A.R. Møller (Ed.)
Progress in Brain Research, Vol. 157
ISSN 0079-6123

CHAPTER 14

Novel cell therapy approaches for brain repair

Svitlana Garbuzova-Davis[1,*], Alison E. Willing[1], Samuel Saporta[1], Paula C. Bickford[1], Carmelina Gemma[1], Ning Chen[1], Cyndy D. Sanberg[3], Stephen K. Klasko[2], Cesario V. Borlongan[4] and Paul R. Sanberg[1]

[1]*Center of Excellence for Aging and Brain Repair, Department of Neurosurgery, University of South Florida, College of Medicine, MDC 78, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA*
[2]*University of South Florida Health, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA*
[3]*Saneron CCEL Therapeutics Inc., 3802 Spectrum Blvd., Suite 145, Tampa, FL 33612, USA*
[4]*Department of Neurology, Medical College of Georgia, 15th Street, Augusta, GA 30912, USA*

Abstract: Numerous reports elucidate that tissue-specific stem cells are phenotypically plastic and their differentiation pathways are not strictly delineated. Although the identity of all the epigenetic factors which may trigger stem cells to make a lineage selection are still unknown, the plasticity of adult stem cells opens new approaches for their application in the treatment of various disorders. There is increasing researcher interest in hematopoietic stem cells for treatment of not only blood-related diseases but also various unrelated disorders including neurodegenerative diseases. Human umbilical cord blood (hUCB) cells, due to their primitive nature and ability to develop into nonhematopoietic cells of various tissue lineages, including neural cells, may be useful as an alternative cell source for cell-based therapies requiring either the replacement of individual cell types and/or substitution of missing substances. Here we focus on recent findings showing the robustness of adult stem cells derived from hUCB and their potential as a source of transplant cells for the treatment of diseased or injured brains and spinal cords. Depending upon the pathological microenvironment in which the hUCB cells are introduced, neuroprotective and/or trophic effects of these cells, from release of various growth or anti-inflammatory factors to moderation of immune-inflammatory effectors, may be more likely than neural replacement. These protective effects may prove essential to maintaining restored tissue integrity over the course of various diseases or injuries.

Keywords: neurodegenerative diseases; brain and spinal cord injury; umbilical cord blood cells; transplantation; alternative treatment

Introduction

The brain is a complex organ containing billions of neurons and other cells, specialized by structure and function. A unique cytoarchitecture and neuronal network of rigorous complexity compose the brain, the source of all the qualities that define our humanity. Since neurogenesis in the adult central nervous system (CNS) of all mammalians, including humans, was discovered (reviewed in Cameron and McKay, 1998; Gage, 2000; Gritti et al., 2002; Lie et al., 2004), the traditional view of the inability of mature nervous tissues to renew and reconstruct themselves has been largely debunked. New neurons are constantly generated from neural stem cells throughout life in restricted brain regions which actually contain adult stem cells. These cells can give rise to differentiated progeny comprising the major cell types of the CNS,

*Corresponding author. Tel.: (813) 974-3189; Fax: (813) 974-3078; E-mail: sgarbuzo@health.usf.edu

DOI: 10.1016/S0079-6123(06)57014-1

neurons and glia, assumed to be responsible for nervous tissue homeostasis and repair throughout adulthood to aging. However, a decline of newly generated neurons has been observed with aging (reviewed in Bernal and Peterson, 2004). Although the mechanisms leading to a reduction of endogenous neurogenesis with increasing age are still unclear, functional decline in the ''normal'' aging processes is mainly associated with the loss of synaptic densities in many brain structures and impairment of cellular metabolism due to increased oxidative damage to DNA and proteins (reviewed in Limke and Rao, 2002; Brazel and Rao, 2004). Such alterations could be even more critical in age-related neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) which afflict more than 6.5 million people in the United States alone. Since current therapies for these devastating disorders merely treat the symptoms but do not provide cures, it is important to develop new therapeutic strategies to replace cells lost in neurodegenerative diseases through neural implantation. In cell-based therapeutics, neural stem cells offer hope for repair of the diseased or injured brain by their endogenous or exogenous (i.e., transplantation) activation. The rise of this neural stem cell concept has raised many questions in these cells therapeutic applications. It has been shown that the fate of adult neural stem cells is under specific environmental control and their proliferation and differentiation biased by different epigenetic signals, such as cytokines and various growth factors (reviewed in Gritti et al., 2002; Baizabal et al., 2003). The changes in microenvironment during disease or injury could influence either endogenous or exogenous repair. For example, Parent (2003) showed increased neurogenesis in the persistent germinative zones (subventricular zone and hippocampal dentate gyrus) using experimental epilepsy and stroke in the adult rodent. Moreover, as recently reported, neurogenesis was elevated in brains of patients with either Huntington's disease (Curtis et al., 2003) or AD (Jin et al., 2004). These results indicate the great regenerative potential of the human brain, but this compensatory mechanism of self-repair as a response to brain insult does not provide complete healing. Limke and Rao (2002) point out, ''As with any disease, the development of new therapies relies heavily on a thorough knowledge of the biology of the system being studied, and the ramifications of alterations of that system during disease.'' The complexities and specificity of CNS diseases make cell replacement therapy a challenging but potentially very rewarding area for research.

Similar to primitive stem cells, adult neural stem cells have capacity for self-renewal and can generate cells other than themselves through asymmetric cell division. Recent findings indicate that neural stem cells display broader than expected multipotentiality. It has been shown that they can differentiate into non-CNS derivates, such as blood cells (Bjornson et al., 1999) or skeletal muscle (Galli et al., 2000). Controversially, bone marrow stem cells can give rise to muscle (Ferrari et al., 1998), hepatic (Petersen et al., 1999), endothelial cells, osteoblasts (Dennis and Charbord, 2002), and neural cells (Sanchez-Ramos et al., 2000; Woodbury et al., 2000). Additionally, the muscle precursors can turn into blood cells (Jackson et al., 1999). The ability of tissue-specific stem cells (neural stem cells and somatic stem cells) to give rise to unrelated cell types may define these cells as pluripotent in nature. In this context, Slack's (2000) overview of studies on stem cells in epithelial tissues suggested that ''all types of stem cell are the same.'' Although which epigenetic factors may trigger stem cells to make a lineage selection are still unknown, the plasticity of adult stem cells opens new approaches for their application in treatment of various neurodegenerative disorders. However, in practical view, the use of human neural stem cells as well as embryonic stem cells for brain therapy (i.e., transplantation) is currently obstructed due to ethical and other issues (Vescovi et al., 1999; Henon, 2003; Daar et al, 2004; Sanberg, 2005), in addition to difficulties in ensuring purity of neural cultures and harvesting needed populations for transplantation (Riaz et al., 2002; Linazasoro, 2003; Sathananthan and Trounson, 2005). Owing to these limitations, exploration of new sources of stem cells for therapeutic perspectives is necessary. It seems that tissue-specific stem cells are phenotypically plastic

and their differentiation pathways are not strictly delineated. Increasing interest of many researchers in plasticity of hematopoietic stem cells in treatment of not only blood-related diseases but also various unrelated disorders, encourages investigations into the possibilities of using these cells to treat neurodegenerative diseases.

Here we focus on recent findings, including ours, showing the robustness of adult stem cells derived from hematopoietic tissue, particularly, human umbilical cord blood (hUCB), in identifying their specific potential as a transplantable cell source for the treatment of diseased or injured brain and spinal cord.

Umbilical cord blood cells: new vision or novel assets

Numerous reports elucidate the many advantages of hUCB cells for cellular therapies. These cells are easily accessible in unlimited supply, avoiding ethical and other issues, and hUCB cells may be preferable to other potential cell sources (Sanberg et al., 2001, 2002). Hematopoietic progenitors from hUCB are rich in the most primitive stem cells and are capable of long-term repopulation of blood lineages (Broxmeyer et al., 1989, 1992; Mayani and Lansdorp, 1998; Todaro et al., 2000; Nayar et al., 2002). The number of myeloid progenitor cells in hUCB is similar to the number in bone marrow (Broxmeyer et al., 1992), however, hUCB cells have a greater colony-forming ability (Nakahata and Ogawa, 1982) and can be expanded in long-term cultures in vitro using different growth factors and have longer telomeres than adult cells (Vaziri et al., 1994).

