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3) To support continuation of federal laws prohibiting the federal funding of research that requires the destruction of human life, including the human embryo.

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POTENTIAL APPLICATIONS of ADULT STEM CELLS—RESEARCH NEWS

NEURAL STEM CELLS

Neural stem cells able to be isolated and grown in culture from cadavers. Brain tissue up to 20 hours after death was harvested and adult neural stem cells grown in culture. Cells differentiated in culture into various neuronal types.

Reference:

106. Palmer, TD, Schwartz, PH, Taupin, P, Kaspar, B, Stein, SA, Gage, FH; "Progenitor cells from human brain after death"; Nature 411, 42-43; May 3, 2001

Genetic mechanisms regulating CNS progenitor function and differentiation are not well understood. We have used microarrays derived from a representational difference analysis (RDA) subtraction in a heterogeneous stem cell culture system to systematically study the gene expression patterns of CNS progenitors. This analysis identified both known and novel genes enriched in progenitor cultures. Several genes were also enriched in hematopoietic stem cells, suggesting an overlap of gene expression in neural and hematopoietic progenitors.

Reference:

107. Geschwind DH *et al.*; "A genetic analysis of neural progenitor differentiation"; Neuron 29(2), 325-339; Feb. 2001

Infusion of transforming growth factor-alpha into damaged rat brains induced rapid proliferation of neural stem cells, followed by migration of neuronal and glial progenitors. Subsequent increases in numbers of differentiated neurons occurred. Treated rats, whose brain damage resembled that seen in Parkinson's disease, had decreased symptoms. Thus, the brain contains stem cells capable of being stimulated by growth factors to proliferate, migrate in a directed manner, and differentiate into neurons. "This finding has significant implications with respect to the development of treatments for both acute neural trauma and neurodegenerative diseases." "The data predicts an alternative strategy to the current cell transplant methodologies for the treatment of neurodegenerative diseases."

Reference:

108. Fallon J *et al.*; "*In vivo* induction of massive proliferation, directed migration, and differentiation

of neural cells in the adult mammalian brain"; Proc. Natl. Acad. Sci. USA 97, 14686-14691; December 19 2000
**Progenitors from adult rat spinal cord using bFGF alone show stem cell properties including selfrenewal. Cultures from single cells generate neurons, astrocytes, and oligodendrocytes.
Transplantation into adult rat spinal cord resulted in differentiation into glial cells. Transplantation into hippocampus resulted in integration in the granular cell layer and differentiation of cells with astroglial and oligodendroglial phenotypes. Can generate region-specific neurons in vivo when exposed to appropriate environmental cues.

Reference

109. Shihabuddin S *et al.*; "Adult spinal cord stem cells generate neurons after transplantation in the adult dentate gyrus"; J Neuroscience 20, 8727-8735; December 2000
Updated June 25, 2001 Research—Adult Neural Stem Cells David A. Prentice
Able to directly isolate human central nervous system stem cells from fresh human fetal brain tissue, cultures could be grown from single cells, and transplanted into mouse brain where they engrafted, proliferated, migrated, and differentiated into neurons; 7-12 months after transplant the cells still responded to environmental cues and were not tumorigenic. The authors note they were unable to obtain fresh human adult brain tissue, but speculate that the same cells reside in adult brain.

Reference:

110. Uchida N *et al.*; "Direct isolation of human central nervous system stem cells"; Proc. Natl. Acad. Sci. USA 97, 14720-14725; December 19, 2000
**Implanted neural stem cells infiltrate brain tumors. The neural stem cells show the ability to migrate extensively throughout the brain to reach sites of damage. The results "suggest that NSC migration can be extensive, even in the adult brain and along nonstereotypical routes."

Reference:

111. Aboody KS, Brown A, Rainov NG, Bower KA, Liu S, Yang W, Small JE, Herrlinger U, Ourednik V, Black PM, Breakefield XO, Snyder EY ; "From the cover: neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas"; Proc Natl Acad Sci U S A 97, 12846-12851; Nov 7 2000
**Characterized CCg, glycosylated form of cystatin C; required for FGF-2's mitogenic activity on neural stem cells. Combined delivery of FGF-2 and CCg to adult dentate gyrus stimulated neurogenesis.

Reference

112. Taupin P *et al.*; "FSF-2-responsivie neural stem cell proliferation required CCg, a novel autocrine/paracrine cofactor"; Neuron 28, 385-397; February 2001
**Review of plasticity in neural tissues and possibilities for repair.

Reference:

113. Hodge CJ Jr. and Boakye M; "Biological Plasticity: The future of science in neurosurgery"; Neurosurgery 48, 2-16; Jan 2001
Updated June 25, 2001 Research—Adult Neural Stem Cells David A. Prentice
HUMAN and mouse adult neural stem cells could be reprogrammed to form skeletal muscle.
Italian researchers have transformed adult neural stem cells from humans and mice, changing the cells into muscle. The transformation to muscle not only took place in culture, but also after injection into mice. Dr. Luigi Vescovi, co-director of the Stem Cell Research Institute in Milan, said that the most obvious possibility for therapeutic development was in the area of muscular dystrophy. In its statement, the Institute noted, "With adult stem cells there would also be the possibility of autotransplantation, eliminating all the problems of immunological compatibility and rejection."
Transplant rejection would be a significant problem if using embryonic stem cells.

Reference

114. Galli, R. *et al.*, "Skeletal myogenic potential of human and mouse neural stem cells", Nature Neuroscience 3, 986-991, October, 2000.
Adult neural stem cells from rat were shown to form various types of functional nerve connections in culture.

Reference

115. Toda H *et al*.; "Neurons generated from adult rat hippocampal stem cells form functional glutamatergic and GABAergic synapses *in vitro*"; Experimental Neurology 165, 66-76; September 2000.

Mitogens in the cell culture medium confer conditional immortalization; removal of mitogens results in differentiation to the 3 fundamental cell types in the central nervous system

Reference

116. Villa A *et al*.; "Establishment and properties of a growth factor-dependent, perpetual neural stem cell line from the human CNS"; Exp. Neurol. 161, 67-84; January 2000

Adult Stem Cells from Brain Able to Form Virtually Any Tissue

Research with mice indicates that adult stem cells from brain can grow into a wide variety of organs—heart, lung, intestine, kidney, liver, nervous system, muscle, and other tissues. The study by Swedish scientists, reported in the June 2, 2000 issue of *Science,* confirms that adult stem cells are in fact much more adept at redefining themselves than previously thought. The study involved growing adult stem cells from brain with embryonic cells and within an embryo. Even lone neural adult stem cells had the ability to differentiate into various cell types. The authors observe that the "most striking indication" of this complete cellular redefinition was the finding of apparently normal and beating embryonic mouse hearts that contained very large amounts of the stem cells.

According to Dr. Ihor Lemischka, professor of developmental biology at Princeton University, "This is a very exciting and interesting result," and if the research can be confirmed in human cells it would "nip in the bud" the moral and ethical concerns that now block federal funding of human embryonic stem cell research. The authors of the study state that "This demonstrates that an adult neural stem cell has a very broad developmental capacity and may potentially be used to generate a variety of cell types for transplantation in different diseases." They also note that "…these studies suggest that stem cells in different adult tissues may be more similar than previously thought and perhaps in some cases have a developmental repertoire close to that of ES cells."

Reference

117. Clarke *et al*.; "Generalized potential of adult neural stem cells"; Science 288, 1660-1663, June 2, 2000.

Updated June 25, 2001 Research—Adult Neural Stem Cells David A. Prentice

Adult Stem Cells in Brain Stimulated to Grow and Replace Damaged Brain Tissue

Studies in mice show that adult stem cells in the brain can be stimulated to grow and replace damaged neural tissue. The re-growth could take place even in regions of adult mammalian brain that do not normally undergo any new cell growth, and the neurons were able to re-form appropriate connections within the adult brain. The authors state that "Our results indicate that neuronal replacement therapies for neurodegenerative disease and CNS injury may be possible through manipulation of endogenous neural precursors *in situ*." Commenting on the report, Drs. Anders Bjorklund and Olle Lindvall of Lund University in Sweden noted that learning how to activate stem cells in the brain "might eventually lead to a powerful tool for brain repair in human disorders of the central nervous system." Scientists have already used implants of adult neural stem cells to cure mice of severe brain disorders.

