Umbilical Cord Blood–Derived Stem Cells and Brain Repair

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ABSTRACT: Human umbilical cord blood (HUCB) is now considered a valuable source for stem cell–based therapies. HUCB cells are enriched for stem cells that have the potential to initiate and maintain tissue repair. This potential is especially attractive in neural diseases for which no current cure is available. Furthermore, HUCB cells are easily available and less immunogenic compared to other sources for stem cell therapy such as bone marrow. Accordingly, the number of cord blood transplants has doubled in the last year alone, especially in the pediatric population. The therapeutic potential of HUCB cells may be attributed to inherent ability of stem cell populations to replace damaged tissues. Alternatively, various cell types within the graft may promote neural repair by delivering neural protection and secretion of neurotrophic factors. In this review, we evaluate the preclinical studies in which HUCB was applied for treatment of neurodegenerative diseases and for traumatic and ischemic brain damage. We discuss how transplantation of HUCB cells affects these disorders and we present recent clinical studies with promising outcome.

KEYWORDS: stem cell; cord blood; neurogenesis; brain repair

INTRODUCTION

The last few years have witnessed an expansion in stem cell research and its potential for therapy following the revolutionary experiments in mammalian cloning. In addition to the controversial embryonic stem cell research, adult stem cell sources like hematopoietic stem cells, mesenchymal stem cells, epidermal stem cells, pancreatic stem cells, and several other organ stem cells are currently identified and characterized in laboratories all over the world. Clinical stem cell therapy dates back

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to the first bone marrow transplant experiments in the middle of the past century. Nonetheless, hematopoietic stem cell therapy (HSCT) has been reserved for life-threatening or advanced illness because of associated complications in the form of graft rejection, graft-versus-host disease (GVHD), and infections. These post-transplant bacterial and viral infections caused by conditioning regimens like chemotherapy, irradiation, cytokines, and the use of anti-T cell antibodies are considered the primary impediment for survival after stem cell transplantation.[1] Manipulation of the stem cell graft by enriching for desirable stem cells, deleting mature lymphocytes, and including additional stem cell sources like umbilical cord blood and peripheral blood stem cells has significantly improved the outcome of this form of stem cell therapy.[2,3]

Peripheral blood stem cells are rapidly becoming the standard care in autologous transplantation after the use of hematopoietic growth factors G-CSF and GM-CSF to recruit stem cells into peripheral blood. In the allogeneic setting, however, a major limitation to stem cell therapy has been the higher incidence of acute and chronic GVHD and its potential negative impact on survival.[4,5] During the past two decades, human umbilical cord blood (HUCB) has emerged as a novel valuable source for stem cells next to bone marrow and peripheral blood. Only 20 to 25% of patients are expected to find an HLA-matched sibling from the bone marrow donor pool. Since its first successful transplant for Fanconi's anemia in 1988, HUCB was found to be a highly enriched source for immature stem and blood cells that are less immunogenic than adult marrow and blood cells. Compared with bone marrow recipients, cord blood recipients from related or unrelated donors experience a decreased incidence of acute graft-versus-host disease and a rather delayed hematopoietic recovery.[6–8] Lower immunogenicity of cord blood is credited to abundant immature progenitors with longer telomeres than adult marrow stem cells.[9] T lymphocytes in the cord blood demonstrate a healthy proliferative response to alloantigen stimulation, but their cytotoxic lytic function seems to be depressed.[10,11] Cord blood–expanded dendritic cells (DCs) are less alloreactive in a mixed lymphocyte reaction than peripheral bloods DCs, which may account for the lower GVHD observed in cord blood. Decreased cytokine production like IL-12 and interferon gamma have been associated with lower immunogenicity.[13]

ENGRAFTMENT OF CORD BLOOD STEM CELLS

Unlike bone marrow transplants, limited data are available on HUCB engraftment and the dynamics of its contribution to reconstitution of the lymphohematopoietic system. Impressive evidence for engraftment was demonstrated when unrelated HUCB cells were transplanted into children with immune deficiency. Donor stem cells were engrafted, and induced rapid and reliable recovery of immune functions. In addition, these children suffered lower risk of GVHD and post-transplant infections.[14] In their elegant study, Traggiai *et al.*[15] injected $CD34^+$ HUCB cells via the intrahepatic route into conditioned newborn immune-deficient $Rag2^{-/-}gammac^{-/-}$ mice. Cord blood cells engrafted and reconstituted primary and secondary lymphoid organs. *De novo* development of donor origin B and T lymphocytes, and dendritic cells was associated with production of normal functional immune responses.