Cord blood transplants have already been used to reconstitute bone marrow and blood cell lineages in children with various hematological malignant and nonmalignant diseases (Lu et al., 1996; Sirchia and Rebulla, 1999). Since 1988, when the first transplant was performed in a patient with Fanconi anemia (Gluckman et al., 1989), cord blood transplantations have increased; more than 3000 cases have been reported worldwide (Gluckman, 2000; Broxmeyer, 2004). Moreover, hUCB transplants from unrelated donors (vs. autologous transplantation) have been successfully used for children and adult patients (Gluckman et al., 1997, 1999; Rubinstein et al., 1998; Ooi et al., 2002; Wagner et al., 2002). Recently, it has been shown that hUCB transplants, versus adult bone marrow stem cells, better restore the host hematopoietic progenitor cell reservoir (Frassoni et al., 2003). Some studies indicate that a single hUCB sample supplies enough hematopoietic stem cells to provide both short- and long-term engraftment (Lu et al., 1996; Sirchia and Rebulla, 1999). This advantage is due to the immune-immaturity of the hUCB cells, which reduces the risk and severity of graft-versus-host disease (GVHD) after transplantation (Madrigal et al., 1997; Gluckman, 2000; Thomson et al., 2000). In the first electron microscopic comparison of cord blood, peripheral blood and bone marrow cells, hUCB cells had a more immature morphology of the myelo-monocytic cells with small numbers of mature neutrophils and unique ultrastructure elements, such as nuclear pockets in the neutrophils, which accelerated the transport of RNA to the cytoplasm (Mikami et al., 2002).

The immaturity of immunological properties in hUCB cells is believed to cause a prolonged immunodeficient state after hUCB transplantation (Roncarolo et al., 1994; Garderet et al., 1998; Thomson et al., 2000). However, it has been shown that hUCB cells contain a fully constituted T-cell repertoire (Garderet et al., 1998). When comparing the characteristics of B-cell differentiation in vitro from $CD34^+$ cord blood cells with those of peripheral blood, it was found that B-cell precursors differentiated from cord blood are more immature (Hirose et al., 2001); this may cause the delay in mature B-cell production. Moreover, cord blood lymphocytes expressed cytokine receptor profiles (IL-2, IL-4, IL-6, IL-7, TNF-α, and interferon-γ) at lower levels than in adult blood cells (Zola et al., 1995) and produced great amounts of the anti-inflammatory cytokine IL-10 (Rainsford and Reen, 2002). Recently, it has been demonstrated that human $CD34^+$ cord blood cells can completely reconstitute the immune system in NOD/SCID mice with functionally competent cells, as indicated by IgM, IgA, and IgG expression (Hiramatsu et al., 2003). These human lymphocytes also formed organized structures in mouse spleens and thymi.

Current interest in the transplantation field for clinical applications focuses on the ex vivo expansion of cord blood precursor/progenitor/stem cells to provide a sufficient amount of stem cells for adult transplantation (Conrad and Emerson, 1998; Liesveld, 2003). Major questions arise on selection of an optimal stem cell population for expansion and definition of desired characteristics of the expanded stem cells to be used for engraftment (Shih et al., 2000). Human AC133 (CD133) antigen has been identified as a hematopoietic stem cell marker that may provide an alternative to CD34 for the selection and expansion of hematopoietic cells for transplantation (Yin et al., 1997; Kobari et al., 2001). It has been shown that about 80% of $CD34^+$ cells express CD133 and more than 97% of $CD133^+$ cells are $CD133^+CD34^+$ in fresh cord blood (Hao et al., 2003). Although $CD133^+$ cells comprised 0.67% of the total mononuclear hUCB cells (Ma et al., 2002), expansion of $CD133^+$ and $CD133^+CD34^+$ cells was significantly higher than those from the $CD34^+$ cells (Hao et al., 2003). These findings suggest that $CD133^+$ may be more primitive hematopoietic progenitor/stem cells than $CD34^+$. In the first clinical trial of autologous transplantation of CD133 selected progenitors in a pediatric patient with relapsed leukemia, complete remission was reported at follow-up, 11 months after transplantation (Koehl et al., 2002).

To advance the usefulness of the hUCB cells in treating neurodegenerative diseases, an in vitro study was first conducted by our research group (Sanchez-Ramos et al., 2001). We showed that mononuclear hUCB (MNC hUCB) cells treated with retinoic acid (RA) and nerve growth factor (NGF) expressed molecular markers usually associated with neurons and glia, as determined by immunocytochemistry, Western blot, and DNA microarray. The MNC hUCB cells under neuralization-inducing (RA + NGF) media expressed specific markers for early neural precursors (musashi-1, nestin, TuJ1), mature neurons (NeuN, MAP2), and astrocytes (GFAP). Moreover, cells exposed to RA + NGF treatment increased TuJ1 and GFAP expressions by approximately two times. Similar to this study, we have demonstrated that in standard growth (i.e., DMEM) media, MNC hUCB cells express neural markers, such as nestin, TuJ1, MAP2, and GFAP (Garbuzova-Davis et al., 2003). Colocalization of nestin and MAP2 and various cytoplasmic expressions of TuJ1 by cells at 2 weeks after plating were also observed; findings that we suggest may depend upon cell cycle or cell development. Additionally, the increased number of cells expressing CD133 antigen, a marker of primitive hematopoietic progenitor/stem cells, in 7 days cultured cells probably gives rise to cells which show immature and mature neural characteristics at the same time.

This novel benefit of hUCB cells, that they can express antigens typical of neural lineages within the CNS, has been confirmed by other researchers. Bicknese et al. (2002) and Buzanska et al. (2002) demonstrated that these cells could be induced to express class III β-tubulin, GFAP, and GalC (a marker of oligodendrocytes). Similarly, Ha et al. (2001) showed that these cells can express the neurofilament microtubule associated protein 2 (MAP2) using immunohistochemistry and RT-PCR. In a study reported by Goodwin et al. (2001), a subset of cells from MNC hUCB, which had been maintained in continuous culture for more than 6 months, was described without antigen expression for hematopoietic differentiation. When these cells were exposed to osteogenic, adipogenic agents or basic fibroblast and epidermal growth factors, they expressed bone, fat and neural markers, respectively. According to these data, hUCB contains a cell population, which is capable of expressing antigens of multiple lineages, demonstrating the plasticity of these cells that is very important for cellular therapeutics with the goal of system repair. However, the authors raise critical questions about the nature of this cell population. They cannot conclude that these cells are "a stem cell population, multiple differentiated progenitors, or cells with transdifferentiation capacity" (Goodwin et al., 2001). More investigation needs to be performed using various testing of these cells, including in vivo studies. Confirming the presence of a cell population in hUCB with multipotent ability, McGuckin's research team developed a negative immunomagnetic selection method that depletes hUCB from hematopoietic lineage marker-expressing cells, therefore isolating a discrete lineage negative (LinNeg) stem cell

population (0.1% of MNC hUCB) (Forraz et al., 2004; McGuckin et al., 2004). These selected LinNeg hUCB cells expanded primitive nonadherent hematopoietic progenitors (up to 47-fold) and simultaneously produced adherent cells with neuroglial progenitor cell morphology over 8 weeks. Gene expression analysis showed upregulation of primitive neuroglial progenitor cell markers for GFAP, nestin, musashi-1, and necdin.

Recently, we characterized in vitro two different subpopulations of MNC hUCB cells – adherent and floating (Chen et al., 2005). We found that there were a significant number of progenitor/stem and neural cell antigen expressions on cells in the floating population. The adherent cell population mainly contained lymphocytes (over 50%) expressing hematopoietic antigens. These results suggest that a nonhematopoietic subpopulation of cells exists within MNC hUCB cells and seems to have the potential to become neural cells. Although this study is continuing, it is clear that hUCB has a primitive stem cell population that may give rise to both hematopoietic and neural cells.

Thus, hUCB cells due to their primitive nature, with ability to transdifferentiate or become nonhematopoietic cells of various tissue lineages, including neural cells, may be useful for cell-based therapies requiring either the replacement of individual cell types and/or substitution of missing substances. Using hUCB cells as an alternative cell source in the treatment of neurodegenerative disorders is therefore an attractive approach.

Umbilical cord blood cells in treatment of diseased brain and spinal cord

Stroke

Stroke is the most common age-related cerebrovascular disease, which is the third leading cause of morbidity and mortality in the United States (Gorelick et al., 1999). Care for this disease is problematic due to certain disease attributes. Stroke can involve multiple anatomical brain structures, affecting different neuronal cell populations, and disrupting various neuroanatomical pathways. Ischemic injury may be an ongoing process increasing cell and/or tissue damage; so timing to begin therapeutic course is critical. Currently, the only effective treatment for stroke (tissue plasminogen activator or TPA) must be delivered within a restricted time frame from the initiation of the stroke. These disease outcomes should be taken under consideration in developing any therapeutic intervention, especially, in cell-based therapy for stroke. A recent review (Savitz et al., 2004) discussed preclinical and clinical studies on potential cell therapy for stroke. In on-going clinical trials, patients with intrastriatal neuronal transplantation of the immortalized cell line NT2N (LBS neurons) showed a trend toward improvement in functional outcomes on several scales, compared with baseline measurements before transplantation. Some transplanted patients improved on a test of memory 6 months after transplantation. Another pilot study on intrastriatal transplantation of fetal cells from the pig into patients with basal ganglia infarcts demonstrated that the patients developed no new neurological deficits in the acute setting. But at 2 years, no patients showed improvement on the modified Rankin scale and the study was terminated by the FDA. Although clinical trials on neuronal cell transplantation are feasible, using multipotent stem cells from a different source might be more beneficial.