References

118. Magavi *et al*.; "Induction of neurogenesis in the neocortex of adult mice"; Nature 405, 951-955, June 22, 2000.

119. Bjorklund A and Lindvall O; "Self-repair in the brain"; Nature 405, 892-893, June 22, 2000.

Brain cells called "oligodendrocytes" could be "reprogrammed", forming complete adult neural stem cells which could generate all cell types of the brain.

Reference

120. Kondo, T. and Raff, M. "Oligodendrocyte precursor cells reprogrammed to become multipotent CNS stem cells"; Science 289, 1754-1757; Sept. 8, 2000.

Adult neural stem cells isolated from different regions of the human brain (lateral ventricle wall and hippocampus).

Reference

121. Johansson CB *et al*.; "Neural stem cells in the adult human brain"; Exp. Cell Res. 253, 733-736; December 1999.

Adult neural stem cells identified in additional sites within the brain. The cells migrate to other regions as well (ependymal cells, migrate to olfactory bulb.)

Reference

122. Johansson CB *et al*.; "Identification of a neural stem cell in the adult mammalian central nervous system"; Cell 96, 25-34; January 1999

Turning Brain Into Blood

Adult neural stem cells can be "retrained" for a new occupation—as blood stem cells. It has been known since 1997 that adult neural stem cells can regenerate the three major cell types in the brain. Working together, scientists in Canada and Italy now have shown that neural stem cells from mice can also form numerous blood cell types. The results are surprising because it was previously thought that adult stem cells were restricted to forming only cell types from the tissue in which they were found. Given that human neural stem cells can be expanded in culture for extended periods of time, the results open possibilities for future treatment of a number of disorders.

Reference

123. Bjornson *et al*.; "Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo"; Science 283, 534-537; January 22, 1999

Updated June 25, 2001 Research—Adult Neural Stem Cells David A. Prentice

Adult Stem Cells Possible for Repair of Spinal Cord Damage

Researchers in the UK announced that they have isolated a human adult stem cell which can function in repair of nerve damage, for example in spinal cord repair or other parts of the central nervous system (CNS). The human adult stem cell, known as an "olfactory ensheathing cell" (OEC), was able to repair nerve axons in damaged rat spinal cord. The scientists noted that "Thus, the human OEC represents an important new cell for the development of transplant therapy of CNS diseases."

Reference

124. Barnett *et al*.; "Identification of a human olfactory ensheathing cell that can effect transplantmediated remyelination of demyelinated CNS axons"; Brain 123, 1581-1588, August 2000

Adult neural stem cells identified in a relatively accessible part of the human brain, allowing easier removal. The cells can be expanded, established in continuous cell lines and differentiated into the three classical neuronal phenotypes (neurons, astrocytes, and oligodendrocytes). Also, after exposition to leukemia inhibitory factor, we are able to improve the number of neurons, an ideal biological source for transplantation in various neurodegenerative disorders.

"similar to human embryonic stem cells" "The fact that this revolutionary strategy uses autologous neuronal material means that it has all of the advantages of biosafety, histocompatibility, and neurophysiological efficiency. Furthermore, it does not raise the ethical and moral questions associated with the use of embryonic or heterologous material."

Reference

125. Pagano S *et al*.; "Isolation and Characterization of Neural Stem Cells from the Adult Human Olfactory Bulb"; Stem Cells 18, 295-300; July 2000

**Marrow stem cells injected into mouse brain migrated through forebrain and cerebellum without disrupting host brain structure. The marrow stem cells populated various regions of the brain, and differentiated into astrocytes. These stem cells are proposed as methods for treating a variety of central nervous system disorders.

Reference:

126. Kopen GC *et al*.; "Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains"; Proc. Natl. Acad. Sci. USA 96, 10711-10716; Sept 14 1999

Review of methods which now enable cell immortalization, purification and safety mechanisms, and genetic therapy using neural stem cells.

Reference

127. Foster GA, Stringer BM; "Genetic regulatory elements introduced into neural stem and progenitor cell populations"; Brain Pathol. 9, 547-567; July 1999.
Adult neural stem cells transplanted into mice which have a condition similar to Parkinson's disease. The cells migrated through the brain, repairing tissue and decreasing tremors in the mice.
Reference
128. Yandava BD *et al*.; " 'Global' cell replacement is feasible via neural stem cell transplantation: evidence from the dysmyelinated shiverer mouse brain"; Proc. Natl. Acad. Sci. USA 96, 7029-7034; June 8, 1999
Updated June 25, 2001 Research—Adult Neural Stem Cells David A. Prentice
Treatment of damaged spinal cord with added growth factors allowed re-growth of damaged spinal cord neurons in rats.
Reference
129. Ramer MS *et al*.; "Functional regeneration of sensory axons into the adult spinal cord"; Nature 403, 312-316; January 20, 2000
Adult neural stem cells could treat retinal problems. Researchers have found that adult neural stem cells may be useful in treating blindness due to problems with the retina. The eyes of rats that had degradation of their retinas were injected with adult neural stem cells. The cells migrated to the retina and began to take on characteristics of retinal cells. Interestingly, this only occurred if the retina was damaged and not in undamaged retinas. Dr. Michael Young of the Schepens Eye Research Institute, who led the study, said "These cells somehow sense that they are needed and begin to differentiate into cells that could take on the job of retinal neurons." The finding raises the possibility of using adult stem cells for patients with macular degeneration and glaucoma.
Reference
130. Young MJ *et al*., "Neuronal differentiation and morphological integration of hippocampal progenitor cells transplanted to the retina of immature and mature dystrophic rats", Molecular and Cellular Neurosciences 16, 197-205; Sept., 2000.
Development of Stable Neural Stem Cell Lines. Stable clones of neural stem cells (fetal-derived); cells are self-renewing. Transplanted into mouse they migrate along established pathways to CNS regions, differentiate into multiple types, intersperse with host cells. Can be genetically engineered, cryopreservable.
Reference
131. Flax JD *et al.*, "Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes", Nature Biotechnol. 16, 1033; November, 1998
Used NTERA-2 (EC line, from teratocarcinoma) to demonstrate developmental regulation of neurogenesis.
Reference
132. Przyborski SA *et al.*; "Developmental regulation of neurogenesis in the pluripotent human embryonal carcinoma cell line NTERA-2"; Eur. J. Neurosci. 12, 3521-3528; Oct. 2000
**"infused intraparenchymally, NGF rescues basal forebrain cholinergic neurons, alters the topography of axonal sprouting responses, and does not induce adverse affects over a 2-week infusion period. Intraparenchymal NGF delivery merits further study at longer term time points as a means of treating the cholinergic component of neuronal loss in Alzheimer's disease."
Reference:
133. Tuszynski MH; "Intraparenchymal NGF infusions rescue degenerating cholinergic neurons"; Cell Transplant 9; 629-636; Sept-Oct 2000
**Study identified reversible cellular atrophy as a potential aging mechanism in the brain; used neurotrophin gene transfer as potential effective method to prevent neural degeneration.
Reference:
134. Smith DE, Roberts J, Gage FH, Tuszynski MH; "Age-associated neuronal atrophy occurs in the primate brain and is reversible by growth factor gene therapy"; Proc Natl Acad Sci U S A 96, 10893-10898; Sept 14 1999
Updated June 25, 2001 Research—Adult Neural Stem Cells David A. Prentice

Establishment of human neural cell lines. Established immortalized human CNS cell lines, can differentiate into functional sensory neurons.

Reference

135. Raymon HK *et al.*, "Immortalized human dorsal root ganglion cells differentiate into neurons with nociceptive properties", J. Neurosci 19, 5420; July 1, 1999

Updated June 25, 2001 Research—Adult Retinal Stem Cells David A. Prentice

RETINAL STEM CELLS

Neural Stem Cells in Adult Mammalian Eye. Researchers at University of Nebraska Medical Center have isolated neural stem cells from adult mammalian eye. In culture the cells show the ability for self-renewal, and can differentiate showing characteristics of retina, neurons, and glia.