Reconstitution studies show that short-term recovery of neutrophils is delayed compared to bone marrow and peripheral blood stem cells, but long-term recovery of T, B lymphocytes, and natural killer cells seem to be satisfactory.[16] The composition of cord blood cells may contribute to these dynamics. HUCB cells correlate with bone marrow cells with the exception of the lymphocyte content, which tends to be lowest in CB grafts.[17] The percentage of $CD34^{+}CD38^{-}$ hematopoietic stem cells (HSCs) seems to be higher in cord blood as compared to bone marrow, as well as the natural killer (NK) cell populations.[18] Superior functions of cord blood stem cells have also been reported in hematopoietic colony functional assays. Pluripotent HSCs within cord blood had a higher cloning efficiency, were more proliferative in response to cytokine stimulation, and generated approximately seven-fold progeny when compared to bone marrow cells.[19] This enrichment was clinically supported by studies that showed the efficiency of fewer number of cord blood HSCs used in transplantation as compared to bone marrow.[20] Considering that one limitation of cord blood therapy in adults is the inadequate volume obtained from a single cord, this clinical efficacy lead Broxmeyer *et al.*[21] to suggest that a single collection of cord blood could be sufficient for adult transplantation.

EXPANSION OF CORD BLOOD STEM CELLS

In preclinical studies, cord blood transplantation (CBT) has rescued lethally irradiated mice and reconstituted their bone marrow.[22,23] After these leading experiments, clinical trials using cord blood transplantations have been applied to more than 2500 patients, mostly children, thus far. Insufficient number of stem cells obtained from a single cord blood has hampered extensive applications in adults, who required compilation of blood cells from several umbilical cords. Predictably, a primary goal in the field of cord blood transplantation has been *ex vivo* expansion and amplification of its stem cell content by various manipulations.

Similar to the case in bone marrow stem cells, expansion of cord blood stem cells by cocktails of growth factors has been attempted and reported with variable degrees of success.[24,25] These factors included cytokines like stem cell factor (SCF), FLT3L, thrombopoietin, and chemokines like IL-8, MIP1a, and VEGF, in addition to glycoaminoglycan.[26] Expanded cells following any of these protocols are assessed by both phenotypes and culture characteristics *in vitro,* by functional assays like colony-forming cell assay (CFC assay), and long-term culture–initiating cell assay (LTC-IC assay). Expansion of cord blood stem cells by co-culture on feeder layers of marrow stromal cells, or more recently, cord blood mesenchymal stem cells has also been reported.[27] Likewise, this co-stimulatory function of stromal cells has been shown *in vivo;* co-transplantation of mesenchymal stem cells has facilitated engraftment of cord blood stem cells in various mouse models.[28,29]

PLASTICITY OF CORD BLOOD CELLS

Several successful CBTs have been performed for both malignant and non-malignant diseases of blood and other organs.[30] Diseases of the nervous system are

an especially attractive target for stem cell therapy, since neurodegeneration is considered an end-stage illness and treatment for the most part is symptomatic. In addition, the discovery of neural stem cells and the potential of other stem cells to transdifferentiate into neural tissues have expanded neuroscience research in that direction. Studies that showed differentiation of embryonic stem (ES) cells *in vitro* into all neurons and glia[31] generated justifiable excitement about their therapeutic potential to replace degenerating neural cells. Ethical and medical concerns have directed stem cell research nationwide to alternative sources for plastic stem cells including HUCB stem cells. Many reports have followed from our laboratories and several others that show that HUCB stem cells could differentiate across tissue lineage boundaries into neural and other tissue lineages.

Sanchez-Ramos *et al.*[32] have demonstrated that the culture of mononuclear fraction of HUCB in a proliferating medium supplemented with all-*trans*-retinoic acid (RA) and nerve growth factor (NGF) promoted the expression Musashi-1 and TUJ-1 neural markers, and GFAP astrocyte marker (glial fibrillary acidic protein). In addition, mRNA for neuronal markers nestin and necdin was detected. Likewise, Ha *et al.*[33] have shown that HUCB cultured in beta-mercaptoethanol differentiated into neural phenotype as determined by positive immunocytochemical expression of neural nuclear antigen (NeuN), neurofilament, and GFAP, and by RT-PCR mRNA for nestin, neurofilament and microtubule-associated protein (MAP2). McGuckin *et al.*[34] have recently demonstrated that HUCB cells could expand in liquid culture supplemented with thrombopoietin, flt-3 ligand, and c-kit ligand (TPOFLK) into both hematopoietic and neuroglial progenitors.