A series of published reports by Chopp and colleagues (Chen et al., 2001a, 2003; Chen et al., 2002; Li et al., 2002) on using bone marrow stromal cells as a source of transplantable cells for treatment of stroke demonstrated functional improvement in rats after focal cerebral ischemia. Transplanted cells migrated to areas of ischemic infarcts and differentiated into neuronal and glial cell types. Authors suggested that recovery mechanisms are likely due to trophic factors released by cells which may promote endogenous neurogenesis and angiogenesis rather than a result of neuronal replacement.

The hUCB is another source of multipotential stem cells that has shown promising effects in preclinical studies for treatment of stroke. The intravenous administration of MNC hUCB cells (3×10^6) at 24 h or 7 days after middle cerebral artery occlusion (MCAO) in a rat model of stroke significantly improved neurological function (Chen

et al., 2001b). Upon histological examination of the brains, MNC hUCB cells were observed mainly in the cortex and striatum of the injured hemisphere in the ischemic boundary zone. Few cells were found in the contralateral hemisphere. Using immunohistochemistry, it was determined that some of these MNC hUCB cells were immunoreactive for neuronal markers NeuN (2%) and MAP2 (3%), the astrocytic marker GFAP (6%), and the endothelial cell marker FVIII (8%). The transplanted cells were also detected outside the brain in bone marrow (3%), spleen (1%), muscle, heart, lung, and liver (0.01%–0.5%).

Recently, in our study (Willing et al., 2003), we compared the effect of intravenous versus intrastriatal injection of MNC hUCB cells to assess which produced the greatest behavioral recovery in rats with permanent MCAO. It was found that spontaneous activity was significantly less when cells were transplanted 24 h after stroke compared with nontreated stroke animals. Furthermore, behavioral recovery was similar with both cell delivery routes. However, in one functional test (step test) at 2 months after transplant, significant improvements were found only after intravenous delivery of the MNC hUCB cells. Also, in the passive avoidance test, transplanted animals learned the task faster than nontransplanted rats. These results suggest that intravenous delivery of MNC hUCB cells may be more effective than direct striatal delivery in producing long-term functional benefits to the stroke animal.

In continuing studies of our research team lead by Dr. Willing, the effect of increasing doses of MNC hUCB cells after MCAO on the behavioral recovery and stroke infarct volume in rats (Vendrame et al., 2004) was examined. Twenty-four hours after induced stroke, rats were intravenously infused with 10^4–3–5×10^7 MNC hUCB cells. Results showed that, at 4 weeks after infusion, there was a significant recovery in behavioral performance (spontaneous activity, step test, elevated body swing test) when 10^6 or more MNC hUCB cells were delivered. Infarct volume measurements revealed an inverse relationship between cell dose and damage volume, which reached significance at the higher doses of MNC hUCB cells (10^7 cells, $p < 0.01$; 3–5×10^7 cells, $p < 0.05$). Moreover, transplanted cells were localized by immunofluorescence for human nuclei antigen expression and PCR analysis only in the injured brain hemisphere and spleen. These results extend previous observations of MNC hUCB cell infusion in the MCAO rat stroke model by demonstrating a dose relationship between introduced transplanted cells, behavioral improvement, and neuronal sparing.

Lately, we showed (Vendrame et al., 2005) that in the brain of rats with permanent MCAO, there was an increase in the number of $CD45^+/CD11b^+$ cells (lymphocytes). After MNC hUCB cell transplantation, the number of $CD45^+/CD11b^+$ cells returned to the level observed in the normal brain. There was a decrease in the number of $CD45^+/CD11b^+$ cells (resting microglia) in the MCAO brain that was reversed by hUCB transplantation. After stroke, there was also a large increase in the cell population labeled with $CD45^+/CD11b^+$, which returned toward normal values in the transplanted animals. This population has been previously characterized as mainly granulocytes, macrophage, and activated microglia. These cellular changes were accompanied by decreases in mRNA and protein expression of pro-inflammatory cytokines and in nuclear factor kappaB (NF-kappaB) DNA binding activity in the brain of stroke animals treated with MNC hUCB cells. In addition to modulating the inflammatory response, we demonstrated that the transplanted cells increased neuronal survival through nonimmune mechanisms. Once thought of as "cell replacement therapy," we now propose that cord blood treatment in stroke reduces inflammation and provides neuroprotection. Also, we recently showed that MNC hUCB cells could benefit in stroke by providing protective trophic neuronal support through secretion of glial-derived neurotrophic factor (GDNF) or other growth factors (Sanberg et al., 2004).

Finally, the therapeutic window for treatment of individuals after stroke is narrow, regardless of the treatment regime; extension of this window would provide a major therapeutic advance. Based on our findings that significant improvement occurred in the behavior of rats receiving MNC hUCB cells 24 h after MCAO, another study from our research group (Newman et al., 2005) attempted to determine the optimal time to administer these

cells after stroke. Using ischemic tissue extracts, the migration capability of MNC hUCB cells was investigated. We demonstrated increased migratory activity of MNC hUCB cells toward the extracts harvested at 24–72 h after stroke. The extracts possessed increased levels of certain cytokines and chemokines, suggesting participation of these substances in the cell migration. The results from this study are promising in that the current 3 h therapeutic window for the treatment of stroke victims, using approved anticoagulant treatment, may be extended with the use of MNC hUCB cell therapy to 24–72 h post stroke event. Also, the chemokines present in the supernatant could provide a sound starting point for examining the mechanisms responsible for the in vivo migration of MNC hUCB cells after stroke induction.

However, as concluded by Savitz et al. (2004) "Transplantation is unlikely to succeed if there is a severe arterial occlusion without collateral circulation; inadequate blood supply would not support graft survival." On this point, an other advantage for using hUCB cells for treatment of stroke is potential restoration of vascularity since cord blood contains endothelial progenitor cells which may be of use in proangiogenic neovascularization therapy (Delorme et al., 2005; Shin et al., 2005).

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by a loss of motor neurons throughout the neural axis that clinically manifests as a progressive muscular weakness leading to paralysis and death, usually within 3–5 years of diagnosis. The diffuse degeneration includes damage to upper motor neurons in the motor cortex, lower motor neurons in the spinal cord, and some brainstem nuclei. New therapeutic strategies, including cell replacement, for this disease are difficult to develop due to multifocal and multicausal motor neuron death (Silani and Leigh, 2003). In agreement with Bruijn's (2002) comment concerning neural stem cell replacement therapies for ALS, "it is hard to imagine that transplanted motor neurons would form appropriate connections with target muscle," there are severe limitations to using this treatment in ALS.

Since numerous hypotheses about the etiopathology of ALS have been proposed (reviewed in Cleveland and Rothstein, 2001; Bruijn et al., 2004), increasing evidence points to immune system involvement in the pathogenesis of ALS. If ALS is an autoimmune disease, as some have hypothesized (Kawamata et al., 1992; Alexianu, 1995; Niebroj-Dobosz et al., 1999; Alexianu et al., 2001; Mohamed et al., 2002), hUCB cells may improve disease outcome through immune modulation. Supporting this hypothesis is evidence showing that intravenous administration of a large dose (35×10^6) of MNC hUCB cells into irradiated G93A mice substantially increased lifespan of mice (Ende et al., 2000; Chen and Ende, 2002). While the survival data was impressive, the investigators did not examine motor function in these animals or determine the underlying mechanism(s). Authors suggested that MNC hUCB cells possibly "provide enhanced hematopoietic reconstitution of the irradiated hosts own stem cells" (Ende et al., 2000).

In our study, a single low dose (10^6 cells) of MNC hUCB administered into the systemic circulation of the presymptomatic G93A mice delayed disease progression at least 2–3 weeks, as determined by testing motor function, and modestly prolonged the lifespan (Garbuzova-Davis et al., 2003). Transplanted cells survived long-term (10–12 weeks) post-transplantation and were found widely distributed in the brain, spinal cord, and other organs. Although most cells were associated with blood vessels, some cells migrated into the parenchyma within the brain and spinal cord and expressed early neural (nestin), neuronal (TuJ-1), and astrocytic (GFAP) markers. However, only morphological evidence was found for differentiated astrocytes. The other finding in our study was that a large number of MNC hUCB cells were present in the spleens of treated mice. As this was the first study demonstrating that MNC hUCB cells altered the peripheral immune system, we examined the immune phenotypes (T- and B-cell antigen expression) of intravenously transplanted cells found in the spleen (Desjarlais et al., 2003). Many of the cells expressed CD8 and CD19

while few expressed CD4. Only a few cells were found positive for CD28 and CD80, indicating activation of T- and B-cells. When we analyzed T- and B-cell antigen expression of MNC hUCB cells, which had migrated to the spinal cord after their administration into systemic circulation, most cells expressed CD4 and CD8; fewer expressed CD19, CD28, and CD80. These results suggest that the MNC hUCB cells can not only differentiate into immune cells, but are actively involved in the immune response. It is possible that the beneficial effect of MNC hUCB cells may occur through peripheral immunomodulation of immune effectors due to MNC hUCB development into cells with immune regulatory function.