Reference

136. Ahmad I *et al.*; "Identification of neural progenitors in the adult mammalian eye"; Biochem. Biophys. Res. Commun. 270, 517-521; April 13, 2000

Retinal Stem Cells Found in Adult Eye

Researchers at the University of Toronto have identified retinal stem cells in the adult mammalian eye. The adult stem cells were found in humans, cows, and mice. While still in the eye, the cells appear to be under an inhibitory control, but once removed and placed in culture the cells grow. The scientists hope to learn how to stimulate the stem cells inside the eye so that proper function can be restored. The results open the way to possible regeneration of retinal tissue.

Reference

137. Tropepe *et al*.; "Retinal stem cells in the adult mammalian eye"; Science 287, 2032-2036, March 17, 2000.

Updated June 25, 2001 Research—Adult Muscle Stem Cells David A. Prentice

MUSCLE STEM CELLS

The authors report the first intramyocardial transplantation of autologous skeletal myoblasts in a patient with severe ischaemic cardiac failure. The encouraging result after eight months' follow-up underlines the potential of this new approach.

Reference:

138. Menasche P, Hagege A, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JT, Marolleau JP. [Autologous skeletal myoblast transplantation for cardiac insufficiency. First clinical case] [Article in French] Arch Mal Coeur Vaiss 94(3):180-182; Mar 2001

This study assessed the extent to which the initial degree of functional impairment and the number of injected cells may influence the functional improvement provided by autologous skeletal myoblast transplantation into infarcted myocardium. Used rats with heart impairment, injected the rats' own skeletal myoblasts. CONCLUSIONS: Autologous myoblast transplantation is functionally effective over a wide range of postinfarct ejection fractions, including in the sickest hearts provided that they are injected with a sufficiently high number of cells.

Reference:

139. Pouzet B, Vilquin JT, Hagege AA, Scorsin M, Messas E, Fiszman M, Schwartz K, Menasche P. "Factors affecting functional outcome after autologous skeletal myoblast transplantation." Ann Thorac Surg 71(3):844-850; Mar 2001

Intramyocardial skeletal muscle transplantation has been shown experimentally to improve heart function after infarction. We report success with this procedure in a patient with severe ischaemic heart failure. We implanted autologous skeletal myoblasts into the postinfarction scar during coronary artery bypass grafting of remote myocardial areas. 5 months later, there was evidence of contraction and viability in the grafted scar on echocardiography and positron emission tomography. Although this result is encouraging, it requires validation by additional studies.

Reference:

140. Menasche P, Hagege AA, Scorsin M, Pouzet B, Desnos M, Duboc D, Schwartz K, Vilquin JT, Marolleau JP. "Myoblast transplantation for heart failure." Lancet 357(9252):279-280; Jan 27, 2001

Cell transplantation is a potential therapeutic approach for patients with chronic myocardial failure. Experimental transplantation of neonatal and fetal cardiac myocytes showed that the grafted cells can functionally integrate with and augment the function of the recipient heart. Clinical application of this approach will be limited by shortage of donors, chronic rejection, and because it is ethically contentious. By contrast skeletal myoblasts (satellite cells) are abundant and can be grafted successfully into the animal's own heart even after genetic manipulation in vitro. In experimental studies several other cell types have been used to augment cardiac function. In this review we discuss the published results of myocyte transplantation with emphasis on potential sources of cells, the ethics of using donor embryonic and fetal cardiomyocytes, genetic transformation of skeletal myoblasts for myocardial repair, and the functional benefits of cell transplantation to the failing heart.

Reference:

141. El Oakley RM *et al.*; "Myocyte transplantation for cardiac repair: A few good cells can mend a broken heart"; Ann Thorac Surg 71, 1724 –1733; 2001

Multipotent stem cells were isolated from mouse muscle, capable of differentiating into muscle and multiple blood cell types. The adult stem cells were injected into bloodstream of *mdx* mice, a model of Duchenne muscular dystrophy. The stem cells migrated to muscle, participated in formation of

muscle fibers, and helped in regeneration of muscle and restoration of production of dystrophin protein, which is deficient in muscular dystrophy.

Reference:

142. Torrente Y *et al.*; "Intraarterial injection of muscle-derived CD34+Sca-1+ stem cells restores dystrophin in *mdx* mice"; Journal of Cell Biology 152, 335-348; January 22, 2001

**"Transplantation of fetal cardiomyocytes improves function of infarcted myocardium but raises availability, immunologic, and ethical issues that justify the investigation of alternate cell types, among which skeletal myoblasts are attractive candidates." "These results support the hypothesis that skeletal myoblasts are as effective as fetal cardiomyocytes for improving postinfarction left ventricular function. The clinical relevance of these findings is based on the possibility for skeletal myoblasts to be harvested from the patient himself."

Reference:

143. Scorsin M, Hagege A, Vilquin JT, Fiszman M, Marotte F, Samuel JL, Rappaport L, Schwartz K, Menasche P ; "Comparison of the effects of fetal cardiomyocyte and skeletal myoblast transplantation on postinfarction left ventricular function"; J Thorac Cardiovasc Surg 119; 1169-1175; June 2000

**Autologous skeletal myoblast (SM) transplantation improves function of infarcted myocardium in rats.

Reference:

144. Pouzet B, Vilquin JT, Hagege AA, Scorsin M, Messas E, Fiszman M, Schwartz K, Menasche P; "Intramyocardial transplantation of autologous myoblasts : can tissue processing Be optimized?"; Circulation 102; III210-215; Nov 7, 2000

Blood Cells From Muscle

Researchers at Baylor College of Medicine have found that skeletal muscle contains stem cells which can form all the major types of blood cells. Using adult mice, they isolated skeletal muscle cells, grew them in culture, and placed the stem cells into mice whose bone marrow cells were destroyed. The transplanted stem cells took up the job of forming all blood cells for the mice.

Reference

145. Jackson K *et al.*; "Hematopoietic potential of stem cells isolated from murine skeletal muscle"; Proceedings National Academy of Sciences USA 96, 14482-14486; December 7, 1999

Adult stem cells to treat muscular dystrophy

Used a mouse model of Duchenne's muscular dystrophy. Purified adult muscle stem cells from these mice. Intravenous injection of these muscle-derived adult stem cells back into the mice resulted in muscle regeneration and partial restoration of dystrophin expression in the mice. Transplantation of these cells engineered to secrete a bone protein results in their differentiation into bone cells and

acceleration of healing of a skull defect in immunodeficient mice.

Reference

146. Lee JY *et al*.; "Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing"; J. Cell Biology 150, 1085-1100; September 4, 2000

Updated June 25, 2001 Research—Adult Muscle Stem Cells David A. Prentice

An animal model of Duchenne's muscular dystrophy which indicate that the intravenous injection of either normal haematopoietic stem cells or a novel population of muscle-derived stem cells into irradiated animals results in the reconstitution of the haematopoietic compartment of the transplanted recipients, the incorporation of donor-derived nuclei into muscle, and the partial restoration of dystrophin expression in the affected muscle. These results suggest that the transplantation of different stem cell populations, using the procedures of bone marrow transplantation, might provide an unanticipated avenue for treating muscular dystrophy as well as other diseases where the systemic delivery of therapeutic cells to sites throughout the body is critical. Our studies also suggest that the inherent developmental potential of stem cells isolated from diverse tissues or organs may be more similar than previously anticipated.

Reference

147. Gussoni E *et al*.; "Dystrophin expression in the mdx mouse restored by stem cell transplantation"; Nature 401, 390-394; 23 September 1999

Obtained stem cells from skeletal muscle, which in culture could form skeletal myotubes, smooth muscle, bone, cartilage, fat.

Reference

148. Williams JT *et al*.; "Cells isolated from adult human skeletal muscle capable of differentiating into multiple mesodermal phenotypes"; Am. Surg. 65, 22; January 1999

Proposed use of numerous stem cells which have shown promise for cardiac repair, incl. myogenic cell lines, adult skeletal myoblasts, immortalized atrial cells, adult cardiomyocytes, altered fibroblasts, smooth muscle cells, and bone marrow-derived cells. Best developed option is mesodermally derived cells.

Reference

149. Kessler PD, Byrne BJ; "Myoblast cell grafting into heart muscle: cellular biology and potential applications", Ann. Rev. Physiol. 61, 219; 1999

Updated June 25, 2001 Research—Adult Skin Stem Cells David A. Prentice

SKIN STEM CELLS

Further studies showing the skin/hair follicle cell in multipotent and can form epidermis, hair follicles, sebaceous glands, and all structures of the hairy skin.