EXPERIMENTS WITH SELECTED CORD BLOOD STEM CELLS

In most of the aforementioned studies, the mononuclear fraction of cord blood cells was used in the initial culture, without prior purification or selection of a precursor cell of interest. Whether neural differentiation of cord blood cells is the progeny of neural progenitors, or hematopoietic, or mesenchymal, or other stem cells within the cord blood graft was not clear. It became important to characterize a specialized cell fraction or population within the cord blood that is enriched for neural precursors for purposes of targeted therapy, genetic manipulations, and to further understand stem cell biology.

Bicknese *et al.*[35] purified a multipotent HUCB cell subset that is negative for the CD14 monocyte marker, and the CD34 hematopoietic progenitor marker. The culture was supplemented with basic fibroblast growth factor (bFGF) and human epidermal growth factor (hEGF). Immunohistochemistry and Western blot analysis showed differentiation into cells that expressed both GFAP and TUJ1 astrocyte and neural markers after 7 days in culture. In another study, a different HUCB cell fraction that is positive for both CD34 and the leukocyte marker CD45 was isolated by Buzanska *et al.*[36] by means of magnetic cell sorting. These clonic cells were, however, incapable of forming hematopoietic colonies. Upon culture in DMEM and hEGF, cells positive for nestin were produced. After further exposure to retinoic acid and BDNF, cells were immunoposive for TUJ1, MAP2, GFAP, and Gal-C (galactocerebroside) oligodendrocyte marker.

Studies of bone marrow stromal cells have shown that MSCs could be induced under conditions that increase intracellular levels of cAMP, to differentiate into cells of neural phenotype.[37] Under the appropriate culture conditions, both rodent and human marrow MSCs could be induced to differentiate into neural like cells.[38,39] Isolation of MSCs has not been as promising in cord blood as it is in bone marrow. Earlier experiments to separate MSCs from cord blood have either failed or obtained a low yield. However, further studies from various laboratories, including an ongoing study in our lab, have shown that MSCs could be isolated from cord blood, and could engraft immune-deficient mice.[40] In a recent study, Jeong *et al.*[41] isolated adherent cells expressing MSC-related antigens such as SH2, CD13, CD29, and AS-MA, from a mononuclear cell fraction of HUCB. After culture in neurogenic differentiation medium, both immunofluorescence and RT-PCR analyses indicated elevated expression of Tuj1, TrkA, GFAP and CNPases neural markers. Most of these studies agree that MSCs collected from cord blood are somewhat different in both structure, function, and abundance from bone marrow MSCs. Bieback *et al*.[42] have set strict criteria to obtain MSCs from cord blood, such as a time from collection to isolation of less than 15 hours, a net volume of more than 33 mL, and mononuclear cell count of at least 1×10^8 cells.

In view of available literature on cord blood stem cells, it is premature to define the cord blood cell fraction most enriched for neuroprogenitors. Further studies that include clonal analysis of transfected cells, neural and glial functional assays, and *in vivo* transdifferentiation are required before defining and isolating plastic cord blood cell populations. Most of the ongoing research is influenced by the bone marrow system, which may be similar to cord blood in terms of hematpoietic reconstitution, but significantly different in both cellular structure and content.

PRECLINICAL STUDIES UTILIZING HUCB CELLS

In vitro plasticity studies strongly suggest that cord blood stem cell therapy may represent a viable alternative for brain repair. Preliminary *in vivo* investigative studies in our laboratories examined homing, migration, and differentiation of HUCB cells into normal brains.[43] HUCB mononuclear fraction was transplanted into the subventricular zone of neonatal rat pups. Thirty days after transplantation, the pups were euthanized and brains dissected for human cells. HUCB cells were detected in the subventricular zone, the overlying cortex, and corpus callosum. Immunohistochemical phenotyping showed GFAP- and TUJ1-positive cells of donor HUCB origin in the developing brain, indicating differentiation into glial and neural phenotypes. The safety of this form of xenogenic transplantation was determined by absence of histological abnormalities or behavioral deficits in the transplanted rats. In both allo- and xenotransplants, however, the use of immune suppressive therapy enhanced, and at times was necessary, to maintain the survival of donor cells.[44]

In addition to the mononuclear fraction, purified populations of cord blood stem cells were also transplanted crossing the xenogenic barrier. A pluripotent, $CD45^-$ population from HUCB, termed unrestricted somatic stem cells (USSCs), has been recently described by Kogler *et al*.[45] *In vitro*, USSCs differentiated into cells of multiple lineages including osteoblasts, chondroblasts, adipocytes, hematopoietic cells,

and also astrocytes and neurons that express neurofilament, sodium channel protein, and various neurotransmitter phenotypes. When USSCs were transplanted into adult rat brain, human tau-positive cells with a typical neuron morphology were detected for up to 3 months post transplantation and showed neuron-like migratory activity.

