Science

Over One Million Served—with Adult Stem Cells!

Even though it may sound a bit like a long past commercial for fast food, the news is all good and truly something to sink your teeth into when it comes to the number of patients treated with adult stem cells for various ailments. An international team has published the most recent numbers, and surprisingly to some, there have been over one million hematopoietic (blood-forming) adult stem cell transplants.[1] In fact, the one million patient milestone was passed December 2012, and this current tally counted only hematopoietic stem cell transplants and not other sources of adult stem cells already used in patients; so in fact, there are now well over one million people who have experienced the lifesaving advantages of adult stem cells. Many are still confused when they hear about "stem cells," may be unaware of the existence of adult stem cells, and may think that any success is attributable to embryonic stem cells. Yet the truth remains that adult stem cells are the real gold standard for stem cells, meeting the functional needs of patients using an ethical source of cells.

More Progress Treating Spinal Cord Injuries

Regaining physiological function, including feeling and mobility, after a spinal cord injury has long been a goal of regenerative medicine. A Chinese team reports some success in a three-year follow-up of patients in a double-blind study.[2] Eight patients had their own olfactory epithelial cells harvested and injected into the lesion site of their damaged spinal cord (from six to eighty-three months after their injury),

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[1] Alois Gratwohl et al., "One Million Haemopoietic Stem-Cell Transplants: A Retrospective Observational Study," *Lancet Haematology* 2.3 (March 2015): 91–100, doi: 10.1016/S2352–3026(15)00028–9.

[2] Sheng Wang et al., "Autologous Olfactory Lamina Propria Transplantation for Chronic Spinal Cord Injury: Three-Year Follow-Up Outcomes from a Prospective Double Blinded Clinical Trial," *Cell Transplantation*, e-pub April 22, 2015, doi: 10.3727/096368915X688065.

while four other patients received a sham operation with no cell transplantation. After three years, transplanted patients showed improvements in movement and sensation, including one patient who showed significant improvement (two grades on the Abbreviated Injury Scale). The authors note that the limited recovery suggests that the cell transplant alone is not enough to elicit full repair, but points the way for further refinements of the technique. Their results are similar to those observed previously by a group in Portugal who used similar olfactory epithelial tissue transplants,[3] by a group in Australia who used purified olfactory cells,[4] and by a British-Polish collaboration using cultured cells from olfactory epithelium.[5]

A team from Poland reports progress using injections of a different adult stem cell source: bone marrow stem cells.[6] The group isolated and cultured mesenchymal stem cells from the patient, a teenage girl, and then over a two-year period did multiple injections of the adult stem cell preparation into the spinal canal of the patient. They report in this case study that over time the patient showed significant improvement in her movement and sensation, improving two to three levels in the standardized injury scale measurements.

Finally, a Canadian group worked on development of another potential source of cells for treatment of spinal cord injury—induced pluripotent stem cells (iPSCs).[7] The iPSCs, which have characteristics of embryonic stem cells but are created from skin or other normal cells, without the use of embryos, eggs, or cloning techniques, can be produced from a patient and represent a potential source of large volumes of cells for study or cellular therapy. In this study, iPSCs were generated from normal mice (wild type) and also from mice with a genetic defect in their ability to form myelin sheaths around large nerve axons. Neural stem cells were produced from both iPSC lines and then tested for their ability to repair induced spinal cord damage in mice. While both neural stem cell derivatives integrated into the damaged spinal cord, only the wild type neural stem cell transplants provided a functional improvement. This indicates that remyelination is the key regenerative function in this particular

[3] Carlos Lima et al., "Olfactory Mucosal Autografts and Rehabilitation for Chronic Traumatic Spinal Cord Injury," *Neurorehabilitation Neural Repair* 24.1 (January 2010): 10–22, doi: 10.1177/1545968309347685.

[4] Alan Mackay-Sim et al., "Autologous Olfactory Ensheathing Cell Transplantation in Human Paraplegia: A 3-Year Clinical Trial," *Brain* 131.9 (September 2008): 2376–2386, doi: 10.1093/brain/awn173.