An additional advantage of MNC hUCB cells in treatment of ALS could be through altered function of the lymphoid system which critically influences the immune system. Significant lymphopenia and an increase of the CD4/CD8 ratio have been noted in ALS patients even at an early disease stage (Provinciali et al., 1988). This was confirmed by our recent findings that severe lymphopenia accompanied by spontaneous autorosette formation was determined in G93A mice modeling ALS at the terminal stage of disease (Kuzmenok et al., 2006). It is possible that restoration of the lymphoid system by hUCB cell transplantation may elevate ''defense'' for motor neurons.

Umbilical cord blood cells in treatment of injured brain and spinal cord

Traumatic brain injury (TBI) and spinal cord injury (SCI) result from external physical insult and are associated with high morbidity and mortality. There are currently no sufficient treatments. Brain neurotrauma is characterized not only by focal abnormalities, but rather by multifocal, or even global structural and functional disturbances of the brain network. In both TBI and SCI, the impact initially causes necrotic cell death and then apoptotic cell death in the underlying and surrounding tissues due to multiple subsequent events, such as ischemia and excitotoxicity. Stem cells might participate in reconstructing the molecular and cellular milieu of traumatized brains or spinal cords (Brodhum et al., 2004; Kulbatski et al., 2005). However, there is no definitive answer about the ideal cell type for transplantation. A recent retrospective analysis of 70 cases of brain trauma or paraplegia treated with neural stem cell transplantation concluded that the transplantation promoted functional recovery (Zhou et al., 2004). In another clinical trial, Rabinovich et al. (2003) reported that cells from fetal nervous and hemopoietic tissues (gestational age 16–22 weeks) had been subarachnoidally implanted into 15 patients with severe SCI at cervical or thoracic spine level. Each patient received from one to four cell grafts at various time intervals. With cell treatment, six patients improved their neurological status exhibiting incomplete restoration of both motor and sensory function. The status of five other cell-treated patients became consistent and was characterized by appearance of contracting activity in some muscles and incomplete restoration of sensitivity. The remaining four patients did not exhibit any clinical improvements. No serious complications of cell transplantation were noted. These results suggest the clinical relevance of the cell-based approach to treating severe consequences of SCI.

In our study, we showed that when MNC hUCB cells (2×10^6) were delivered to the tail vein of rats with TBI, neurological deficits were reduced (Lu et al., 2002). Wide distribution of administered cells in the brain and peripheral organs was detected. The cells which migrated into the parenchyma of the injured brain expressed neuronal markers NeuN and MAP2 and the astrocytic marker GFAP.

In another of our studies, intravenously delivered MNC hUCB cells (10^6), at 1 day or 5 days postinjury in rats with compression injury of the spinal cord, increased the rate of behavioral recovery (Kim et al., 2002; Saporta et al., 2003). However, rats which received the cells 5 days after injury showed significantly improved recovery of motor function compared to those that received cells on the 1st day postinjury, and both were significantly better than animals upon which only laminectomy was performed. The transplanted cells were observed in injured areas of rat spinal cords and were never seen in corresponding areas of spinal cord of noninjured animals. The results

of our studies are consistent with the hypothesis that cord blood-derived stem cells participate in the healing of neurological defects caused by not only disease but also by traumatic assault.

It is interesting to note that even direct injection of hUCB-derived AC133 (hematopoietic progenitor/stem) cells into a demyelinated lesion of rat spinal cord showed extensive axon remyelination by light and electron microscopic examination (Honmou et al., 2002).

Thus, our studies demonstrated that hUCB cells delivered into the systemic circulation were able to migrate into injured/damaged areas of the brain or spinal cord and express neural-like markers without pre-exposure to specific factors. Although the mechanism of cell migration remains unclear, Lu et al. (2002) suggest that hUCB cells "may enter the brain from blood–brain barrier disruption or in response to signals from cytokines and cell surface receptors and antigens" and "the microenvironment of the brain [spinal cord] after injury may drive hUCB cells into a neural cell phenotype."

Umbilical cord blood cells in treatment of metabolic diseases

The core strategy in developing a therapy for mucopolysaccharidosis (MPS) is replacement or delivery of the missing enzyme. Cell therapy may show promise as a new treatment for this disease. Recently, cord blood transplants from unrelated donors were shown to improve neurocognitive performance and decrease somatic features in patients with Hurler's syndrome (MPS type I) (Staba et al., 2004).

We investigated the prospects of MNC hUCB as a potential cell source for treatment of Sanfilippo syndrome type B (MPS III B). MPS III B is an autosomal recessive disorder caused by a deficiency of α-*N*-acetylglucosaminidase enzyme (*Naglu*). The lack of *Naglu* enzyme leads to accumulation of heparan sulfate, a glycosaminoglycan (GAG), within lysosomes. Clinical symptoms appear after 2 years of normal development and then progressive cerebral and systemic multiple organ abnormalities are seen.

Our examination of *Naglu* enzyme activity of MNC hUCB cells in vitro showed that cells contain and extracellularly release the *Naglu* enzyme, making them a suitable vehicle for use in enzyme replacement therapy (Garbuzova-Davis et al., 2005). When we administered MNC hUCB cells into the cerebral ventricle of *Naglu* mice modeling MPS III B at 1 month of age, transplanted cells survived long-term (7 months), migrated into the parenchyma of the brain and peripheral organs, expressed neural antigens (nestin, NeuN, GFAP), and exhibited neuron and astrocyte-like morphology. Transplant benefits were also demonstrated by stable cytoarchitecture in the hippocampus and cerebellum, and by reduced GAGs in the livers of treated mutant mice. Although all these results indicate the beneficial effects of MNC hUCB cells, mechanisms of cell migration, as well as mechanisms promoting cell integration and differentiation in the host environment are still unknown. Many questions remain concerning the ability of MNC hUCB cells to treat multifaceted diseases, such as MPS III B, with complex factors underlying the pathogenesis. However, these are the first results supporting enzyme replacement by administered MNC hUCB cells and may lead to new strategies for delivery of the missing enzyme. We are currently investigating systemic administration of hUCB cells into *Naglu* mice, which may prove even more effective.

In one of our more recent studies (Garbuzova-Davis et al., 2006), we attempted to take advantage of the passage of maternal cells into the fetus during pregnancy for prenatal delivery of *Naglu* enzyme into the enzyme-deficient mouse model of MPS III B. Enzymatically sufficient MNC hUCB cells were intravenously administered into heterozygote females modeling MPS III B on the 5th day of pregnancy during blastocyst implantation. We found that administered MNC hUCB cells transmigrated and diffused into the embryos (E12.5) and some cells expressed CD34 and CD117 antigens. Additionally, transmigrated cells were found in both the maternal and embryonic parts of placentas and also were extensively distributed in the organs and the blood of heterozygote mothers at 1 week after transplantation. More importantly, MNC hUCB cells corrected *Naglu* enzyme

activity in all embryos. Thus, our results indicate that prenatal delivery of *Naglu* enzyme by MNC hUCB cell administration into mothers of enzyme-deficient embryonic mice is possible and may present a significant opportunity for new biotechnologies to treat many inherited disorders.

Alternative and/or combined treatment of diseased or injured brain and spinal cord

Recent work in our laboratory lead by Dr. Bickford and others has shown the potential beneficial actions of nutritional approaches to the treatment of aging and neurodegenerative diseases (Joseph et al., 1998; Bickford et al., 1999, 2000; Ferrante et al., 2001; Gemma et al., 2002). The use of fruits or vegetables has the benefit of providing a cocktail of numerous phytochemicals with multiple actions including antioxidant and anti-inflammatory effects and is one reason that many fruits and vegetables have been extensively studied in the field of cancer biology. For example, blueberries are known to contain many phenolic compounds such as anthocyanins that are potent antioxidants; the phenolic content changes with different berry varieties (Zheng and Wang, 2003). Spirulina, a blue green algae used for thousands of years as a food source by the Aztecs, is known to contain large amounts of β-carotene (Annapurna et al., 1991) and several phycocyanins (Bhat and Madyastha, 2001), all with potent antioxidant effects; phycocyanin is also known to have potent COX-2 inhibitory actions (Reddy et al., 2000, 2003) and as COX-2 is increased in ALS (Maihofner et al., 2003) and after stroke (Yokota et al., 2004), foods containing phycocyanin may have dual benefits.

In fact, we have shown that aged rats fed diets supplemented with spirulina or spinach demonstrate improved motor learning in either a rod-running motor learning task or classical eyeblink conditioning (Bickford et al., 2000; Cartford et al., 2002) and show decreases in both markers of oxidative damage and markers of inflammation (Cartford et al., 2002; Gemma et al., 2002).

