Applications of Human Umbilical Cord Blood Cells in Central Nervous System Regeneration

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Abstract: In recent decades, there has been considerable amount of information about embryonic stem cells (ES). The dilemma facing scientists interested in the development and use of human stem cells in replacement therapies is the source of these cells, i.e. the human embryo. There are many ethical and moral problems related to the use of these cells. Hematopoietic stem cells from umbilical cord blood have been proposed as an alternative source of embryonic stem cells. After exposure to different agents, these cells are able to express antigens of diverse cellular lineages, including the neural type. The *In vitro* manipulation of human umbilical cord blood (hUCB) cells has shown their stem capacity and plasticity. These cells are easily accessible, *In vitro* amplifiable, well tolerated by the host, and with more primitive molecular characteristics that give them great flexibility. Overall, these properties open a promising future for the use of hUCB in regenerative therapies for the Central Nervous System (CNS). This review will focus on the available literature concerning umbilical cord blood cells as a therapeutic tool for the treatment of neurodegenerative diseases.

Keywords: Stem cells, umbilical cord blood, neuroregeneration, CNS transplantation, brain injury, neurodegenerative disorders.

INTRODUCTION

Neurogenesis in the adult brain was described in the 1990s. The traditional view that the adult brain was incapable of renewing its cells and repairing its structures has now generally been put aside. Throughout life, new neurons are constantly being formed in certain restricted areas of the brain, known as "neurogenic areas", specifically in the hippocampus and the subventricular zone (SVZ). These cells are able to differentiate into the cell types of the CNS, neurons and glia, which are responsible for tissular homeostasis and regeneration during adult life and old age. Nevertheless, a decrease of new neurons has been observed in old age. The mechanism that produces a decrease in neurogenesis with aging is unknown. The functional loss in "normal" aging is associated to a loss of synaptic contacts in most brain structures, apparently due to a decrease in cellular metabolism and an increase of oxidative stress of DNA and proteins. These changes can be even more critical in diseases such as Parkinson's, Alzheimer's, or Amyotrophic Lateral Sclerosis (ALS). At present, treatments only help to reduce the symptoms, but are not curative. The scientific community is trying to develop new strategies in which lost cells can be replaced by implantation of new cells. In brain cell therapy, neural stem cells (NSC) offer a hopeful scenario for repairing the damaged brain. Currently, many studies are focused on the signals that trigger the mechanisms by which NSC choose a differentiation pathway. Thus, it is well known that the destination of adult NSC depends on the microenvironment that controls their proliferation and differentiation, which includes specific signals such as cytokines or growth factors [1, 2].

Although NSC may have a considerable regeneration capacity in certain areas and could be used for implantation, the difficulty in obtaining them could limit their future use. Therefore, there has been an increase of research interest in the plasticity of hematopoietic stem cells for the treatment of non-hematopoietic diseases, including neurodegenerative diseases [3, 4].

Certain considerations affecting the success of the engraftment have to be taken into account in any type of stem cell transplantation. These include: 1) The compatibility between donor and recipient of the Human Leukocyte Antigen (HLA), the Major Histocompatibility Complex (MHC) in humans, thereby determining the critical cell number required, 2) whether or not factors or accessory cells have to be used, and 3) the time elapsed between the brain damage and the transplantation.

Taking the hematopoietic system as an example, the time factor is very important. It has been shown that a small number of cells are able to survive implantation and to produce the complete hematopoietic system; however this requires a much longer period of time than when a larger number of cells are transplanted [5].

The sources of stem cells chosen should be those that are easily preserved in stem cell banks and have a considerable capacity for proliferation and self-renewal. The cell lines derived from ES cells have a high proliferation capacity and a very good survival rate in cryopreservation. At present, the creation of embryonic stem cell banks covering a large number of ethnically diverse people is hardly feasible due, among other reasons, to the inefficiency in the production of ES cells lines and the limited availability of human blastocysts. Of the potential new stem cell sources, the genuinely useful ones will be those with a similar potential to ES cells and a high proliferation capacity, or those that can be obtained easily in large numbers. Although there is still no clear winner, some possible candidates are emerging, such as cells derived from bone marrow, or "bone-marrow-like" stem cells from the umbilical cord and the peripheral blood.

HUMAN UMBILICAL CORD BLOOD STEM CELLS

Human umbilical cord blood cells were used successfully for the first time in 1989, as a bone marrow transplant in a patient with Fanconi's anemia [6]. Most hematopoietic stem cells are found in fetal circulation, in the placenta and the umbilical cord. Hematopoietic stem cells migrate towards the bone marrow within several hours after birth. Around 120 ml of the blood containing the stem cells remains in the placenta and the umbilical cord after birth, and can be collected without risk to the mother or the baby. It is worth mentioning that this rich source of stem cells is generally discarded.