[5] Pawel Tabakow et al., "Transplantation of Autologous Olfactory Ensheathing Cells in Complete Human Spinal Cord Injury," *Cell Transplantation* 22.9 (September 2013): 1591–1612, doi: 10.3727/096368912X663532.

[6] Danuta Jarocha et al., "Continuous Improvement after Multiple Mesenchymal Stem Cell Transplantations in a Patient with Complete Spinal Cord Injury," *Cell Transplantation* 24.4 (April 2015): 661–672, doi: 10.3727/096368915X687796.

[7] Ryan P. Salewski et al., "Transplantation of Induced Pluripotent Stem Cell-Derived Neural Stem Cells Mediate Functional Recovery following Thoracic Spinal Cord Injury through Remyelination of Axons," *Stem Cells Translational Medicine* 4.7 (July 2015): 743–754, doi: 10.5966/sctm.2014–0236.

spinal cord repair system, and provides an important target for future attempts at treating such injuries.

Promising Early Results Using Adult Stem Cells for ALS

Korean doctors report promising results from a phase I clinical trial, using two injections of a patient's own bone marrow adult stem cells, to test for safety in a therapy for ALS (amyotrophic lateral sclerosis; Lou Gehrig's disease).[8] Seven patients underwent two injections of their own bone marrow mesenchymal stem cells, which had been grown in the lab for several weeks to increase the numbers of cells available for transplant. In a twelve-month follow-up, the patients showed no serious adverse events, and their scores on various tests of neurological and muscular measurements suggested that their condition might have stabilized. Further trials will be necessary to show definitive signs of efficacy, however.

You've Got a Lot of Nerve—in Cord Blood

It can at times be difficult to harvest or grow enough neural cells for experiments or for clinical trials at cellular therapy. Dose can be an important variable in many trials, especially involving neurodegenerative conditions. Now scientists have shown that large numbers of neural stem cells can be generated in the lab—directly from umbilical cord blood.[9] The direct conversion (with no cellular intermediate forms) was accomplished by adding the protein Oct 4, which functions normally as a master transcription factor to increase the growth and the flexibility of cellular differentiation potential. The converted cells showed normal neural stem cell characteristics, could differentiate into all of the normal derivatives seen with neural stem cells, and could survive and differentiate when transplanted into mouse brain. The results suggest a potential abundant source of neural stem cells for future applications.

Growing New Skin with Donor Adult Stem Cells

Recessive dystrophic epidermolysis bullosa is a genetic disease that results in fragile skin, often unanchored to the underlying dermis. The lack of anchoring means that the skin blisters and sloughs off on contact, often resulting in life-threatening infections. Most affected children have shortened lives due to carcinomas. A British group now reports promising results using allogeneic (unrelated donor) mesenchymal stem cells from bone marrow.[10] Ten children were given multiple infusions of the donor adult stem cells, and after a one-year follow-up, the treatment appeared safe. The patients showed decreased pain, decreased severity of skin problems, better

[8] Ki-Wook Oh et al., "Phase I Trial of Repeated Intrathecal Autologous Bone Marrow-Derived Mesenchymal Stromal Cells in Amyotrophic Lateral Sclerosis," *Stem Cells Translational Medicine* 4.6 (June 2015): 590–597, doi: 10.5966/sctm.2014–0212.

[9] Wenbin Liao et al., "Direct Conversion of Cord Blood CD34+ Cells into Neural Stem Cells by OCT4," *Stem Cells Translational Medicine* 4.7 (July 2015): 755–763, doi: 10.5966/sctm.2014–0289.