Reference:

150. Oshima H *et al*.; "Morphogenesis and renewal of hair follicles from adult multipotent stem cells"; Cell 104, 233-245; January 2001

A common stem cell replenishes both skin and hair follicles, and resides in the hair follicle.

Reference:

151. Taylor G; "Involvement of follicular stem cells in forming not only the follicle but also the epidermis"; Cell 102, 451-461; August 2000

Updated June 25, 2001 Research—Adult Pancreatic Stem Cells David A. Prentice

PANCREATIC STEM CELLS

**Review of possible stem cell treatments for diabetes. The review notes that "Human pancreatic duct cells have also been grown successfully in vitro and induced to differentiate", and "Not only does the use of adult donor ductal cells avoid the controversy of using fetal cells but there are fewer biological problems associated with making beta cells from duct cells than from, for example, embryonic stem cells." It points out that "…differentiation into endodermal cell types has not yet been reported" for human embryonic stem cells; pancreatic cells are an endodermal cell type. The authors also point out that insulin producing cells had been derived from mouse embryonic stem cells, but "this procedure gives rise to proliferating cells, and thereby potentially malignant cells,

rather than mature, post-mitotic cells." The authors note "When the nature of pancreatic beta cell ontogeny is fully understood we may be able to mimic this process in vitro to propagate beta cells—either starting with duct cells derived from pancreatic donor specimens or by the use of other appropriate human stem cells (such as from bone marrow or even blood samples). This development would clearly be welcome because it would avoid the need for therapeutic cloning, with all the attendant controversy of creating human embryos solely for medical use." The authors conclude that "Of the techniques described above, the most promising is generation of beta cells from pancreatic duct cells. It is inherently a shorter biological step to make a beta cell from a duct cell than it is from other possible cells, such as embryonic stem cells and haemopoietic stem cells."

Reference:

152. Serup P, Madsen OD, Mandrup-Poulsen T; "Islet and stem cell transplantation for treating diabetes"; British Medical Journal 322, 29-32; Jan 6 2001

**"Genetic engineering of non-beta cells to release insulin upon feeding could be a therapeutic modality for patients with diabetes. The workers derived a mouse cell line that could be induced to produce human insulin. Mice expressing this transgene produced human insulin specifically in gut cells. This insulin protected the mice from developing diabetes and maintained glucose tolerance after destruction of the native insulin-producing beta cells in their pancreas.

Reference:

153. Cheung AT, Dayanandan B, Lewis JT, Korbutt GS, Rajotte RV, Bryer-Ash M, Boylan MO, Wolfe MM, Kieffer TJ; "Glucose-dependent insulin release from genetically engineered K cells"; Science 290; 1959-1962; Dec 8 2000

Evidence for Human Adult Pancreatic Stem Cells

Researchers in France have found further evidence for pancreatic stem cells in humans. The pancreatic cells from healthy donors, when placed into culture, proliferated and expressed characteristics critical for production and secretion of insulin. The results are another step toward treatment of diabetes using adult stem cells.

Reference

154.V Gmyr *et al.*, "Adult human cytokeratin 19-positive cells reexpress insulin promoter factor 1 in vitro: Further evidence for pluripotent pancreatic stem cells in humans", Diabetes 49, 1671-1680; Oct. 2000

Updated June 25, 2001 Research—Adult Pancreatic Stem Cells David A. Prentice

**Cultured human pancreatic ductal cells under specific conditions. The cells formed islet buds and secreted insulin. "Thus, duct tissue from human pancreas can be expanded in culture and then be directed to differentiate into glucose responsive islet tissue in vitro. This approach may provide a potential new source of pancreatic islet cells for transplantation."

Reference:

155. Bonner-Weir S *et al.*; "In vitro cultivation of human islets from expanded ductal tissue"; Proc Natl Acad Sci USA 97, 7999-8004; July 5, 2000

Were able to reverse diabetes in mice using the animals' own adult stem cells; after treatment, the mice no longer needed insulin shots to survive.

Reference

156. Ramiya VK *et al*.; "Reversal of insulin-dependent diabetes using islets generated in vitro from pancreatic stem cells"; Nature Medicine 6, 278-282; March 2000

Updated June 25, 2001 Research—Adult Bone Marrow/Blood Stem Cells David A. Prentice

BONE MARROW STEM CELLS and PERIPHERAL BLOOD STEM CELLS

Showed the ability of a single adult bone marrow stem cell to repopulate the bone marrow of mice, forming functional marrow and blood cells, and also differentiate into functional cells of liver, lung, gastrointestinal tract (esophagus, stomach, intestine, colon), and skin. Indications that the cell could also form functional cells in heart and skeletal muscle. Possible evidence that the stem cells "home" to sites of tissue damage.

Reference:

157. Krause DS *et al.*; "Multi-Organ, Multi-Lineage Engraftment by a Single Bone Marrow-Derived

Stem Cell"; Cell 105, 369-377; May 4, 2001
Researchers at Baylor College of Medicine showed that adult bone marrow stem cells could form functional heart muscle and blood vessels in mice which had heart damage. They note that their results demonstrate the potential of adult bone marrow stem cells for heart repair, and suggest a therapeutic strategy that eventually could benefit patients with heart attacks. Their results also suggest that circulating stem cells may naturally contribute to repair of tissues.

Reference:

158. Jackson KA *et al.*; "Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells"; *Journal of Clinical Investigation* 107, 1395-1402; June 2001
Used bone marrow stem cells from mice expressing green fluorescent protein to track the cells. Injected the stem cells into area of heart where damage had been induced. Newly formed myocardium occupied 68% of the infarcted portion of the ventricle 9 days after transplanting the bone marrow cells. The developing tissue comprised proliferating myocytes and vascular structures. The studies indicate that locally delivered bone marrow cells can generate de novo myocardium, ameliorating the outcome of coronary artery disease.

Reference:

159. Orlic D *et al.*; "Bone marrow cells regenerate infarcted myocardium"; Nature 410, 701-705; April 5, 2001
Human bone-marrow-derived stem cells were implanted into rats with cardiac damage. The cells participated in formation of new cardiac blood vessels and stimulated existing vessels. The authors note that "The use of cytokine-mobilized autologous human bonemarrow –derived angioblasts for revascularization of infarcted myocardium (alone or in conjunction with currently used therapies) has the potential to significantly reduce morbidity and mortality associated with left ventricular remodeling."

Reference:

160. Kocher AA *et al.*; "Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function"; Nature Medicine 7, 430-436; April 2001.
MSCs delivered to ischemic brain tissue through an intravenous route in rats provide therapeutic benefit after stroke. MSCs may provide a powerful autoplastic therapy for stroke.

Reference:

161. Chen J *et al.*; "Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats"; Stroke 32, 1005-1011; April 2001
Updated June 25, 2001 Research—Adult Bone Marrow/Blood Stem Cells David A. Prentice
These data suggest that intracerebral transplantation of bone marrow could potentially be used to induce plasticity in ischemic brain.

Reference:

162. Li Y *et al.*; "Adult bone marrow transplantation after stroke in adult rats"; Cell Transplant 10(1), 31-40; Jan-Feb 2001
This study confirms that, in the context of the severe combined immunodeficiency disease (SCID) mouse model, culture-expanded, cryopreserved human Mesenchymal Stem Cells have osteogenic potential and demonstrates that implanted cell gene expression can reveal the early onset of bone formation.

Reference:

163. Cooper LF *et al.*; Incipient analysis of mesenchymal stem-cell-derived osteogenesis";;J Dent Res 80(1), 314-320; Jan. 2001
Developed a regulated stem cell-based system for controlling bone regeneration, utilizing genetically engineered mesenchymal stem cells (MSCs) harboring a tetracycline-regulated expression vector encoding the osteogenic growth factor human BMP-2. We show that doxycycline (a tetracycline analogue) is able to control hBMP-2 expression and thus control MSC osteogenic differentiation both in vitro and in vivo. Showed increased angiogenesis accompanied by bone formation whenever genetically engineered MSCs were induced to express hBMP-2 in vivo. Thus, our results demonstrate

that regulated gene expression in mesenchymal stem cells can be used as a means to control bone healing.