Umbilical cord blood is richer in hematopoietic stem cells, by unit of volume, than peripheral blood or bone marrow. In addition, it appears to be more tolerant to a lack of histocompatibility to HLA [7]. Another advantage over bone marrow is that is easily obtained

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in a non-invasive way. Although hematological reconstitution using cord cells is slower or delayed, because of the limited number of CD34+ cells present, recently a two-step umbilical cord blood cell collection method has been described, in which sufficient nucleated cells were obtained for transplantation into adult patients [8].

One characteristic of these cells is that, although they are adult stem cells they still have certain properties of ES cells, such as the expression of: transcription factors Oct-4, Rex-1, Sox-2 and Nanong; stage-specific embryonic antigens SSEA-3 and SSEA-4; and typical embryonic stem cell markers, such as TRA-1-60 and TRA-1-81. Another characteristic is low immunogenicity, revealed by weak expression of MHC and by a lack of stimulating capacity on the proliferation of allogeneic lymphocytes [9].

In addition to hematopoietic stem cells, umbilical cord blood contains other cell populations, such as mesenchymal stem cells [10], very small embryonic-like stem cells [11], unrestricted somatic stem cells [12] and endothelial precursor cells [13].

Hematopoietic stem cells derived from umbilical cord blood are easily obtained and have relatively low costs for their collection and storage. This makes it possible for people to preserve their own cord cells for possible future use, if cell therapy is indicated.

Neural Aspects of Umbilical Cord Cells

In 2001, Sánchez-Ramos's group at the University of South Florida published the first study describing how mononuclear hUCB (MNChUCB) cells, treated with retinoic acid (RA) and nerve growth factor (NGF), expressed molecular markers typically associated to neurons and glia [14]. They detected early expressed markers for neural precursors (Musashi-1, nestin and Tuj1), for mature neurons (NeuN and MAP2), and for astrocytes (GFAP). Cord blood cells cultured with normal medium (DMEM) also expressed certain neural markers, such as nestin, TuJ1, MAP2 and GFAP [3]. Sixty percent of cells treated with RA+NGF showed immunoreactivity to BrdU, indicating that they continued to proliferate. These proliferating cells expressed the CD133 antigen, a primitive marker for hematopoietic progenitor/stem cells. After 7 days in culture, they generated cells that simultaneously showed characteristics of mature and immature neural cells.

It has been shown that the developing brain is a favorable environment for the development of neural phenotypes. Cord cells treated with RA+NGF were transplanted to the developing brains of newborn rats (1 day old). They were unilaterally injected in the anterior part of the SVZ, and after a month the brains were studied. Approximately 20% of the implanted cells survived in the brains of the neonates. Most of them were found in the SVZ with some dispersion to the cerebral cortex and the adjacent corpus callosum. Around 2% of these cells expressed the glial marker GFAP, and a very small amount (<0.2%) expressed the neuronal marker β Tubulin III [15].

In a gene expression study, using microarray technology, cells treated with RA+NGF showed higher expression of 322 genes out of 12,600 human genes represented in a DNA Chip (Affymetrix). Most of the genes with increased expression were not directly related to the process of neurogenesis; nevertheless, at least 20 of these genes could be linked to products found in neurons, glia or developing neural cells [14]. For example, the highest level of upregulation (44 times higher) was found in the mRNA of pleiotrophin, the neurite growth-promoting factor 1. Other transcripts associated to early neural development, neuronal growth or early markers of neural precursors also increased, but by a much smaller amount (1.5 times higher). A decrease in many of the genes associated with the development of blood cell lines was detected simultaneously to the expression of markers indicating neural development. Other authors have confirmed these findings and have demonstrated that cord cells can be induced to express β-Tub III, GFAP and GalC (an oligodendrocyte marker) [16,17].

In 2001, Goodwin et al., showed that a subgroup of UCB cells maintained in culture for 6 months lost the expression of the antigen for hematopoietic differentiation. When these cells were exposed to osteogenic or adipogenic agents or to the basic fibroblast and epidermal growth factors, they expressed bone, fat and nervous tissue markers [18].

Taken together, these findings suggest that umbilical cord blood contains cells that, under certain conditions, are able to express the antigens of various cell lineages, thereby demonstrating their plasticity.

A methodology based on immunomagnetic negative selection has been developed to confirm the presence of multipotent cells. Using this method, a cell population was isolated representing 0.1% of all the mononuclear cells of the human umbilical cord. When cultured, these cells produced non-adherent hematopoietic cells and, at the same time, adherent cells with the morphology of neural progenitor cells. An increase in the genetic expression of neuronal and glial markers has been found after 8 weeks of culture [19, 20].