In an animal model of PD, the blueberry or spirulina diet was neuroprotective in that the size of the 6-hyrdoxydopamine (6-OHDA) induced dopaminergic terminal loss was significantly smaller at 30 days postlesion (Stromberg et al., 2005). At this time, there was a significant reduction in MHC class II positive microglia, suggesting that part of the beneficial mechanism may be via reducing inflammation. At 4 weeks after the dopamine depletion by 6-OHDA injection, a significant increase in GFAP-positive profiles was found in lesioned animals given the control diet, while blueberry- and spirulina-treated animals showed no changes compared to sham-injected rats or to the 1 week time point.

In a following study, we showed the protective effects of three antioxidant diets: blueberry, spinach and spirulina against stroke in rats (Wang et al., 2005). Our data indicate that these diets have different effects in reducing ischemia-induced caspase-3 activity and cerebral infarction. Animals were put on a control, blueberry, spinach, or spirulina diet for 4 weeks prior to the insult. We used a 60 min occlusion of the middle cerebral artery and at 24 h examined the size of the infarct using TTC staining. We found a 70% protection in infarct size in the spirulina treated rats and a 50% protection in both the blueberry and spinach treated rats. In these same animals, we observed a significant decrease in caspase-3 activity and in the number of TUNEL positive cells, indicating that a reduction of apoptosis was achieved. All groups also showed significant improvement on horizontal and vertical activity measures when compared with controls.

Thus, our results suggest that nutritional supplements (spirulina, blueberries, and spinach) show a broad spectrum of neuroprotection in multiple models of neurodegeneration and can be used as alternative and/or combined treatment of diseased or injured brain and spinal cord. As studies continue using different model of neurodegenerative disease such as ALS, it will be interesting to note changes in the diseased microenvironment by diet supplementations prior to cell transplantation.

Conclusion

Numerous reports by us and others have demonstrated the plasticity of MNC hUCB cells in both

in vivo and in vitro studies. These cells differentiate into blood, immune, or neural cell lineages and seem a promising novel cell source for treatment of various neurodegenerative diseases and the injured brain or spinal cord. The MNC hUCB cells may modulate host hematopoietic and immune systems or even replace dying cells depending upon the pathological microenvironment in which the MNC hUCB cells are placed. Currently, neuroprotective and/or trophic effects of these cells, from release of various growth or anti-inflammatory factors and/or by moderation of immune-inflammatory effectors, seems more likely than neural replacement. These protective effects may be essential to maintaining/restoring tissue integrity over the course of various diseases or injuries. As evidence of MNC hUCB cell benefits continues to increase, so should enthusiasm mount for the use of these cells in new and existing therapies.

The brain is the source of intelligence and memory, responsible for a variety of crucial interconnected systems. Given our present limited understanding of brain processes and interactions, it would be naïve to expect simple solutions to brain diseases and injuries with their inevitably complex pathologies. We expect that adult stem cell and nutritional therapies will increasingly be used in conjunction with other therapies in the treatment of brain injuries and diseases.

Conflict of interest disclosure

S. Garbuzova-Davis, A.E. Willing, S. Saporta, P.C. Bickford, and C.V. Borlongan are consultants and P.R. Sanberg is Co-Founder and Chairman of Saneron CCEL Therapeutics, Inc. (SCTI, Tampa, FL). SCTI is a start-up company from the University of South Florida which is developing cord blood derived treatments for neurodegenerative and cardiovascular disorders.

References

Alexianu, M.E. (1995) The role of immune processes in amyotrophic lateral sclerosis pathogenesis. Rom. J. Neurol. Psychiatry, 33: 215–227.

Alexianu, M.E., Kozovska, M. and Appel, S.H. (2001) Immune reactivity in a mouse model of familial ALS correlates with disease progression. Neurology, 57: 1282–1289.

Annapurna, V., Deosthale, Y.G. and Bamji, M.S. (1991) Spirulina as a source of vitamin A. Plant Foods Hum. Nutr., 41: 125–134.

Baizabal, J.M., Furlan-Magaril, M., Santa-Olalla, J. and Covarrubias, L. (2003) Neural stem cells in development and regenerative medicine. Arch. Med. Res., 34: 572–588.

Bernal, G.M. and Peterson, D.A. (2004) Neural stem cells as therapeutic agents for age-related brain repair. Aging Cell, 3: 345–351.

Bhat, V. and Madyastha, K.M. (2001) Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis*: protection against oxidative damage to DNA. Biochem. Biophys. Res. Commun., 285: 262–266.

Bickford, P., Gould, T., Briederick, L., Chadman, K., Pollock, A., Young, D., Shukitt-Hale, B. and Joseph, J. (2000) Antioxidant-rich diets improve cerebellar physiology and motor learning in aged rats. Brain Res. Rev., 866: 211–217.

Bickford, P., Shukitt-Hale, B. and Joseph, J. (1999) Effects of aging on cerebellar noradrenergic function and motor learning: nutritional interventions. Mech. Ageing Dev., 111: 141–154.

Bicknese, A.R., Goodwin, H.S., Quinn, C.O., Henderson, V.C., Chien, S.N. and Wall, D.A. (2002) Human umbilical cord blood cells can be induced to express markers for neurons and glia. Cell Transp., 11: 261–264.

Bjornson, C.R.R., Rietze, R.L., Reynolds, B.A., Magli, M.C. and Vescovi, A.L. (1999) Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. Science, 283: 534–537.

Brazel, C.Y. and Rao, M.S. (2004) Aging and neuronal replacement. Ageing Res. Rev., 3: 465–483.

Brodhum, M., Bauer, R. and Patt, S. (2004) Potential stem cell therapy and application in neurotrauma. Exp. Toxicol. Pathol., 56: 103–112.

Broxmeyer, H.E. (2004) Proliferative, self-renewal, and survival characteristics of cord blood hematopoietic stem and progenitor cells. In: Broxmeyer, H.E. (Ed.), Cord Blood: Biology, Immunology, Banking, and Clinical Transplantation. AABB Press, Bethesda, Maryland, pp. 1–21.

Broxmeyer, H.E., Douglas, G.W., Hangoc, G., Cooper, S., Bard, J., English, D., Arny, M., Thomas, L. and Boyse, E.A. (1989) Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc. Natl. Acad. Sci. USA, 86: 3828–3832.

Broxmeyer, H.E., Hangoc, G., Cooper, S., Ribeiro, R.C., Graves, V., Yoder, M., Wagner, J., Vadhan-Raj, S., Benninger, L., Rubinstein, P. and Broun, E.R. (1992) Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation in adults. Proc. Natl. Acad. Sci. USA, 89: 4109–4113.

Bruijn, L.I. (2002) Amyotrophic lateral sclerosis: fromdisease mechanisms to therapies. BioTechniques, 32: 1112–11121.

Bruijn, L.I., Miller, T.M. and Cleveland, D.W. (2004) Unraveling the mechanisms involved in motor neuron degeneration in ALS. Annu. Rev. Neurosci., 27: 723–749.

Buzanska, L., Machaj, E.K., Zablocka, B., Pojda, Z. and Domanska-Janik, K. (2002) Human cord blood-derived cells attain neuronal and glial features in vitro. J. Cell Sci., 115: 2131–2138.

Cameron, H.A. and McKay, R. (1998) Stem cells and neurogenesis in the adult brain. Curr. Opin. Neurobiol., 8: 677–680.

Cartford, M., Gemma, C. and Bickford, P.C. (2002) Eighteen-month-old Fischer 344 rats fed a spinach-enriched diet show improved delay classical eyeblink conditioning and reduced expression of tumor necrosis factor alpha (TNFalpha) and TNFbeta in the cerebellum. J. Neurosci., 22: 5813–5816.

Chen, N., Hudson, J.E., Walczak, P., Misiuta, I., Garbuzova-Davis, S., Jiang, L., Sanchez-Ramos, J., Sanberg, P.R., Zigova, T. and Willing, A.E. (2005) Human umbilical cord blood progenitors: the potential of these hematopoietic cells to become neural. Stem Cells, 23: 1560–1570.

Chen, J., Li, Y., Wang, L., Zhang, Z., Lu, D., Lu, M. and Chopp, M. (2001a) Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. Stroke, 32: 1005–1011.

Chen, J., Sanberg, P.R., Li, Y., Wang, L., Lu, M., Willing, A.E., Sanchez-Ramos, J. and Chopp, M. (2001b) Intravenous administration of human umbilical cord blood reduces behavioral deficit after stroke in rats. Stroke, 32: 2682–2688.

Chen, J., Zhang, Z.G., Li, Y., Wang, L., Xu, Y., Gautam, S.C., Lu, M., Zhu, Z. and Chopp, M. (2003) Intravenous administration of human bone marrow stromal cells induces angiogenesis in the ischemic boundary zone after stroke in rats. Circ. Res., 92: 692–699.

Chen, R. and Ende, N. (2002) The potential for the use of mononuclear cells from human umbilical cord blood in the treatment of amyotrophic lateral sclerosis in SOD1 mice. J. Med., 31: 21–30.