Reference:

164. Moutsatsos IK *et al.*; "Exogenously regulated stem cell-mediated gene therapy for bone regeneration"; Mol Ther 3(4), 449-461; April 2001

Discovered two additional types of adult stem cells in peripheral blood. These two new stem cell types are short-term in their ability to repopulate bone marrow, and are then followed by the longterm repopulating stem cell when engrafted into mice.

Reference:

165. Glimm H *et al.*; "Previously undetected human hematopoietic cell populations with short-term repopulating activity selectively engraft NOD/SCID-beta2 microglobulin-null mice"; J. Clin. Invest. 107, 199-206; January 2001

**Autologous transplantation of marrow stromal stem cells, injected into myocardium of rats. The marrow stromal stem cells showed myogenic differentiation, including indication that the injected stem cells, as well as native cardiomyocytes, were connected. The authors note that "In an appropriate microenvironment they will exhibit cardiomyogenic phenotypes and may replace native cardiomyocytes lost by necrosis or apoptosis. Because marrow stromal cells can be obtained repeatedly by bone marrow aspiration and expanded vastly in vitro before being implanted or used as autologous implants, and because their use does not call for immunosuppression, the clinical use of marrow stromal cells for cellular cardiomyoplasty appears to be most advantageous."

Reference:

166. Wang J-S, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, Chiu Ray C-J; "Marrow stromal cells for cellular cardiomyoplasty: Feasibility and potential clinical advantages"; The Journal of Thoracic and Cardiovascular Surgery 120, 999-1006; Nov 2000

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**Adult stem cells from mouse bone marrow injected into mouse blood stream, could be found developing neuron characteristics in brain. Generation of brain cells from adult bone marrow "demonstrates a remarkable plasticity of adult tissues with potential clinical applications."

Reference:

167. Brazelton TR *et al*.; "From marrow to brain: expression of neuronal phenotypes in adult mice"; Science 290, 1775-1779; Dec 1 2000

**Showed in mice that transplanted adult bone marrow stem cells can migrate into brain and differentiate into neuronal cells. "These findings raise the possibility that bone marrow-derived cells may provide an alternative source of neurons in patients with neurodegenerative diseases or central nervous system injury".

Reference:

168. Mezey E *et al*.; "Turning blood into brain: Cells bearing neuronal antigens generated in vivo from bone marrow"; Science 290, 1779-1782; Dec 1 2000

**Previously reported human stem cell frequencies and their in vivo self-renewal activity have been markedly underestimated.

Reference

169. Cashman JD and Eaves CJ; "High marrow seeding efficiency of human lymphomyeloid repopulating cells in irradiated NOD/SCID mice"; Blood 96, 3979-3981; Dec. 1 2000

**Tested human peripheral blood stem cells injected into mice. Results showed stromal progenitor cells present in human peripheral blood or cord blood, which could be used to re-seed bone marrow.

Reference

170. Goan *et al*.; "Donor stromal cells from human blood engraft in NOD/SCID mice"; Blood 96, 3971-3978; Dec 1 2000

**Transplanted human mesenchymal (bone marrow) stem cells into fetal sheep early in gestation. The cells engrafted and persisted in multiple tissues, and underwent site-specific differentiation into chondrocytes, adipocytes, myocytes, cardiomyocytes, bone marrow stromal cells, and thymic stroma. "Our data support the possibility of the transplantability of mesenchymal stem cells and their

potential utility in tissue engineering, and cellular and gene therapy applications."
Reference:
171. Liechty KW *et al.*; "Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep"; Nature Medicine 6, 1282-1286; Nov 2000
**Intravenous injection of adult bone marrow stem cells in a mouse model of tyrosinemia type I rescued the mouse and restored biochemical function of its liver.
Reference:
172. Lagasse E *et al.*; "Purified hematopoietic stem cells can differentiate into hepatocytes in vivo"; Nature Medicine 6, 1229-1234; Nov 2000
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**Used a mouse model of progressive and ultimately fatal systemic autoimmune disease; these mice develop degenerative coronary vascular disease with myocardial infarctions and hypertension. Transplanted bone marrow stem cells from mice which allowed survival of the recipients, and significant amelioration of degenerative coronary vascular disease.
Reference:
173. Kirzner RP *et al.*; "Prevention of coronary vascular disease by transplantation of T-cell-depleted bone marrow and hematopoietic stem cell preparation in autoimmune-prone w/BF(1) mice"; Biol. Blood Marrow Transplant 6, 513-522; 2000
**Identified role of the *Notch* gene as a signal regulating hematopoietic stem cell self-renewal. "Furthermore, the establishment of clonal, pluripotent cell lines provides the opportunity to assess mechanisms regulating stem cell commitment and demonstrates a general method for immortalizing stem cell populations for further analysis."
Reference:
174. Varnum-Finney B *et al.*; "Pluripotent, cytokine-dependent, hematopoietic stem cells are immortalized by constitutive Notch1 signaling"; Nature Medicine 6, 1278-1281; Nov 2000
**Identification of expression of the *hiwi* gene in human stem cells; gene similar to that expressed in embryonic germline stem cells of Drosophila and shown to be important for stem cell renewal. The gene is not expressed in more differentiated cell populations. Expression also detected in many developing fetal and adult tissues. The *hiwi* gene appears to be an important negative developmental regulator which in part underlies the unique biologic properties associated with progenitor cells.
Reference
175. Sharma AK *et al.*; "Human CD34(+) stem cells express the hiwi gene, a human homologue of the Drosophila gene piwi"; Blood 97, 426-434; Jan 15 2001
**Studied growth factors for stem cell replication in culture. Single-cell replication of self-renewing stem cells achieved with Stem Cell Factor and Thrombopoietin. Regenerated populations could be transplanted into secondary recipients. Study also shows evidence that one hematopoietic stem cell regenerates at least one stem cell in culture.
Reference:
176. Ema H *et al.*; "In vitro self-renewal division of hematopoietic stem cells"; J. Exp. Med. 192, 1281-1288; Nov 6 2000
**Review of techniques to isolate hematopoietic and mesenchymal stem cells from various sources, and expansion and differentiation in culture for potential clinical uses.
Reference:
177. Huss R; "Isolation of primary and immortalized CD34- hematopoietic and mesenchymal stem cells from various sources"; Stem Cells 18, 1-9; 2000
HUMAN and mouse bone marrow stem cells able to form nerve cells. Dr. Juan Sanchez-Ramos, lead scientist, noted that "It's striking that we can generate new kinds of cells from deep within the bone, including cells with the potential to become neurons for brain repair." Layton BioScience, Inc. has licensed the rights to this technology and is developing it for clinical use.
Reference
178. Sanchez-Ramos J *et al.*; "Adult bone marrow stromal cells differentiate into neural cells in

vitro"; Experimental Neurology 164, 247-256; August 2000
Updated June 25, 2001 Research—Adult Bone Marrow/Blood Stem Cells David A. Prentice
Adult human bone marrow stem cells can create a "virtually limitless supply" of nerve cells. According to the published results, the adult stem cells "grow rapidly in culture, precluding the need for immortalization, and differentiate into neurons exclusively with use of a simple protocol". The report also notes that "The marrow cells are readily accessible, overcoming the risks of obtaining neural stem cells from the brain, and provide a renewable population. Autologous transplantation overcomes the ethical and immunological concerns associated with the use of fetal tissue."

Reference

179. Woodbury D *et al*.; "Adult rat and human bone marrow stromal cells differentiate into neurons"; J. Neuroscience Research 61, 364-370; August 15, 2000
Generated large numbers of dendritic cells from HUMAN blood monocytes. Provides example of use for clinical immunotherapy.

Reference

180. Cao H *et al.*; "In vitro generation of dendritic cells from human blood monocytes in experimental conditions compatible for in vivo cell therapy"; J. Hematother. Stem Cell Res. 9, 183-194; April 2000.
HUMAN bone marrow stem cells can form liver. According to Dr. Nick Wright, professor at the Imperial Cancer Research Fund,, since patients could use their own stem cells, "We could avoid problems with current liver transplants where the patient's body rejects the foreign organ." Dr. Markus Grompe, professor of molecular medical genetics at Oregon Health Sciences University, said "This would suggest that maybe you don't need any type of fetal stem cell at all—that our adult bodies continue to have stem cells that can do this stuff."