Marrow stromal cell therapy showed promise in an ischemic cortex model where more marrow-derived cells were shown to migrate to the site of injury,[46] and to promote regeneration of the brain architecture in experimental autoimmune encephalitis.[47] In a promising clinical trial, *ex vivo* expansion of autologous mesenchymal stem cells and transplantation into the spinal cord of humans proved to be safe and well tolerated by ALS patients.[48]

HUCB CELL THERAPY FOR ISCHEMIC BRAIN DAMAGE

Stroke, one of the leading causes of death worldwide, is produced by focal ischemia to the brain and subsequent neural degeneration and damage. The basis for the use of cellular therapy in animal models for stroke is to stimulate neural regeneration and to limit further damage. The beneficial effect of HUCB transplantation in stroke-affected animals has challenged the dogma of neural rejuvenation and offered a viable system to analyze the cellular and molecular events involved in this regeneration.

There seems to be a consensus that ischemic injury in vital organs like the heart and the brain promotes cell migration to the site of injury to initiate the process of repair. Stimulation of endogenous stem cells that are activated and mobilized in response to various injuries seems to be an exciting strategy to promote endogenous repair of the adult CNS. However, the capacity of these progenitors to migrate and to differentiate into neural or glial cells differs according to the lesion type and the germinative zone from which they arise (reviewed by Picard-Riera *et al.*[49]). Studies in rodent models of stroke suggested that this process may be maintained by intrinsic stem cells that reside in the subventricular zone. Similar stem cells depicted as subventricular astrocytes have been recently proposed as a source for neural stem cells in adult humans.[50]

Ischemic neural injury stimulates inflammatory processes associated with recruitment and release of mediators crucial for initiating repair and supporting regeneration. This was demonstrated by *in vitro* studies using migration assays, in which extracts of ischemic tissues promoted migration of HUCB as compared to results in healthy controls.[51] Neurotransmitters involved in this inflammatory process included NGF (neurotrophic growth factor), BDNF (brain-derived neurotrophic factor), EGF (epidermal growth factor), FGF-2 (fibroblast growth factor-2), IGF, erythropoietin, and SCF (stem cell factor) (reviewed by Peterson[52]).

The evidence for the effect of HUCB transplantation on neural recovery after stroke first came from studies in Chopp's laboratories,[51,53] where rats were subjected to middle cerebral artery occlusion (MCAO) to induce focal ischemia-like pathology. Systemic delivery of HUCB via lateral tail-vein injection helped improvement in the transplanted animals and were detected in the affected cortex, subcortex, and striatum of the damaged brain. Immnuohistochemical phenotyping showed positive staining for neuronal markers (NeuN and MAP-2), astrocyte marker GFAP, and en-

dothelial marker FVIII. Homing studies showed that tissue damage induced by traumatic brain injury stimulated migration of infused cord blood to the parenchyma of the affected brain tissue. Expression of neural and astrocyte markers was associated with functional improvements and reduction of motor and neuronal deficits.

Studies in stroke models from our laboratories have suggested that this improvement is linked to several factors including the site and extent of the neural damage, timing of the transplant, and the route of cellular administration. In the studies by Willing *et al.*,[54] stroke was similarly induced in rats by MCAO. Transplantation of HUCB into the striatum or the femoral veins of rats resulted in alleviated behavioral deficits. Nonetheless, this improvement was not associated with detection of donor HUCB cells in the brain by immunostaining methods. Cellular administration via the femoral vein was less invasive and associated with recovery of the forelimb. A subsequent study in our laboratories[55] has shown significant improvement in behavioral recovery 4 weeks after MCAO occlusion. Migration of HUCB was observed only in the injured hemisphere, and better recovery was correlated with higher doses of infused cord blood.

Another study by Saporta *et al.*[56] tested the effect of intravenous injection of cord blood on the recovery from spinal cord injury. HUCB was injected into rats 1 and 5 days after compression spinal cord injury. HUCB cells were localized around the site of injury, but not in the healthy spinal cord tissues. Behavioral open-field test scores were improved in the rat group undergoing transplantation 5 days after the injury. All of these studies have demonstrated that tissue injury is a critical factor in attracting donor cord blood cells and initiating the process of repair. Release of trophic factors at the site of injury may simultaneously promote selective migration of donor stem cells, and also accelerate healing and tissue repair.