Chen, X., Li, Y., Wang, L., Katakowski, M., Zhang, L., Chen, J., Xu, Y., Gautam, S.C. and Chopp, M. (2002) Ischemic rat brain extracts induce human marrow stromal cell growth factor production. Neuropathology, 22: 275–279.

Cleveland, D.W. and Rothstein, J.D. (2001) From Charcot to Lou Gehrig: deciphering selective motor neuron death in ALS. Nat. Rev. Neurosci., 2: 806–819.

Conrad, P.D. and Emerson, S.G. (1998) Ex vivo expansion of hematopoietic cells from umbilical cord blood for clinical transplantation. J. Leukoc. Biol., 64: 147–155.

Curtis, M.A., Penney, E.B., Pearson, A.G., van Roon-Mom, W.M., Butterworth, N.J., Dragunow, M., Connor, B. and Faull, R.L. (2003) Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. Proc. Natl. Acad. Sci. USA, 100: 9023–9027.

Daar, A.S., Bhatt, A., Court, E. and Singer, P.A. (2004) Stem cell research and transplantation: science leading ethics. Transplant. Proc., 36: 2504–2506.

Delorme, B., Basire, A., Gentile, C., Sabatier, F., Monsonis, F., Desouches, C., Blot-Chabaund, M., Uzan, G., Sampol, J. and Dignat-George, F. (2005) Presence of endothelial progenitor cells, distinct from mature endothelial cells, within human CD146+ blood cells. Thromb. Haemost., 4: 1270–1279.

Dennis, J.E. and Charbord, P. (2002) Origin and differentiation of human and murine stroma. Stem Cells, 20: 205–214.

Desjarlais, T., Garbuzova-Davis, S., Willing, A.E., Zigova, T., Saporta, S., Davis, C. and Sanberg, P.R. (2003) Putative immunomodulation of human umbilical cord blood cells in a mouse model of ALS. Exp. Neurol., 181: 90.

Ende, N., Weistein, F., Chen, R. and Ende, M. (2000) Human umbilical cord blood effect on SOD mice (amyotrophic lateral sclerosis). Life Sci., 67: 53–59.

Ferrante, R.J., Klein, A.M., Dedeoglu, A. and Beal, M.F. (2001) Therapeutic efficacy of EGb761 (*Gingko biloba* extract) in a transgenic mouse model of amyotrophic lateral sclerosis. J. Mol. Neurosci., 17: 89–96.

Ferrari, G., Cusella-De Angelis, G., Coletta, M., Paolucci, E., Stornaiuolo, A., Cossu, G. and Mavilio, F. (1998) Muscle regeneration by bone marrow-derived myogenic progenitors. Science, 279: 1528–1530.

Forraz, N., Pettengell, R. and McGuckin, C.P. (2004) Characterization of a lineage-negative stem-progenitor cell population optimized for ex-vivo expansion and enriched for LTC-IC. Stem Cells, 22: 100–108.

Frassoni, F., Podesta, M., Maccario, R., Giorgiani, G., Rossi, G., Zecca, M., Bacigalupo, A., Piaggio, G. and Locatelli, F. (2003) Cord blood transplantation provides better reconstitution of hematopoietic reservoir compared with bone marrow transplantation. Blood, 102: 1138–1141.

Gage, F.H. (2000) Mammalian neural stem cells. Science, 287: 1433–1438.

Galli, R., Borello, U., Gritti, A., Minasi, M.G., Bjornson, C., Coletta, M., Mora, M., De Angelis, M.G., Fiocco, R., Cossu, G. and Vescovi, A.L. (2000) Skeletal myogenic potential of human and mouse neural stem cells. Nat. Neurosci., 3: 986–991.

Garbuzova-Davis, S., Gografe, S.J., Davis Sanberg, C., Willing, A.E., Saporta, S., Cameron, D.F., Desjarlais, T., Daily, J., Kuzmin-Nichols, N., Chamizo, W., Klasko, S.K. and Sanberg, P.R. (2006) Maternal transplantation of human umbilical cord blood cells provides prenatal therapy in Sanfilippo type B mouse model. FASEB, 20: 485–487.

Garbuzova-Davis, S., Willing, A.E., Desjarlais, T., Davis Sanberg, C. and Sanberg, P.R. (2005) Transplantation of human umbilical cord blood cells benefits an animal model of Sanfilippo syndrome type B. Stem Cells Dev., 14: 384–394.

Garbuzova-Davis, S., Willing, A.E., Zigova, T., Saporta, S., Justen, E.B., Lane, J.C., Hudson, J.E., Chen, N., Davis, C.D. and Sanberg, P.R. (2003) Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. J. Hematother. Stem Cell Res., 12: 255–270.

Garderet, L., Dulphy, N., Douay, C., Chalumeau, N., Schaeffer, V., Zilber, M.T., Lim, A., Even, J., Mooney, N., Gelin, C., Gluckman, E., Charron, D. and Toubert, A. (1998) The umbilical cord blood alphabeta T-cell repertoire: characteristics of a polyclonal and naive but completely formed repertoire. Blood, 91: 340–346.

Gemma, C., Mesches, M.H., Sepesi, B., Choo, K., Holmes, D.B. and Bickford, P.C. (2002) Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. J. Neurosci., 22: 6114–6120.

Gluckman, E. (2000) Current status of umbilical cord blood hematopoietic stem cell transplantation. Exp. Hematol., 28: 1197–1205.

Gluckman, E., Broxmeyer, H.A., Auerbach, A.D., Friedman, H.S., Douglas, G.W., Devergie, A., Esperou, H., Thierry, D., Socie, G. and Lehn, P. (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N. Engl. J. Med., 321: 1174–1178.

Gluckman, E., Rocha, V., Boyer-Chammard, A., Locatelli, F., Arcese, W., Pasquini, R., Ortega, J., Souillet, G., Ferreira, E., Laporte, J.P., Fernandez, M. and Chastang, C. (1997) Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N. Engl. J. Med., 337: 373–381.

Gluckman, E., Rocha, V. and Chastang, C. (1999) Peripheral stem cells in bone marrow transplantation. Cord blood stem cell transplantation. Baillieres Best Pract. Res. Clin. Haematol., 12: 279–292.

Goodwin, H.S., Bicknese, A.R., Chien, S.N., Bogucki, B.D., Oliver, D.A., Quinn, C.O. and Wall, D.A. (2001) Multilineage differentiation activity by cells isolated from umbilical cord blood: expression of bone, fat, and neural markers. Biol. Blood Marrow Transpl., 7: 581–588.

Gorelick, P.B., Sacco, R.L., Smith, D.B., Alberts, M., Mustone-Alexander, L., Rader, D., Ross, J.L., Raps, E., Ozer, M.N., Brass, L.M., Malone, M.E., Goldberg, S., Booss, J., Hanley, D.F., Toole, J.F., Greengold, N.L. and Rhew, D.C. (1999) Prevention of a first stroke: a review of guidelines and a multidisciplinary consensus statement from the National Stroke Association. JAMA, 281: 1112–1120.

Gritti, A., Vescovi, A.L. and Galli, R. (2002) Adult neural stem cells: plasticity and developmental potential. J. Physiol. Paris., 96: 81–90.

Ha, Y., Choi, J.U., Yoon, D.H., Yeon, D.S., Lee, J.J., Kim, H.O. and Cho, Y.E. (2001) Neural phenotype expression of cultured human cord blood cells in vitro. Neuroreport, 12: 3523–3527.

Hao, S.G., Sun, G.L., Wu, W.L. and Wu, Y.L. (2003) Studies on the dynamics of biological characteristics of CD133+ cells from human umbilical cord blood during short-term culture. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 11: 569–575.

Henon, P.R. (2003) Human embryonic or adult stem cells: an overview on ethics and perspectives for tissue engineering. Adv. Exp. Med. Biol., 534: 27–45.

Hiramatsu, H., Nishikomori, R., Heike, T., Ito, M., Kobayashi, K., Katamura, K. and Nakahata, T. (2003) Complete reconstitution of human lymphocytes from cord blood CD34+ cells using the NOD/SCID/{gamma}cnull mice model. Blood, 102: 873–880.

Hirose, Y., Kiyoi, H., Itoh, K., Kato, K., Saito, H. and Naoe, T. (2001) B-cell precursors differentiated from cord blood CD34+ cells are more immature than those derived from granulocyte colony-stimulating factor-mobilized peripheral blood CD34+ cells. Immunology, 104: 410–417.

Honmou, O., Oka, S., Houkin, K., Hashi, K. and Kocsis, J.D. (2002) AC133 – positive cells derived from human cord blood remyelinate demyelinated axons in the adult rat spinal cord. Program No. 34.2, 2002 Abstracts Viewer/Itinerary Planner. Washington DC, Society for Neuroscience.

Jackson, K.A., Mi, T. and Goodell, M.A. (1999) Hematopoietic potential of stem cells isolated from murine skeletal muscle. Proc. Natl. Acad. Sci. USA, 96: 14482–14486.