[10] Gabriela Petrof et al., "Potential of Systemic Allogeneic Mesenchymal Stromal Cell Therapy for Children with Recessive Dystrophic Epidermolysis Bullosa," *Journal of Investigative Dermatology* (September 2015), in press, doi:10.1038/jid.2015.158.

healing, and overall positive benefits. The promising outcomes using adult stem cells for treatment of this genetic condition reflected similar, previous results using donor bone marrow or umbilical cord blood adult stem cells.[11] While this current study is only a preliminary, unblinded study, the results show significant promise for future treatments.

Growing a Bioartificial Limb

Growing new limbs is no trick for a salamander, but for humans it is currently not possible. Current prosthetic limbs are increasingly sophisticated, and great progress has been made in construction of bionic replacements for damaged limbs.[12] But even the best prosthetics are still not an authentic replacement for the 1.5 million Americans who have lost a limb. Now scientists at Massachusetts General Hospital have shown the tantalizing possibility for growing a bioartificial limb—a limb constructed in the laboratory from the patient's own cells and biological substrates.[13] Previous work has generated organs such as liver and heart, but the three-dimensional architecture of a limb adds an additional layer of complexity. To test the process, cellular material was slowly removed from a rat limb, while preserving the primary vasculature and nerve matrix. These matrix materials provided a structural guide for growth and modeling of the various cells and tissue structures needed for a complete limb. Muscle and vascular progenitor cells were used to repopulate the matrix structure, as well as an electrical current to stimulate muscle growth. When transplanted onto rat hosts, blood circulation was renewed. Electrical stimulation showed flexion of wrists and paws. The results indicate potential for regeneration of replacement limbs using the patient's own cells.

Caring for Premature Babies Increases Their Survival

While it might seem self-evident, a recent study notes that very premature infants survive at much higher rates when given simple care. A new study in the *New England Journal of Medicine* emphasizes that premature babies born earlier and earlier can indeed live, as well as thrive, and at significantly higher rates if the attending physicians take an attitude toward these young patients as one of care, rather than resignation due to their extreme prematurity. A survey of various hospitals across the United States examined the survival of very premature newborns, as well as the attitudes and interventions of hospital staff toward these young individuals. The new study clearly demonstrates that babies delivered as young as twenty weeks post-fertilization (twenty-two weeks gestation) can survive, and even thrive, and active intervention for treatment greatly improves their rate of survival and decreases

[11] John E. Wagner et al., "Bone Marrow Transplantation for Recessive Dystrophic Epidermolysis Bullosa," *New England Journal of Medicine* 363.7 (August 12, 2010): 629–639, doi: 10.1056/NEJMoa0910501.

[12] Oskar C. Aszmann et al., "Bionic Reconstruction to Restore Hand Function after Brachial Plexus Injury: A Case Series of Three Patients," *Lancet* 385.9983 (May 2015): 2183–2189, doi: 10.1016/S0140–6736(14)61776–1.

[13] Bernhard J. Jank et al., "Engineered Composite Tissue as a Bioartificial Limb Graft," *Biomaterials* 61 (August 2015): 246–256, doi: 10.1016/j.biomaterials.2015.04.051.

later impairment of the preemies as they develop.[14] Doctors who consider these preterm babies as patients demonstrate that active treatment significantly benefits these young babies.

Babies Sense Pain Like Adults

There continues to be debate on the ability of preborn or newborn babies to sense pain. A recent study using sensitive measurement of brain responses indicates that newborns sense pain in similar manner as adults. An Oxford University team used fMRI (functional magnetic resonance imaging) to measure pain response in the brains of newborns (one to six days old), comparing the brain response regions to those seen in adults (twenty-three to thirty-six years old).[15] The authors noted that "brain regions that encode sensory and affective components of pain are active in infants, suggesting that the infant pain experience closely resembles that seen in adults." In fact, eighteen out of twenty brain regions in the newborns showed responses similar to the adults. Moreover, the newborns showed greater sensitivity to even a mild pain stimulus, responding at a level that required four times the pain stimulus in adults to achieve the same response.