Despite detection of donor cells at the site of injury, which cells within the cord blood graft contribute to the observed recovery and how this repair is achieved remain critical questions in neural transplantation. In a recent study, Borlongan *et al.*[57] were not able to detect HUCB in the brains of rats transplanted with low doses via the intravenous route after induction of stroke. Despite that a blood–brain barrier permeabilizer (mannitol) was co-infused with the cord blood cells, human cells were not engrafted. Reduced cerebral infarct size and increased levels of neuroprotectant factors led the investigators to suggest that the observed recovery after stroke was mediated by trophic factors and molecules induced by the cord blood cells, regardless of the availability of these cells at the site of the damage. A different mechanism was suggested by a study by Taguchi *et al.*,[58] who used the $CD34^+$ cells within the cord blood to study the effect of this stem cell–enriched population on recovery from stroke. Immunocompromised mice underwent intravenous transplantation with $CD34^+$ cells 48 hours after the induction of stroke. Recovery was associated with enhanced neovascularization on the borders of the ischemic zone. Interestingly, when this neovasculaization was suppressed by antiangiogenic agents, neurogenesis was impaired. This study suggests that systemic administration of cord blood $CD34^+$ cells stimulated neovascularization, which in turn stimulated endogenous neurogenesis. Despite these encouraging data, the role of stem cell therapy in stroke is still elusive. Several factors like the type of injected cells (whole cord blood versus purified stem cells), the route of transplantation (systemic versus local, and intra-arterial versus intravenous), and most importantly, the window of time for successful therapy (less than 13 hours to up to 1 week) remains to be determined.

NEURODEGENERATIVE DISEASES

Neurodegenerative diseases include a group of brain disorders characterized by slow onset and progressive course of deteriorating neural functions. Nerve cell dysfunction caused by degeneration of specific brain regions affects memory and movement, usually in middle-aged and older populations. Several years usually elapse between the onset of the pathology and the clinical symptoms of disorders like Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS). While advances in the treatment of these illnesses have dramatically improved the quality of life and elongated life span for afflicted patients, no cure is currently available. The impact of cellular therapy approaches on neurodegenerative diseases has been both encouraging and intangible. An important obstacle has been the scarcity of animal models to provide the appropriate *in vivo* opportunities to study and design more effective forms of therapy.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by progressive degeneration of the motor neurons. Patients with ALS suffer weakness and progressive wasting and paralysis of the muscles that affect their ability to move, speak, swallow, and eventually breathe. Progressive paralysis could lead to death, usually within 5 years of disease onset. Currently, there is no cure for ALS, but treatment focuses on symptoms to improve the quality of life and delay complications. Recently, animal studies show that cell therapy could be a viable option to treatment of ALS by means of replacing diseased cells, stimulating neural cell regeneration, and delaying neural and motor atrophy.

The SOD-1 transgenic (B6SJL-TgN[SOD1-G93A]1GUR) mouse has a mutation of the human transgene, CuZn superoxide dismutase gene SOD1, which has been associated with amyotrophic lateral sclerosis. Ende *et al*.[59] and Chen *et al*.[60] attempted the cell therapy approach by transplanting large doses of HUCB mononuclear cells into SOD mice by intravenous administration. HUCB transplantation caused considerable delay in the onset of symptoms and death of the ALS mouse model.

In a recent study in our laboratories, Garbuzova-Davis *et al*.[61] have administered MNC fraction of HUCB via jugular vein injection into a presymptomatic G93A ALS mouse model prior to the onset of behavioral impairments. Significant benefits were observed in hind-limb extension and gait. Mice undergoing transplantation maintained their weight better and had a significantly longer life span than diseased nontransplanted mice. Cord blood graft has provided protection of motor neurons and perhaps replacement of damaged neurons in ALS-affected mice. The mechanism of repair after cord blood transplantation is, however, not well understood. FIGURE 1 shows immunophenotyping of HUCB in the brain after intravenous injections. Donor cord blood cells, detected in the parenchyma of the brain and spinal cord (FIG. 2), were positive for neural and astrocyte phenotype markers (TUJ1 and GFAP). Donor cells were also detected in the spleen, kidneys, liver, lungs, and heart.

CLINICAL STUDIES WITH CORD BLOOD

An expanding list of disorders currently treated with cord blood transplantation include hemoglobinopathies like sickle cell anemia and thalassemia, leukodystro-