References

181. Theise N *et al*.; "Liver from bone marrow in humans"; Hepatology 32, 11-16; July 2000
Alison M *et al*.; "Cell differentiation: hepatocytes from non-hepatic adult stem cells"; Nature 406, 257; July 20, 2000
Bone marrow cells able to form liver.

Reference

182. Theise N *et al*.; "Derivation of hepatocytes from bone marrow cells in mice after radiationinduced myeloablation"; Hepatology 31, 235-240; January 2000
Bone marrow able to form liver.

Reference

183. Petersen B *et al*.; "Bone marrow as a potential source of hepatic oval cells"; Science 284, 1168-1170; May 14, 1999
Bone-specific expression of gene in marrow cells, showing targeted gene therapy for transplantation.

Reference

184. Lian JB, Stein GS, Stein JL, van Wijnen AJ; "Marrow transplantation and targeted gene therapy to the skeleton"; Clin Orthop 379 Suppl, S146-155; Oct. 2000.
Review of bone marrow as a source of cells for nervous system.

Reference

185. Mezey E, Chandross, KJ; "Bone marrow: a possible alternative source of cells in the adult nervous system"; Eur. J. Pharmacol. 405, 297-302; Sept. 29, 2000
Conditions have been identified to allow large-scale expansion of adult stem cells in culture, making these cells an almost unlimited source. Able to achieve a billion-fold increase in cell number in just a few weeks.
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Reference

186. Colter D *et al*.; "Rapid Expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow"; Proc. Natl. Acad. Sci. USA 97, 3213-3218; March 28, 2000
Able to achieve a significant increase in number of human hematopoietic stem cells in culture.

Reference

187. Ueda T *et al*.; "Expansion of human NOD/SCID-repopulating cells by stem cell factor, Flk2/Flt3 ligand, thrombopoietin, IL-6, and soluble IL-6 receptor"; J. Clin. Invest. 105, 1013-1021; April 2000
Description of potential mechanism to direct bone marrow (mesenchymal) stem cells to differentiate into specific lineages.
Reference
188. Jaiswal RK *et al.*; "Adult huma mesenchymal stem cell differentiation to the osteogenic or adipogenic lineage is regulated by mitogen-activated protein kinase"; J. Biol. Chem. 275, 9645-9652; Mar. 31, 2000
In culture, the cells were stimulated to form either bone, cartilage, or fat cells. The cells appear to have the potential to form other tissues as well, including tendon and muscle.
Reference
189. Pittenger MF *et al*.; "Multilineage potential of adult human mesenchymal stem cells"; Science 284, 143-147; April 2, 1999
**Marrow stem cells injected into mouse brain migrated through forebrain and cerebellum without disrupting host brain structure. The marrow stem cells populated various regions of the brain, and differentiated into astrocytes. These stem cells are proposed as methods for treating a variety of central nervous system disorders.
Reference:
190. Kopen GC *et al*.; "Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains"; Proc. Natl. Acad. Sci. USA 96, 10711-10716; Sept 14 1999
Human peripheral (circulating) blood contains stem cells for endothelial (blood vessel) cells.
References
191. Asahara T *et* al.; "Isolation of Putative Progenitor Endothelial Cells for Angiogenesis"; Science 275, 964-967; February 14, 1997
192. Shi Q *et al.*; "Evidence for Circulating Bone Marrow-Derived Endothelial Cells"; Blood 92, 362-367; July 15, 1998
Long, possibly unlimited lifespan of hematopoietic stem cells in culture. Using mouse bone marrow, a SINGLE stem cell could repopulate the marrow of a lethally-irradiated mouse.
Reference
193. Yagi M *et al*.; "Sustained ex vivo expansion of hematopoietic stem cells mediated by thrombopoietin"; Proc. Natl. Acad. Sci. USA 96, 8126–8131; July 1999
Updated June 25, 2001 Research—Adult Bone Marrow/Blood Stem Cells David A. Prentice
Able to repopulate bone marrow of mice with ONE transplanted stem cell.
Reference
194. Bhatia M *et al*.; "Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice"; Proc. Natl. Acad. Sci. USA 94, 5320–5325; May 1997
Circulating blood contains stem cells which are from bone marrow (study done in dogs.)
Reference
195. Huss R *et al*.; "Evidence of Peripheral Blood-Derived, Plastic-Adherent CD34 –/low Hematopoietic Stem Cell Clones with Mesenchymal Stem Cell Characteristics"; Stem Cells 18, 252-260, 2000
Using rat system, transplanted cells migrate to ischemic cortex.
Reference
196. Eglitis MA *et al*.; "Targeting of marrow-derived astrocytes to the ischemic brain"; Neuroreport 10, 1289; April 26, 1999
Multiple tissue types can be derived from bone marrow stem cells, with many potential clinical uses.
Reference
197. Deans, RJ and Moseley, AB, "Mesenchymal stem cells. Biology and potential clinical uses", Experimental Hematology 28, 875-884, August, 2000.
Human Bone Marrow Can Help Repair Brain Tissue

Human marrow stromal cells transplanted into rat. Cells engrafted, no evidence of inflammatory response or rejection. Useful for autotransplantation, gene therapy for variety of CNS diseases incl Parkinson's.

Reference

198. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ, "Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats-similarities to astrocyte grafts", Proc. Natl. Acad. Sci. USA 95, 3908; March, 1998

Bone Marrow Stem Cells Can Regenerate New Bone

Human mesenchymal stem cells, expanded in culture, regenerate human bone implanted in rats.

Reference

199. Bruder SP, Kurth AA, Shea M, Hayes WC, Jaiswal N, Kadiyala S, "Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells", J Orthop Res 16, 155; 1998

Allogeneic peripheral blood stem cell transplants as good or better than bone marrow

Reference

200. Ringden O *et al.*, “Peripheral blood stem cell transplantation from unrelated donors: a comparison with marrow transplantation”, Blood 94, 455; July 15, 1999

Human Bone Marrow Cells Induced To Form Bone In Culture

Reference

201. Jaiswal N, Haynesworth SE, Caplan AI, Bruder SP, "Osteogenic differentiation of purified, culture-expanded human mesenchymal stem cells in vitro", J Cell Biochem 64:295-312; 1997

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Bone Marrow Cells Maintain Potential After Long-Term Cryopreservation

Reference

202. Bruder SP, Jaiswal N, Haynesworth SE, "Growth kinetics, self-renewal, and the osteogenic potential of purified human mesenchymal stem cells during extensive subcultivation and following cryopreservation", J Cell Biochem 64, 278; 1997

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LIVER STEM CELLS

Adult stem cells from liver form heart tissue

Scientists at Duke University Medical Center showed that adult stem cells from liver could transform into heart tissue when injected into mice. They say that “Recent evidence suggests that adult-derived stem cells, like their embryonic counterparts, are pluripotent”, and that “These results demonstrate that adult liver-derived stem cells respond to the tissue microenvironment of the adult heart in vivo and differentiate into mature cardiac myocytes.”

Reference:

203. Malouf NN *et al.*; “Adult-derived stem cells from the liver become myocytes in the heart in vivo”, *American Journal of Pathology* 158, 1929-1935; June 2001

Developed culture and separation system for liver stem cells. When isolated liver stem cells were transplanted in mouse spleen, the cells migrated to the recipient liver and differentiated into mature liver cells. The authors suggest this approach could be used to isolate human liver stem cells and supplant whole organ transplant.

Reference:

204. Suzuki A *et al.*; “Flow-cytometric separation and enrichment of hepatic progenitor cell sin the developing mouse liver”; Hepatology 32, 1230-1239; Dec 2000

**Commentary re: Suzuki *et al.* article on treatment of liver disease by “repopulation of the diseased liver by cell transplantation.” “It should be noted that stem cells have also been found in other tissues and when transplanted, these cells differentiate into different mature phenotypes de-pending on the organ environment in which they are en-grafted. Thus, it is clear that liver stem/progenitor cells, their hematopoietic cousins, and perhaps other stem-cell rel-atives, have a bright future in the treatment of liver, as well as other diseases.”