Editing the Human Genome

An area that has been in the news as well as in science journals of late is the idea of editing the human genome, especially at the embryonic stage of development. When such genetic modifications occur so early in development, whether in the nuclear DNA genome or in the mitochondrial genome, the alterations are contained in virtually every cell of the developing organism and are known as "germline engineering" or "heritable modifications," that is, able to be passed to future generations. The science supporting successful genetic engineering of any mammal is at this point very thin. Nonetheless, proposals have come forth to proceed with human trials in the US and are being debated, and one proposal has already been approved in the UK. This note is not meant to give a comprehensive review of the science, or of the ethical arguments, related to the genetic engineering of humans. It is important, however, to become aware of the debate and some points regarding the proposed technologies.[16]

A Chinese laboratory announced in April that they had created the first genetically modified human embryos using the CRISPR-Cas9 system.[17] This genetic engineering enzyme system is the latest to be developed and perhaps the most specific at

[14] Matthew A. Rysavy et al., "Between-Hospital Variation in Treatment and Outcomes in Extremely Preterm Infants," *New England Journal of Medicine* 372.19 (May 7, 2015): 1801–1811, doi: 10.1056/NEJMoa1410689.

[15] Sezgi Goksan et al., "fMRI Reveals Neural Activity Overlap between Adult and Infant Pain," *eLife* 4 (April 21, 2015): e06356, doi: 10.7554/eLife.06356.001.

[16] For a primer describing various proposed methods, see "'3-Parent Embryos' and 'Gene-Edited Babies': A Visual Aid," Charlotte Lozier Institute, June 26, 2015, https://www.lozierinstitute.org/.

[17] Puping Liang et al., "CRISPR/Cas9-Mediated Gene Editing in Human Tripronuclear Zygotes," *Protein Cell* 6.5 (May 18, 2015): 363–372, doi: 10.1007/s13238–015–0153–5.

targeting specific DNA replacements via a cut-and-paste system. The targeting relies on a guide RNA to find very specific DNA sequences.[18]

The Chinese group found that they could target and effectively cleave the beta-globin locus in human embryos. However, their results showed that repair of the DNA strands occurred at very low efficiencies, resulting in mosaic embryos (embryos in whom some cells contained the DNA alteration and some did not). Their results also showed off-target cleavage of DNA, potentially leading to creation of unintended mutations at various sites within the genome. While it is obvious that the technology is at this point unsuccessful and immature, the attempt at gene editing of human embryos has raised significant concerns regarding the ethics of such research.

One view of socioethical implications seems to be that genetic engineering of humans, and particularly of embryos, is inevitable.[19] In this viewpoint, genetically modified children are a certain future, perhaps an ethical good, and the best we can hope to do is regulate such technology to control the outcomes. This attitude seems true not only in the attitudes toward recent genetic engineering of nuclear DNA in human embryos by the Chinese lab, but also in the headlong rush to approve and implement germline modification of mitochondrial DNA, advocating regulation and creating so-called "three-parent embryos."[20] However, there are certainly other viewpoints which view any human germline gene editing as unwise and even illicit.[21] Much remains to be debated about the wisdom of editing the human genome and altering ourselves, as well as future generations. Stand by for more.

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[18] Jennifer A. Doudna and Emmanuelle Charpentier, "The New Frontier of Genome Engineering with CRISPR-Cas9," *Science* 346.6213 (November 28, 2014): 1–9, doi: 10.1126/science.1258096.

[19] Tetsuya Ishii, "Germline Genome-Editing Research and Its Socioethical Implications," *Trends in Molecular Medicine* 21.8 (August 2015): 473–481, doi: 10.1016/j.molmed.2015.05.006.

[20] I. Glenn Cohen et al., "Transatlantic Lessons in Regulation of Mitochondrial Replacement Therapy," *Science* 348.6231 (April 10, 2015): 178–180, doi: 10.1126/science.aaa8153.

[21] Edward Lanphier et al., "Don't Edit the Human Germ Line," *Nature* 519.7544 (March 26, 2015): 410–411, doi: 10.1038/519410a.