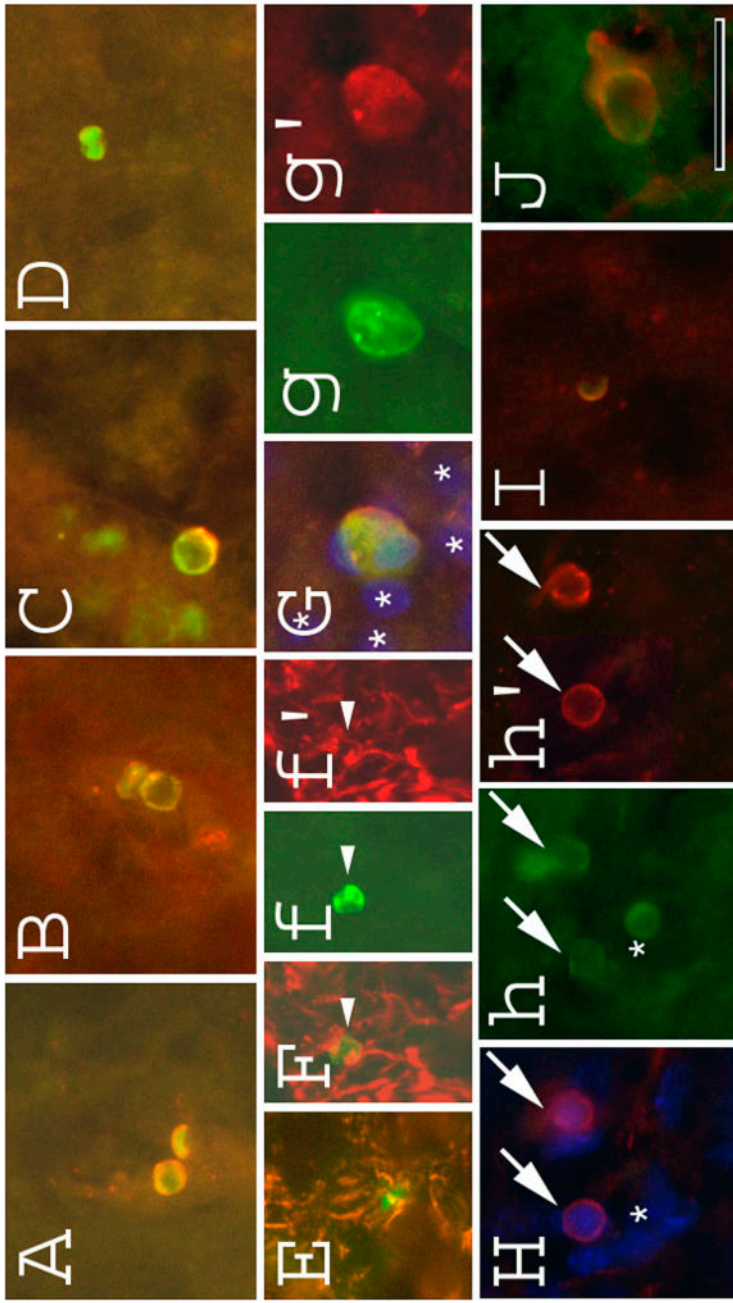

FIGURE 1. Immunohistochemical staining of hUCB cells in the brain. Most cells were found in blood vessels (**A,B,C**) in many structures of the brain. They were (**A**) HuNu/CD45-positive (orange/red, double staining) or (**B,C**) only HuNu-positive (green). Some cells were found (**C**) parenchymally at a distance from the blood vessel in (**D**) corpus callosum, (**E**) ventral tegmental area, (**F**) pons, (**G**) frontal cortex, (**H**) olfactory bulb,(**I**) lateral olfactory tract, and (**J**) hippocampus. The cells were also found in the striatum, cerebellar lobules, ventromedial thalamus, and spinal trigeminal area. Some cells were double-stained with HuNu and GFAP (**F**) and TuJ1 (**H,I,J**). The nuclei in **H** and **J** are shown with DAPI. Scale bar in **A, C, D, E, F, G, H, I,** and **J** is 25 mm. Scale bar in **B** is 50 mm. [*Illustration is in color online.*]