Reference:

205. Shafritz DA; "Rat liver stem cells: Prospects for the future"; Hepatology 32, 1399-1400; Dec 2000

**Intravenous injection of adult bone marrow stem cells in a mouse model of tyrosinemia type I rescued the mouse and restored biochemical function of its liver.

Reference:

206. Lagasse E *et al.*; "Purified hematopoietic stem cells can differentiate into hepatocytes in vivo"; Nature Medicine 6, 1229-1234; Nov 2000

First purification and expansion of adult hepatic stem cells accomplished. "The ability of these hepatic stem cells to expand extensively, even at single cell seeding densities, contrasts with the limited expansion potential of the majority of mature liver cells, which typically undergo only a few cell divisions and require high seeding densities in culture to survive," according to Dr. Reid. In addition to the antigenic profile and methods of purification of the cells, novel culture conditions were described that permit expansion of a single hepatic stem cell to a colony of cells containing both hepatocytes and bile duct cells, the most rigorous proof of the clonality and bipotentiality of the cells. Incara Pharmaceuticals Corporation has license to the technique and is applying discoveries in the field of liver stem cells to the development of cell therapies for liver diseases.

Reference

207. Kubota H, Reid LM; "Clonogenic hepatoblasts, common precursors for hepatocytic and biliary lineages, are lacking classical major histocompatibility complex class I antigen"; Proc. Natl. Acad. Sci. USA 97, 12132-12137; Oct. 24, 2000

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General reference on liver stem cells

208. Strain AJ, Crosby HA; "Hepatic stem cells"; Gut 46, 743-745; 2000

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HEART/BLOOD VESSELS/HEART VALVES

Heart tissue may be regenerated from a heart stem cell

Researchers at New York Medical College, Valhalla, NY, report results that show regeneration of heart muscle is possible after heart attack. The research indicates that the heart may contain its own adult stem cell, which could possible be stimulated to grow and repair damage after a heart attack.

Reference:

209. Beltrami AP *et al.*; "Evidence That Human Cardiac Myocytes Divide after Myocardial Infarction", *New England Journal of Medicine* 344, 1750-1757; June 7, 2001

Engineered replacement aorta using a matrix onto which were seeded the sheep's own cells. Previous work had shown this technique also works for heart valves.

Reference

210. Shum-Tim D *et al*.; "Tissue engineering of autologous aorta using a new biodegradable polymer"; Ann. Thorac. Surg. 68, 2298-2304; December 1999

Human peripheral (circulating) blood contains stem cells for endothelial (blood vessel) cells.

References

211. Asahara T *et* al.; "Isolation of Putative Progenitor Endothelial Cells for Angiogenesis"; Science 275, 964-967; February 14, 1997

212. Shi Q *et al.*; "Evidence for Circulating Bone Marrow-Derived Endothelial Cells"; Blood 92, 362-367; July 15, 1998

FAT STEM CELLS

Isolated adult stem cells from HUMAN fat. Cells could be expanded and maintained in culture for extended periods, and could be differentiated into fat, cartilage, muscle, and bone. Characteristics similar to bone marrow stem cells.

Reference:

213. Zuk PA *et al.*; "Multilineage cells from human adipose tissue: Implications for cell-based therapies"; Tissue Engineering 7, 211-228; 2001

Adult Stem Cells from Fat

Scientists from the University of Pennsylvania have been able to isolate stem cells from fat and convert them into bone cells. “This is a potentially unlimited source of cells to turn into mature cells of different types,” said Dr. Louis P. Bucky. He said that other researchers were investigating forming muscle from fat stem cells. Dr. Bucky noted that with fat, there is an ample supply of cells and it is easy to get at. The work was reported at a meeting of the American Society of Plastic Surgeons in Los Angeles.

Reference

214. Amy Norton, “Stem cells from body fat—limitless supply,” Reuters Health, Oct. 18, 2000

LUNG STEM CELLS

215. Emura M; “Stem cells of the respiratory epithelium and their in vitro cultivation”; In Vitro Cell Dev. Biol. Anim. 33, 3; January 1997

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DENTAL STEM CELLS

**Identification and isolation of stem cells from human dental pulp. The stem cells could be induced to differentiate into tooth structures.

Reference:

216. Gronthos S *et al*.; “Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*”; Proc Natl Acad Sci USA 97, 13625-13630; Dec 5 2000

MAMMARY GLAND

Evidence using rats of subpopulation of epithelial cells from mammary gland with large proliferation and differentiation potentials; results support conclusion that rat mammary clonogens are multipotent mammary stem cells.

Reference

217. Kim ND *et al.*; “Stem cell characteristics of transplanted rat mammary clonogens”; Exp. Cell Res. 260, 146-159; Oct. 10, 2000

SPERMATOGONIAL

“The development of the spermatogonial transplantation technique has given new impetus to research on spermatogonial stem cells. Possibilities opened by this technique include: (a) New ways to study fundamental aspects of spermatogenesis; (b) Generation of transgenic large domestic animals; (c) Protection of (young) male cancer patients from infertility due to chemotherapy or radiotherapy. Spermatogonial stem cell transplantation for the above purposes encompasses a number of steps. First, the stem cells have to be isolated and possibly purified. Second, it should be possible to cryopreserve the stem cells, for example till the children have reached puberty. Third. it should be possible to culture spermatogonial stem cells for a prolonged period of time which would also allow transfection and subsequent selection of stably transfected cells. Fourth, in case of animal studies. the host testis should be emptied from endogenous stem cells. This is probably best done by local irradiation. Finally, the stem cells will have to be transplanted.”

Reference:

218. Izadyar F, Creemers LB, van Dissel-Emiliani FM, van Pelt AM, de Rooij DG; “Spermatogonial stem cell transplantation”; Mol Cell Endocrinol 169, 21-26; Nov 27 2000

Review of advances since the initial report of transplantation in 1994.

Reference

219. Johnston DS *et al.*; “Advances in spermatogonial stem cell transplantation”; Rev. Reprod. 5, 183-188; Sept. 2000

STEM CELLS FROM PLACENTA

220. Anthrogen, Inc. in a press release reports that they can isolate stem cells from placenta after delivery, and that these stem cells so far have been induced to form bone, nerve, cartilage, bone marrow, muscle, tendon, and blood vessel.

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GENERAL

“The committed stem and progenitor cells have been recently isolated from various adult tissues,

including hematopoietic stem cell, neural stem cell, mesenchymal stem cell and endothelial progenitor cell. These adult stem cells have several advantages as compared with embryonic stem cells as their practical therapeutic application for tissue regeneration."

Reference:

221. Asahara T, Kalka C, Isner JM; "Stem cell therapy and gene transfer for regeneration"; Gene Ther 7; 451-457; March 2000

**Mammalian stem cell transformation similar to the transdetermination seen in Drosophila.

Reference:

222. Wei G *et al.*; "Stem cell plasticity in mammals and transdetermination in Drosophila: Common themes?"; Stem Cells 18, 409-414; Nov 2000

Potential Treatment for Stroke Using Umbilical Cord Blood

223. Researchers at the University of South Florida have reported at the meeting of the American Association for the Advancement of Science (Jan 2001) and the American Academy of Neurology meeting (May 2001) that human cord blood stem cells can be induced to form neurons. When injected into the bloodstream of rats which had suffered stroke, the adult stem cells found their way to the brain and repaired much of the damage. Rats which were previously paralyzed showed 80% recovery. (From meeting press releases)

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EMBRYONIC STEM CELLS

Used human ES cells, added mixes of growth factors to try to get specialized cell types formed in culture. Got factors which induce mesoderm, ectoderm+mesoderm, or all 3 germ layers. No specific tissues derived. "The work presented here shows that none of the eight growth factors tested directs a completely uniform and singular differentiation of cells."

Reference:

224. Schuldiner M *et al.*; "Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells"; Proc. Natl. Acad. Sci. USA 97, 11307-11312; Oct. 10, 2000

Formed embryoid bodies (EB's) from embryonic germ (EG) cells, isolated and cultured cells from EB's. Cells show long-term population doubling (PD), normal karyotypes (checked at 20 PD, but not in the long-term cultures), can be stably transfected with extra genes for gene therapy. The cells are relatively uncommitted precursor or progenitor cells. "EB-derived cells may be suited to studies of human cell differentiation and may play a role in future transplantation therapies." "Although a compelling demonstration of the potential of human EG cells, the limited growth characteristics of differentiated cells within EB's and difficulties associated with their isolation would make extensive experimental manipulation difficult and limit their use in future cellular transplantation therapies." "For PSCs [pluripotent stem cells] to be of practical use, methods to generate large numbers of homogeneous cell types must be developed."