Jin, K., Peel, A.L., Mao, X.O., Xie, L., Cottrell, B.A., Henshall, D.C. and Greenberg, D.A. (2004) Increased hippocampal neurogenesis in Alzheimer's disease. Proc. Natl. Acad. Sci. USA, 101: 343–347.

Joseph, J., Denisova, N., Fisher, D., Bickford, P., Prior, R. and Cao, G. (1998) Age-related neurodegeneration and oxidative stress: putative nutritional intervention. Neurol. Clin., 16: 747–755.

Kawamata, T., Akiyama, H., Yamada, T. and McGeer, P.L. (1992) Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. Am. J. Pathol., 140: 691–707.

Kim, J.J., Saporta, S., Willing, A.E., Fu, E.S., Colina, L.O. and Sanberg, P.R. (2002) Behavioral recovery following intravenous administration of human umbilical cord blood cells in rats with spinal cord injury. Exp. Neurol., 175: 443.

Kobari, L., Giarratana, M.C., Pflumio, F., Izac, B., Coulombel, L. and Douay, L. (2001) CD133+ cell selection is an alternative to CD34+ cell selection for ex vivo expansion of hematopoietic stem cells. J. Hematother. Stem Cell Res., 10: 273–281.

Koehl, U., Zimmermann, S., Esser, R., Sorensen, J., Gruttner, H.P., Duchscherer, M., Seifried, E., Klingebiel, T. and Schwabe, D. (2002) Autologous transplantation of CD133 selected hematopoietic progenitor cells in a pediatric patient with relapsed leukemia. Bone Marrow Transpl., 29: 927–930.

Kulbatski, I., Mothe, A.J., Nomura, H. and Tator, C.H. (2005) Endogenous and exogenous CNS derived stem/progenitor cell approaches for neurotrauma. Curr. Drug Targets, 6: 111–126.

Kuzmenok, O.I., Sanberg, P.R., Desjarlais, T., Bennett, S.P. and Garbuzova-Davis, S.N. (2006) Lymphopenia and spontaneous autorosette formation in SOD1 mouse model of ALS. J. Neuroimmunol., 172: 132–136.

Li, Y., Chen, J., Chen, X.G., Wang, L., Gautam, S.C., Xu, Y., Katakowski, M., Zhang, L.J., Lu, M., Janakiraman, N. and Chopp, M. (2002) Human marrow stromal cell therapy for stroke in rat: neurotrophins and functional recovery. Neurology, 59: 514–523.

Lie, D.C., Song, H., Colamarino, S.A., Ming, G.L. and Gage, F.H. (2004) Neurogenesis in the adult brain: new strategies for central nervous system diseases. Annu. Rev. Pharmacol. Toxicol., 44: 399–421.

Liesveld, J.L. (2003) When what you have is not enough: optimizing cord blood transplantation in adults. Leuk. Res., 27: 197–199.

Limke, T.L. and Rao, M.S. (2002) Neural stem cells in aging and disease. Cell Mol. Med., 6: 475–496.

Linazasoro, G. (2003) Stem cells: solution to the problem of transplants in Parkinson's disease? Neurologia, 18: 74–100.

Lu, D., Sanberg, P.R., Mahmood, A., Li, Y., Wang, L., Sanchez-Ramos, J. and Chopp, M. (2002) Intravenous administration of human umbilical cord blood reduces neurological deficit in the rat after traumatic brain injury. Cell Transpl., 11: 275–285.

Lu, L., Shen, R.N. and Broxmeyer, H.E. (1996) Stem cells from bone marrow, umbilical cord blood and peripheral blood for clinical application: current status and future application. Crit. Rev. Oncol. Hematol., 22: 61–78.

Ma, Y., Zou, P., Xiao, J. and Huang, S. (2002) The expression and functional characteristics of AC133 antigen in cord blood hematopoietic cells. Zhonghua Nei Ke Za Zhi, 41: 798–800.

Madrigal, J.A., Cohen, S.B., Gluckman, E. and Charron, D.J. (1997) Does cord blood transplantation result in lower graft-versus-host disease? It takes more than two to tango. Hum. Immunol., 56: 1–5.

Maihofner, C., Probst-Cousin, S., Bergmann, M., Neuhuber, W., Neundorfer, B. and Heuss, D. (2003) Expression and localization of cyclooxygenase-1 and -2 in human sporadic amyotrophic lateral sclerosis. Eur. J. Neurosci., 18: 1527–1534.

Mayani, H. and Lansdorp, P.M. (1998) Biology of human umbilical cord blood-derived hematopoietic stem/progenitor cells. Stem Cells, 16: 153–165.

McGuckin, C.P., Forraz, N., Allouard, Q. and Pettengell, R. (2004) Umbilical cord blood stem cells can expand hematopoietic and neuroglial progenitors in vitro. Exp. Cell Res., 295: 350–359.

Mikami, T., Eguchi, M., Kurosawa, H., Sato, Y., Sugita, K., Suzumura, H., Tadokoro, N., Watanabe, H. and Inaba, N. (2002) Ultrastructural and cytochemical characterization of human cord blood cells. Med. Electron Microsc., 35: 96–101.

Mohamed, H.A., Mosier, D.R., Zou, L.L., Siklos, L., Alexianu, M.E., Engelhardt, J.I., Beers, D.R., Le, W.D. and Appel, S.H. (2002) Immunoglobulin Fc gamma receptor promotes immunoglobulin uptake, immunoglobulin-mediated calcium increase, and neurotransmitter release in motor neurons. J. Neurosci. Res., 69: 110–116.

Nakahata, T. and Ogawa, M. (1982) Hemopoietic colony-forming cells in umbilical cord blood with extensive capability to generate mono- and multipotential hemopoietic progenitors. J. Clin. Invest., 70: 1324–1328.

Nayar, B., Raju, G.M. and Deka, D. (2002) Hematopoietic stem/progenitor cell harvesting from umbilical cord blood. Int. J. Gynaecol. Obstet., 79: 31–32.

Newman, M.B., Willing, A.E., Manresa, J.J., Davis-Sanberg, C. and Sanberg, P.R. (2005) Stroke-induced migration of human umbilical cord blood cells: time course and cytokines. Stem Cells Dev., 14: 576–586.

Niebroj-Dobosz, I., Jamrozik, Z., Janik, P., Hausmanowa-Petrusewicz, I. and Kwiecinski, H. (1999) Anti-neural antibodies in serum and cerebrospinal fluid of amyotrophic lateral sclerosis (ALS) patients. Acta Neurol. Scand., 100: 238–243.

Ooi, J., Iseki, T., Takahashi, S., Tomonari, A., Nagayama, H., Ishii, K., Ito, K., Sato, H., Takahashi, T., Shindo, M., Sekine, R., Ohno, N., Uchimaru, K., Nagamura, F., Shirafuji, N., Tojo, A., Tani, K. and Asano, S. (2002) A clinical comparison of unrelated cord blood transplantation and unrelated bone marrow transplantation for adult patients with acute leukaemia in complete remission. Br. J. Haematol., 118: 140–143.

Parent, J.M. (2003) Injury-induced neurogenesis in the adult mammalian brain. Neuroscientist, 9: 261–272.

Petersen, B.E., Bowen, W.C., Patrene, K.D., Mars, W.M., Sullivan, A.K., Murase, N., Boggs, S.S., Greenberger, J.S. and Goff, J.P. (1999) Bone marrow as a potential source of hepatic oval cells. Science, 284: 1168–1170.

Provinciali, L., Laurenzi, M.A., Vesprini, L., Giovagnoli, A.R., Bartocci, C., Montroni, M., Bagnarelli, P., Clementi, M. and Varaldo, P.E. (1988) Immunity assessment in the early state of amyotropfic lateral sclerosis: a study of virus antibodies and lymphocyte subsets. Acta Neurol. Scand., 78: 449–454.

Rabinovich, S.S., Seledtsov, V.I., Poveschenko, O.V., Senuykov, V.V., Taraban, V.Y., Yarochno, V.I., Kolosov, N.G., Savchenko, S.A. and Kozlov, V.A. (2003) Transplantation treatment of spinal cord injury patients. Biomed. Pharmacother., 57: 428–433.

Rainsford, E. and Reen, D.J. (2002) Interleukin 10, produced in abundance by human newborn T cell, may be the regulator of increased tolerance associated with cord blood stem cell transplantation. Br. J. Haematol., 116: 702–709.

Reddy, C., Bhat, V.B., Kiranmai, G., Reddy, M.N., Reddanna, P. and Madyastha, K.M. (2000) Selective inhibition of cyclooxygenase-2 by C-phycocyanin, a biliprotein from Spirulina platensis. Biochem. Biophys. Res. Commun., 277: 599–603.

Reddy, M., Subhashini, J., Mahipal, S.V., Bhat, V.B., Srinivas, R.P., Kiranmai, G., Madyastha, K.M. and Reddanna, P. (2003) C-Phycocyanin, a selective cyclooxygenase-2 inhibitor, induces apoptosis in lipopolysaccharide-stimulated RAW 264.7 macrophages. Biochem. Biophys. Res. Commun., 304: 385–392.