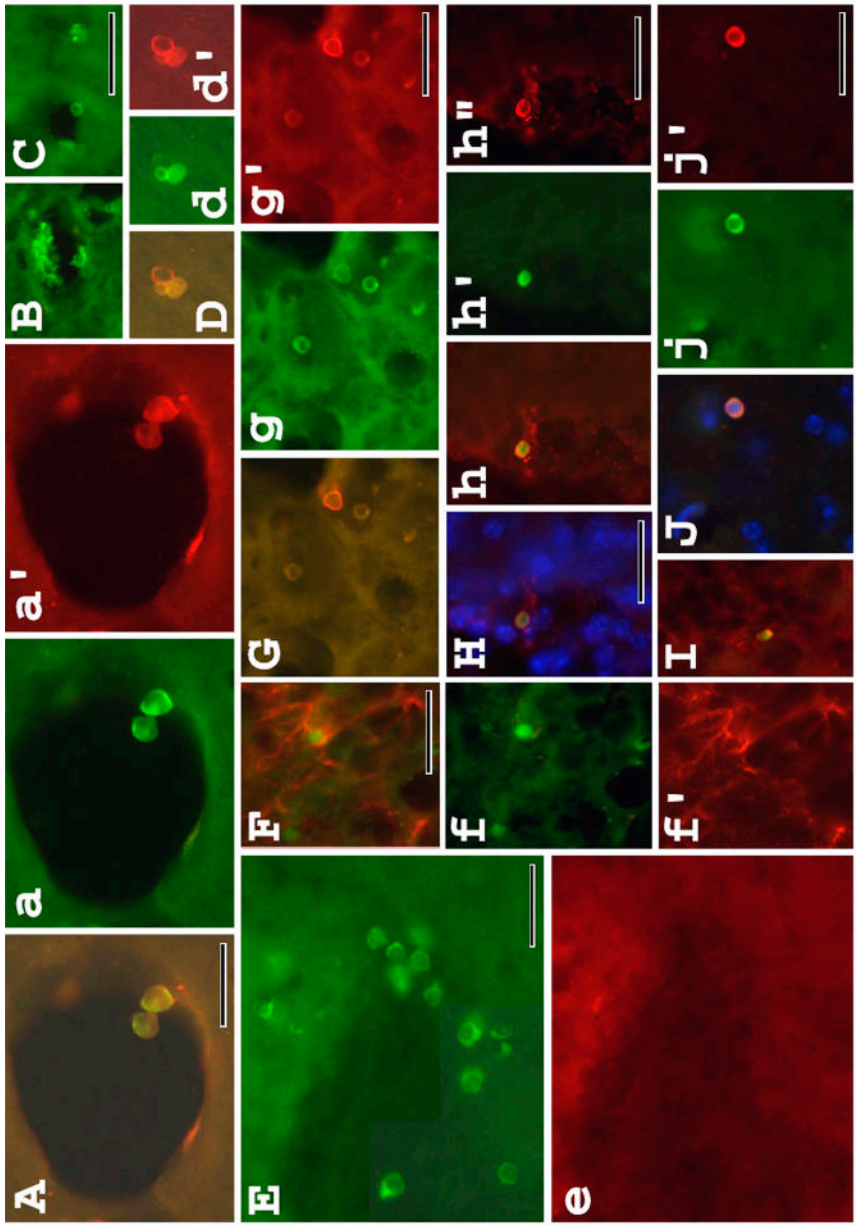

FIGURE 2. *See following page for legend.*

phies, severe combined immune deficiency, aplastic anemia, Fanconi's anemia, glycogen storage diseases like Hurler's syndrome and the Hunter syndrome, erythrocyte enzyme deficiencies and errors of metabolism. Additionally, cord blood cell therapy was promising in the treatment of acute lymphoblastic leukemia[62,63] and acute myeloid leukemia.[64] One hundred per cent donor engraftment and 5 years' disease-free survival was accomplished in a 2-month old infant suffering from beta-thalassemia after transplantation of partially MHC-matched HUCB from an unrelated donor.[65]

Progress of cell therapy applications for neurological disorders has been slower, Wenger *et al.*[66] have utilized cord blood transplantation to treat Krabbe's disease. This enzymatic disorder is caused by deficiency of galactocerebrosidase (CAL-C), which results in deficiency of myelin formation in both the central and the peripheral nervous system. Prognosis of this disease is grave in infants, but better in older patients. HUCB transplantation lessened the disease manifestations, but a cure was not achieved.

Another metabolic disease that seriously affects the nervous system is Hurler's syndrome. It is a severe form of mucopolysaccharidosis type I that affects children, and causes progressive deterioration of the central nervous system, which leads to death. In a study by Staba *et al.*[67] cord blood transplantation from unrelated donors was used to treat young children with Hurler's syndrome. A myeloablative preparative regimen that did not involve total-body irradiation was followed by cord blood transplantation. The children showed a high survival rate and were durably engrafted with donor cord blood.

CONCLUSION AND FUTURE PERSPECTIVES

Stem cell therapy for otherwise fatal diseases has greatly advanced since the first bone marrow transplant for severe combined immune deficiency.[68] Manipulation of the marrow stem cell graft by deleting mature GVHD-causing cells and enriching for the hematopoietic stem cell populations, and improvement of the myeloablative conditioning therapy prior to the transplant have considerably enhanced the outcome of stem cell therapy.[69] While autologous stem cell transplants provide the best engraftment outcome, allogeneic transplantation proved superior in delaying relapse in malignancies and in experimental autoimmunities.