Reference:

225. Shamblott MJ, Axelman J, Littlefield JW, Blumenthal PD, Huggins GR, Cui Y, Cheng L, Gearhart JD; "Human embryonic germ cell derivatives express a broad range of developmentally distinct markers and proliferate extensively in vitro"; Proc Natl Acad Sci USA 98, 113-118; Jan 2 2001

EBDs reproduce readily and are easily maintained, Gearhart said, and thus eliminate the need to use fetal tissues each time as a source – a step that should quell many of the political and ethical concerns that swirl around stem cell studies. **"We thought from the first that problems would arise using hPSCs [human pluripotent stem cells] to make replacement tissues," says molecular biologist Michael Shamblott, Ph.D. The early-stage stem cells are both difficult and slow to grow. "More important," says Shamblott, "there's a risk of tumors. If you're not very careful when coaxing these early cells to differentiate – to form nerve cells and the like -- you risk contaminating the newly differentiated cells with the stem cells. "Injected into the body, stem cells can produce tumors.** The EBDs bypass all this." EBDs readily divide for up to 70 generations, producing millions of cells without any apparent chromosomal abnormalities typical of tumor cells. No tumors appeared in three cancer-prone test mice injected with the new cells. Moreover, EBD cells appear to

accept "foreign" genes readily – a necessity, Shamblott says, for scientists to produce large quantities of differentiated "replacement" cells for human transplants.

226. Johns Hopkins Medical Institutions Office of Communications and Public Affairs; "New Lab-Made Stem Cells May Be Key To Transplants"; Dec. 25 2000.

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EMBRYONIC STEM CELL DIFFERENTIATION

The following quotes are from an article in Science describing first exciting new results with adult stem cells, transforming bone marrow stem cells in brain and liver. The article then goes on to contrast the successes of adult stem cell research with the following description of human embryonic stem cell research.

Reference:

227. Vogel G; "Stem cells: New excitement, persistent questions"; Science 290, 1672-1674; Dec 1 2000

In contrast, the human embryonic stem cells and fetal germ cells that made headlines in November 1998 because they can, in theory, develop into any cell type have so far produced relatively modest results. Only a few papers and meeting reports have emerged from the handful of labs that work with human pluripotent cells, whose use has been restricted by legal and commercial hurdles. Last month, a group led by Nissim Benvenisty of The Hebrew University in Jerusalem, in collaboration with Douglas Melton of Harvard University, reported in the Proceedings of the National Academy of Sciences that they could nudge human embryonic stem cells toward a number of different cell fates. But the results did not produce easy answers; some cells expressed markers from several kinds of lineages.

The work suggests that it will not be simple to produce the pure populations of certain cell types that would be required for safe and reliable cell therapies—much less the hoped-for replacement organs, says stem cell researcher Oliver Brüstle of the University of Bonn in Germany. Brüstle was one of the first to show that mouse embryonic stem cells could help treat an animal disease model, in which neurons lack their insulating coat of myelin. Even so, he is cautious about the near-term prospects in humans. Says Brüstle: "At present, it looks like it is really difficult to differentiate these [human] cells into more advanced cell types." Melton agrees. "It's unlikely anyone will ever find a single growth factor to make a dopaminergic neuron," as some might have hoped, but the work provides "a starting place," he says.

Simply keeping human embryonic stem cells alive can be a challenge, says Peter Andrews of the University of Sheffield in England. For more than a year, he and his colleagues have been experimenting with embryonic stem cell lines that James Thomson derived at the University of Wisconsin, Madison. "They're tricky," Andrews says. It took several false starts--and a trip to Wisconsin --before the researchers learned how to keep the cells thriving, he says. Melton uses almost the same words: Human embryonic stem cells "are trickier than mouse," he says. "They're more tedious to grow."

Researchers from Geron Corp. in Menlo Park, California, are having some luck. Company researchers have been working with human embryonic stem cells as long as any team has, because Geron funded the derivation of the cells and has an exclusive license for their commercial use. They reported in the 15 November issue of Developmental Biology that cell lines derived from a single embryonic stem cell continue to replicate in culture for 250 generations. This is important, says Geron researcher Melissa Carpenter, because it means that a single human embryonic stem cell, which might be modified in the lab, could produce an essentially unlimited supply of cells for therapy. That was known for mouse embryonic stem cells but had not been shown in humans before.

Even so, **Geron researchers seem no closer than other groups to devising therapeutic uses for stem cells. Geron researchers reported last month at the annual meeting of the Society of Neuroscience that they had attempted to transplant human embryonic stem cells into rats. When they injected undifferentiated cells into the brain, they did not readily differentiate into brain cells, the researchers found. Instead, they stayed in a disorganized cluster, and brain cells near them began to die. Even partially differentiated cells, the team reported, tended to clump**

together; again, nearby brain cells died.
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Excerpt from article in *Science*
"Can Adult Stem Cells Suffice?" by Gretchen Vogel
228. Science vol. 292, pp. 1820-1822, 8 Jun 2001
In one tissue, at least, scientists agree that the results are encouraging. In the past few months, a series of papers has strengthened the idea that cells in the bone marrow can respond to cues from damaged tissue and help repair it. Until recently, doctors had only attempted to use bone marrow stem cells to reconstitute the blood or immune system.
But late last year, two teams reported that mouse cells derived from bone marrow could become neuronlike cells (Science, 1 December 2000, pp. 1775 and 1779). In April, another two groups reported that bone marrow-derived cells could help repair damaged heart muscle. In one study, Piero Anversa of New York Medical College in Valhalla and Donald Orlic of the National Human Genome Research Institute in Bethesda, Maryland, induced heart attack-like damage in 30 mice. They then injected the bone marrow cells into surviving heart tissue. Nine days after the injection, the transplanted cells were forming new heart tissue--muscle cells as well as blood vessels--in 12 of the 30 mice, the team reported in the 5 April issue of Nature.
In the other study, Silviu Itescu of Columbia University in New York City and his colleagues isolated cells from the bone marrow of human volunteers, then injected the cells into the bloodstream of rats in which the team had induced heart attacks. Signals from the damaged heart evidently attracted the transplanted cells, the team reported in the April issue of Nature Medicine; 2 weeks after the injection, capillaries made of human cells accounted for up to a quarter of the capillaries in the heart. Four months after the operation, rats that received the blood vessel precursors had significantly less scar tissue--and better heart function--than control rats.
Perhaps most impressive, in the 4 May issue of Cell, scientists reported that a single cell from the bone marrow of an adult mouse can multiply and contribute to the lung tissue, liver, intestine, and skin of experimental mice. Researchers knew that a tiny subset of cells purified from bone marrow had the potential to multiply and give rise to all the blood cell types, but isolating those cells has been very difficult. To increase their chances of capturing the elusive cells, Diane Krause of Yale University School of Medicine and Neil Theise of New York University Medical School and their colleagues performed a double bone marrow transplant. They first injected bone marrow cells from a male mouse, tagged with green fluorescent protein, into the bloodstream of female mice that had received a lethal dose of radiation. Two days later, they killed the recipient mice and isolated a handful of green-tagged cells that had taken up residence in the bone marrow. (Previous studies had suggested that the most primitive transplanted cells lodge in bone marrow.) They then injected irradiated mice with just one of the green-tagged cells accompanied by untagged, female-derived bone marrow cells that survive about a month. When the scientists killed the surviving mice 11 months after the second transplant, they found progeny from the cells in lung, skin, intestine, and liver as well as bone and blood. "Bone marrow stem cells can probably form any cell type," says Harvard's Melton.

Further excerpt from article

"But ES cells have plenty of limitations, too. For one, murine ES cells have a disturbing ability to form tumors, and researchers aren't yet sure how to counteract that. And so far reports of pure cell populations derived from either human or mouse ES cells are few and far between--fewer than those from adult cells."