Riaz, S.S., Jauniaux, E., Stern, G.M. and Bradford, H.F. (2002) The controlled conversion of human neural progenitor cells derived from foetal ventral mesencephalon into dopaminergic neurons in vitro. Brain Res. Dev. Brain Res., 136: 27–34.

Roncarolo, M.G., Bigler, M., Ciuti, E., Martino, S. and Tovo, P.A. (1994) Immune responses by cord blood cells. Blood Cells, 20: 573–585.

Rubinstein, P., Carrier, C., Scaradavou, A., Kurtzberg, J., Adamson, J., Migliaccio, A.R., Berkowitz, R.L., Cabbad, M., Dobrila, N.L., Taylor, P.E., Rosenfield, R.E. and Stevens, C.E. (1998) Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N. Engl. J. Med., 339: 1565–1577.

Sanberg, P.R. (2005) Stem cell research's reversal of fortune. Scientist, 19: 12.

Sanberg, P.R., Willing, A.E. and Cahill, D.W. (2001) Neurosurgery for the 21st century. What does neurobiology justify for repairing neurodegenerative disorders? Clin. Neurosurg., 48: 113–126.

Sanberg, P.R., Willing, A.E. and Cahill, D.W. (2002) Novel cellular approaches to repair of neurodegenerative disease: from Sertoli cells to umbilical cord blood stem cells. Neurotox. Res., 4: 95–101.

Sanberg, P.R., Willing, A.E., Davis Sanberg, C., Kazi, Z. and Borlongan, C.V. (2004) Different functional recovery and neurostructural repair of the stroke brain produced by volume-reduced and mononuclear fractioned human umbilical cord blood. Program No. 617.4 Abstract Viewer/Itinerary Planner. Society for Neuroscience, Washington, DC.

Sanchez-Ramos, J., Song, S., Cardozo-Pelaez, F., Hazzi, C., Stedeford, T., Willing, A., Freeman, T.B., Saporta, S., Janssen, W., Patel, N., Cooper, D.R. and Sanberg, P.R. (2000) Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp. Neurol., 164: 247–256.

Sanchez-Ramos, J.R., Song, S., Kamath, S.G., Zigova, T., Willing, A., Cardozo-Pelaez, F., Stedeford, T., Chopp, M. and Sanberg, P.R. (2001) Expression of neural markers in human umbilical cord blood. Exp. Neurol., 171: 109–115.

Saporta, S., Kim, J.J., Willing, A.E., Fu, E.S., Davis, C.D. and Sanberg, P.R. (2003) Human umbilical cord blood stem cells infusion in spinal cord injury: engraftment and beneficial influence on behavior. J. Hematother. Stem Cell Res., 12: 271–278.

Sathananthan, A.H. and Trounson, A. (2005) Human embryonic stem cells and their spontaneous differentiation. Ital. J. Anat. Embryol., 110: 151–157.

Savitz, S.I., Dinsmore, J.N., Wechsler, L.R., Rosenbaum, D.M. and Caplan, L.R. (2004) Cell therapy for stroke. NeuroRx., 1: 406–414.

Shih, C.C., DiGiusto, D. and Forman, S.J. (2000) Ex vivo expansion of transplantable human hematopoietic stem cells: where do we stand in the year 2000? J. Hematother. Stem. Cell Res., 9: 621–628.

Shin, J.W., Lee, D.W., Kim, M.J., Song, K.S., Kim, H.S. and Kim, H.O. (2005) Isolation of endothelial progenitor cells from cord blood and induction of differentiation by ex vivo expansion. Yonsei. Med. J., 46: 260–267.

Silani, V. and Leigh, N. (2003) Stem therapy for ALS: hope and reality. Amyotrop. Lateral Scler. Other Motor Neuron. Disord., 4: 8–10.

Sirchia, G. and Rebulla, P. (1999) Placental/umbilical cord blood transplantation. Haematologica, 84: 738–747.

Slack, J.M.W. (2000) Stem cells in epithelial tissues. Science, 287: 1431–1433.

Staba, S.L., Escolar, M.L., Poe, M., Kim, Y., Martin, P.L., Szabolcs, P., Allison-Thacker, J., Wood, S., Wenger, D.A., Rubinstein, P., Hopwood, J.J., Krivit, W. and Kurtzberg, J. (2004) Cord-blood transplants from unrelated donors in patients with Hurler's syndrome. N. Engl. J. Med., 350: 1960–1969.

Stromberg, I., Gemma, C., Vila, J. and Bickford, P.C. (2005) Blueberry- and spirulina-enriched diets enhance striatal dopamine recovery and induce a rapid, transient microglia activation after injury of the rat nigrostriatal dopamine system. Exp. Neurol., 196: 298–307.

Thomson, B.G., Robertson, K.A., Gowan, D., Heilman, D., Broxmeyer, H.E., Emanuel, D., Kotylo, P., Brahmi, Z. and Smith, F.O. (2000) Analysis of engraftment, graft-versus-host disease, and immune recovery following unrelated donor cord blood transplantation. Blood, 96: 2703–2711.

Todaro, A.M., Pafumi, C., Pernicone, G., Munda, S., Pilastro, M.R., Russo, A., Farina, M. and Messina, A. (2000) Haematopoietic progenitors from umbilical cord blood. Blood Purif., 18: 144–147.

Vaziri, H., Dragowska, W., Allsopp, R.C., Thomas, T.E., Harley, C.B. and Lansdorp, P.M. (1994) Evidence for a mitotic clock in human hematopoietic stem cells: loss of telomeric DNA with age. Proc. Natl. Acad. Sci. USA, 91: 9857–9860.

Vendrame, M., Cassady, J., Newcomb, J., Butler, T., Pennypacker, K.R., Zigova, T., Sanberg, C.D., Sanberg, P.R. and Willing, A.E. (2004) Infusion of human umbilical cord blood cells in a rat model of stroke dose-dependently rescues behavioral deficits and reduces infarct volume. Stroke, 35: 2390–2395.

Vendrame, M., Gemma, C., de Mesquita, D., Collier, L., Bickford, P.C., Sanberg, C.D., Sanberg, P.R., Pennypacker, K.R. and Willing, A.E. (2005) Anti-inflammatory effects of human cord blood cells in a rat model of stroke. Stem Cells Dev., 14: 595–604.

Vescovi, A.L., Parati, E.A., Gritti, A., Poulin, P., Ferrario, M., Wanke, E., Frolichsthal-Schoeller, P., Cova, L., Arcellana-Panlilio, M., Colombo, A. and Galli, R. (1999) Isolation and cloning of multipotential stem cells from the embryonic human CNS and establishment of transplantable human neural stem cell lines by epigenetic stimulation. Exp. Neurol., 156: 71–83.

Wagner, J.E., Barker, J.N., DeFor, T.E., Baker, K.S., Blazar, B.R., Eide, C., Goldman, A., Kersey, J., Krivit, W., MacMillan, M.L., Orchard, P.J., Peters, C., Weisdorf, D.J., Ramsay, N.K. and Davies, S.M. (2002) Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood, 100: 1611–1618.

Wang, Y., Chang, C.F., Chou, J., Chen, H.L., Deng, X., Harvey, B.K., Cadet, J.L. and Bickford, P.C. (2005) Dietary supplementation with blueberries, spinach, or spirulina reduces ischemic brain damage. Exp. Neurol., 193: 75–84.

Willing, A.E., Lixian, J., Milliken, M., Poulos, S., Zigova, T., Song, S., Hart, C., Sanchez-Ramos, J. and Sanberg, P.R. (2003) Intravenous versus intrastriatal cord blood administration in a rodent model of stroke. J. Neurosci. Res., 73: 296–307.

Woodbury, D., Schwarz, E.J., Prockop, D.J. and Black, I.B. (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. J. Neurosci. Res., 61: 364–370.

Yin, A.H., Miraglia, S., Zanjani, E.D., Almeida-Porada, G., Ogawa, M., Leary, A.G., Olweus, J., Kearney, J. and Buck, D.W. (1997) AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood, 90: 5002–5012.

Yokota, C., Kaji, T., Kuge, Y., Inoue, H., Tamaki, N. and Minematsu, K. (2004) Temporal and topographic profiles of cyclooxygenase-2 expression during 24 h of focal brain ischemia in rats. Neurosci. Lett., 357: 219–222.

Zheng, W. and Wang, S.Y. (2003) Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. J. Agric. Food Chem., 51: 502–509.

Zhou, Q., Zhang, S.Z., Xu, R.X. and Xu, K. (2004) Neural stem cell transplantation and postoperative management: report of 70 cases. Di Yi Jun Yi Da Xue Xue Bao, 24: 1207–1209.

Zola, H., Fusco, M., Macardle, P.J., Flego, L. and Roberton, D. (1995) Expression of cytokine receptors by human cord blood lymphocytes: comparison with adult blood lymphocytes. Pediatr. Res., 38: 397–403.