FIGURE 2. Immunohistochemical staining of hUCB cells in the lumbar spinal cord. (**A,B**) Double-labeled HuNu/CD45 hUCB cells are found in or (**C**) outside the blood vessels (HuNu, green; CD45, red/orange). (**D**) Some cells in parenchymal location were negative for CD45 and only stained with HuNu (green). (**E**) Double-labeled cells expressing GFAP (red) and HuNu (green) or (**F**) surrounded by astrocytes (*arrowhead*). (**f**) same view as **F** with fluorescein and rhodamine (**f′**) filters. (**G**) Nestin expression (red) in double-labeled cell with HuNu (green). Stars indicate nuclei (DAPI) of mouse cells in tissue. (**g**) same view as **G** with fluorescein and (**g'**) rhodamine filters. (**H**,**I**,**J**) Cells double-labeled for TuJ1 (red/orange, *arrows*) and HuNu (green). (**h**) same view as **H** with fluorescein and (**h'**) rhodamine filters. The nuclei in **H** are shown with DAPI and stars (**H**) and (**h**) indicates negative stain for TuJ1. NOTE: Nestin-positive and TuJ1-positive cells differ morphologically from any administered hUCB cells identified within the spinal cord. Scale bar in **A–J** is 25 mm. [*Illustration is in color online*.]

Human umbilical cord blood is a highly promising source for cell therapy in a variety of diseases currently treated with bone marrow transplantation. This promise is particularly valuable for patients suffering from neural disorders for which no cure is available. *In vitro* studies that showed plasticity of HUCB cells, and *in vivo* studies that achieved not only delay or halt of neural degeneration, but also active restoration of neural functions, have created justifiable excitement in neural research. Unique qualities of HUCB like availability, immature cellular phenotype, enrichment for hematopoietic progenitors, plasticity, and lower incidence of graft versus host disease all appropriate its use as an ideal source for cell therapy (reviewed by Newman *et al.*[70]).

HUCB transplantation is a particularly attractive strategy for neurological disorders, because of the grave prognosis and current absence of a cure for most of these diseases, and the promising data from basic research and preclinical studies. Many practical factors influence the outcome of the therapy with CBT. For example the route of cord blood infusion is particularly critical in the CNS when we consider the blood–brain barrier. In animal models, intra-bone transplantation of cord blood has shown higher seeding efficiency than has the intravenous route.[71] This strategy could be especially beneficial when the number of cord blood stem cells is limited, or when direct delivery of stem cells to the site of damage is believed to initiate earlier repair within the critical hours after the ischemic or traumatic damage.

The abundance of *in vitro* data showing differentiation of various cord blood stem cells into neural and glial cells has tempted researchers to suggest that cord blood–induced brain repair is mediated by a transdifferentiation process. Plasticity of stem cells, however, has been a subject of intense debate.[72] Despite evidence for neural differentiation both *in vitro* and *in vivo*, limited evidence suggests that this phenotypic delineation involves functional neural cells. This lack of functional assays has directed most preclinical studies to gauge improvement by behavioral testing.

The promising data of improved behavior, delayed disease onset, and prolonged survival, coupled with an immense desire to find a cure for debilitating and devastating CNS diseases stimulated a renewed interest in stem cell therapy for neural disorders. The critical question to be pursued by researchers is what possible mechanisms are involved in neural repair by cell therapy. Replacement of diseased cells with functional "new" stem cells may be an accepted resolution for cases like leukemia cured by bone marrow transplantation; however, limited evidence in neuroscience studies suggests that this is the case.

While pursuing the inevitable leap of stem cell therapy from the bench top to the clinic, many questions need continued investigation, for example:

- What fraction of cord blood cells provides the maximum benefit with the fewest side effects? Are purified populations of stem cells superior to unseparated or mononuclear cell fractions?
- What is the most efficient way to deliver HUCB stem cells? Is local implantation superior to systemic injection?
- What is the optimal dose of HUCB cells, and are multiple injections required to maintain the desired therapeutic effect?
- How could the stem cell graft be manipulated so that minimal immune suppression is required without graft rejection?

- Which cells within the cord blood graft induce GVHD, and how could the graft be manipulated to deplete such undesired populations?
- How do growth factors and cellular mediators produced by the cord blood cells affect the progress of disease and how do these factors influence the production of intrinsic neurotransmitters and mediators?
- Which diseases of the CNS are most promising as targets for stem cell therapy and what pathology associated with these disorders permits a curative effect mediated by stem cells?
- How does local pathology influence migration of donor stem cells to the site of injury, and promote differentiation into specialized, functional neural cells?
- How are behavioral functions affected by stem cell therapy and how do these functions relate to neural recovery?

And last but not least,

- How do cord blood cells initiate and maintain neural repair?

Modulation of the host immune responses, stimulation of endogenous host stem cells, and production of various neural mediators and epigenic growth factors have all been suggested as mechanisms to be explored by scientists.

For patients and clinicians, however, the hope sparked by case reports of neural recovery after stem cell transplants makes the cell therapy approach for neurological disorders well worthy of attention.

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