereas some early work suggested possible use of ES cells for generation of insulin-secreting cells and diabetes treatment,[17,18] more recent studies indicate that the previously observed insulin secretion was an artifact of insulin imbibed from the culture medium[19,20] and that insulin-expressing cells derived from ES cells were not true beta cells, although they were still tumorigenic.[12] Thus far, it has been difficult to obtain a pure culture of ES-derived functional differentiated cells and to get physiologic integration into damaged tissues.

Another hurdle yet to be overcome in potential therapeutic use of ES cells is immune rejection. Animal studies have usually relied on immunosuppression or injection into immunoprivileged sites, such as the brain, and it is likely that such protocols would need to be followed for any human trials. Several possibilities have been proposed by Odorico and colleagues for overcoming potential rejection of ES cells, including genetic engineering of major histocompatibility complex (MHC) genes, induced hematopoietic chimerism, establishing "banks" of ES cell lines to match potential recipients, and somatic cell nuclear transfer (SCNT; so-called "therapeutic cloning").[21] Zwaka and Thomson demonstrated that it is possible to do homologous recombination in human ES cells, similar to that routinely done in mouse ES cells, opening the possibility of engineering ES cells to match the MHC antigens of different patients.[22] Transplant of ES-derived hematopoietic cells, producing an immune system chimerism, could potentially overcome immune rejection; the concept has already been demonstrated using adult stem cell bone marrow transplants followed by solid organ transplant.[23] Banks of human ES cells to match any patient might also be possible, although it is uncertain just how many ES cell lines would be required, with estimates ranging from 250 to 10,000 potential lines needed.

Therapeutic cloning has been hailed as a potential panacea for overcoming immune rejection. Theoretically, by creating an embryonic clone of the patient, from which matching ES cells could be harvested, patient-specific cell lines could be generated that would not be rejected. South Korean researchers recently claimed creation of scores of cloned human embryos from patients and production of 11 ES cell lines.[24] These claims have now been proven fraudulent and the published paper withdrawn. It is still uncertain whether the cells would actually be accepted by the patient's immune system, and prominent ES cell researchers have questioned the efficiency of using therapeutic cloning for clinical use.[25,26] In a previous experiment in mice, the cells from cloned embryos were rejected by the genetically matched host.[27,28] Reports of successful matching of cells derived by SCNT cloning are so far dubious; the best results to date in animal studies actually come from gestating cloned animals to the fetal stage and then harvesting tissue stem cells.[29–31]

ADULT STEM CELLS

Traditional dogma maintains that there are few adult (tissue or postnatal) stem cells present in the body and that they are difficult to isolate and grow in culture and extremely limited in their capacity to generate new cell types, being limited to forming more cells from their tissue of origin. However, an explosion in publications in the last few years is overturning this dogma and showing a remarkable flexibility for these cells.[32] In a 2001 publication, evidence was presented that a single adult bone marrow stem cell could contribute not only to marrow and blood but also to formation of liver, lung, digestive tract, skin, heart, and muscle.[33] Several examples now exist of some adult stem cells with pluripotent flexibility, including cells from bone marrow,[34–36] peripheral blood,[37] the inner ear,[38] umbilical cord blood,[39,40] nasal mucosa,[41] amniotic fluid,[42] and the placental amniotic membrane.[43] Many of these published studies also document that these particular pluripotent adult stem cells can multiply in culture for extensive periods of time while still retaining their ability to differentiate and providing sufficient numbers of cells for clinical treatments.

Relevant to their potential use in clinical therapies, there have been numerous reports of the effectiveness of adult stem cells in treating animal models of disease. In stroke models, adult stem cells have provided therapeutic benefit.[44–46] Interestingly, in some experiments, the cells showed a "homing" ability to the site of tissue damage. There is some evidence that c-kit ligand (stem cell factor) may be important for this homing behavior[46]; although this phenomenon is still not completely understood, it provides an intriguing possibility for targeting of regenerative stem cells. For spinal cord injury, adult stem cells have promoted neuronal growth and therapeutic benefit in rodent models.[48–50] A recent result that brings into focus some of the unexpected problems potentially faced with regenerative medicine was the discovery that, in successful transplants, the new nerve growth could result in increased pain; however, this could be managed by directed differentiation of the stem cells before trans-

plant.[51] Initial clinical trials in Portugal are under way with approximately 36 patients.[52] In animal models of Parkinson's disease, adult stem cells have shown effectiveness at stimulating dopamine secretion and decreasing behavioral symptoms.[53,54] One patient received a transplant of his own neural stem cells, resulting in decreasing the symptoms of Parkinson's disease.[55] In a study designed not to transplant stem cells but rather to stimulate endogenous adult stem cells for repair, five patients were injected with glial cell–derived neurotrophic factor, resulting in an average 61% decrease in symptomatology.[56] Follow-up pathology with one patient showed that the growth factor stimulated sprouting of new neurons.[57]

Adult stem cells have also been effective at ameliorating retinal degeneration in animal models,[58–60] raising hopes for possible treatments for diabetic retinopathy and age-related macular degeneration. Regarding diabetes, several examples now exist showing generation of insulin-secreting cells from various adult stem cells, including the liver,[60] bone marrow,[62,63] and pancreas.[64] In some experiments, it appears that it is not the adult stem cells that form new beta cells but rather that the injected cells stimulated endogenous precursors within the pancreas to accomplish regeneration.[65] Using spleen cells, one group was able to achieve permanent disease reversal and now has approval from the US Food and Drug Administration to begin human trials for juvenile diabetes.[66]

Use of adult stem cells from bone marrow or mobilized into peripheral blood has become relatively common as an adjunct for cancer chemotherapy to replace the patient's hematopoietic system or for anemias. Similar techniques to replace the immune system are now being tested with some success in patients for various autoimmune conditions, such as scleromyxedema,[67] multiple sclerosis,[68] and Crohn's disease.[70] Such treatments have also shown promising results for metabolic disorders, such as Krabbe's disease.[71] Adult stem cells have also been used in bone repair protocols.[71] Repair of cardiac damage in patients has also moved to the clinical trials stage, with several reports of early success in repair of infarct damage.[72–74]

The mechanism for these regenerative results is still unclear. Adult stem cells in some cases appear to be capable of interconversion between different tissue types, known as transdifferentiation. In some tissues, adult stem cells appear to fuse with the host tissue and take on that tissue's characteristics, facilitating regeneration. In some studies, the adult stem cells do not directly contribute to the regenerating tissue but instead appear to stimulate the endogenous cells of the tissue to begin repair. Whatever the mechanism, adult stem cells are successful at regenerating damaged tissue.

In summary, a great deal of work remains to be done before widespread clinical application of stem cells for regenerative medicine. Given the scientific hurdles that yet remain to be overcome for ES cells, they may be less well suited for clinical applications than for basic scientific studies. Recent results from animal studies and early clinical trials indicate that adult stem cells, in contrast to previous theories, have significant capacities for repair of damaged cells and tissues, somewhat like a native repair kit. The flexibility and potential of these adult stem cells to impact disease appear to be enormous.

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