Recently, an excellent work by a research group from the University of South Florida [21] has characterized the mononuclear fraction of hUCB cells, before and after cultivating these cells in the presence of serum, without the addition of factors such as neurotrophins, growth factors or cytokines. They specifically studied the expression of hematopoietic and neural antigens during the culture period, and classified the hUCB cells into two different subpopulations: adherent and floating cells. The adherent fraction was mainly lymphocytes, expressing hematopoietic antigens. In the floating subpopulation, they described the presence of a significant number of early antigen markers of stem/progenitor cells and of neural antigens. Studying the expression of neural proteins in cultures of hUCB cells in both subpopulations, they had positive results for A2B5, TuJ1, MAP2, GFAP, S100, NF68KD [NFL] and neurotrophin receptors such as trkB and trkC [21]. They found more glial markers expressed in the adherent subpopulation and more neuronal markers in the floating subpopulation. The authors also noted the co-expression of hematopoietic and neural antigens in some cells, as has been seen in other studies [15, 16, 22]. *In vivo*, this fact could be interpreted as a fusion with host cells, but this is not possible as there are only UCB cells in the cultures. An alternative explanation could be that these cells normally express some of these antigens. Indeed, GFAP has been detected in some bone marrow cells [23]. Finally, there is the possibility that, under certain conditions, specific progenitor cells of a particular adult tissue could differentiate into cells with the characteristics of other tissues or organs. Therefore, transdifferentiation should be considered as an option. Independently of the mechanism by which bone marrow and umbilical cord cells differentiate into neurons and glia, the best demonstration that these cells have acquired a neuronal phenotype would be to use a functional assay, in which the formation of synapses, electrophysiological activity, and the release of neurotransmitters must be shown [21].

ISCHEMIC STROKE

Cerebral infarct is a progressive process, in which brain damage increases with time; therefore the moment when treatment is started is critical. At present, the only effective treatment (tissular plasminogen activator) has to be administered in a very narrow time window after the stroke.

In a number of studies where strokes experimentally induced in rats were treated by intravenous transplantation of human bone marrow stromal cells, functional improvements in the animals have been reported [24-26]. These authors suggest that the improvements are due more to the release of trophic factors, which promote both neurogenesis and angiogenesis, than to neuronal replacement by the implanted cells.

Intravenous administration of hUCB cells to rats, where stroke was induced by occlusion of the middle cerebral artery, promoted the improvement of neurological function [27]. The cells were mainly found in the cortex and the striatum of the lesioned hemisphere and, outside the brain, in bone marrow, spleen, and in very small amounts in muscle, heart, lungs and liver. Using immunohistochemistry techniques, these authors found that some of these injected cells showed neuronal markers (NeuN 2 and MAP2), astrocytic markers (GFAP), and those of endothelial cells (FVIII).

The effect of intravenous injection of human cord cells has been compared to their administration by intrastriatal infusion in an animal stroke model, to determine which of the two methods produces a better recovery, based on the behavioral test. No differences were found between the two methods 24 h after transplantation. However, two months after transplantation, it was shown that the improvements were associated with intravenous injection. These results suggest that, in the long-term studies, intravenous implantation is more effective than intrastriatal for the functional recovery of experimental animals [28]. In another study, this research group showed that, 24 h after the induction of the experimental stroke, the functional recovery was better when there were a larger number of implanted cells. Measurement of infarct volume showed an inverse relationship between the number of cells and the damaged volume. Overall, these results show that, in an experimental stroke model in rats, the number of human cord blood cells implanted is a determinant factor for obtaining improvement in functional behavior and neuronal preservation [3].

An increase in the ratio of CD45+ / CD11b+ cells was produced in the brains of stroke rats, as compared to that in non-stroke animals. After transplantation of human cord blood cells into these animals, this ratio was reversed and reached values similar to those seen in a healthy brain. These cellular changes were accompanied by a reduction of mRNA expression of nuclear factor-kappa β [29]. These authors demonstrated that, besides modulating the inflammatory response, the transplantation of hUCB cells to ischemic rats produces an increase in neuronal survival through non-immune mechanisms. They suggested that cord blood cells reduce inflammation and induce neuroprotection in stroke treatment in rats.

A large research group in Osaka has shown that systemic administration of CD34+ cells, derived from the hUCB, to mice with induced experimental stroke by the occlusion of the middle cerebral artery, induces neovascularization in the ischemic zone and provides a favorable environment for neuronal regeneration. In contrast, the beneficial effect of the CD34+ cells is lost in the presence of antiangiogenic agents. This is the first study that shows clear evidence of the inter-relationship between vasculogenesis and neurogenesis in the regeneration of ischemic brain lesions [30].

Finally, it appears that the narrow time window for treating stroke patients (3-4 h) could be widened by the use of cord blood cells implants.

In an In vitro study, authors investigated the migration of hUCB to ischemic tissue extracts. After ischemic assault, brain tissue was homogenized, and the supernatants were assayed for their ability to attract hUCB cells. The ability of hUCB cells to migrate towards the ischemic cerebral tissue of rats was maintained for a period of between 24 and 72 hours after the infarct, although at present the mechanisms involved are still unknown [31]. As a disadvantage of this technique, it has been suggested that cell grafting is unlikely to succeed if there is a severe arterial occlusion without collateral circulation; inadequate blood supply would not support graft survival [32]. It should be pointed out that, in contrast to this view, cord blood cells contain endothelial progenitor cells, which could perhaps be useful in proangiogenic therapy for neovascularization [33, 34].

AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron loss. Clinically, it is manifested by progressive muscle weakness that can result in paralysis and death. The multicausality of motor neuron death poses a considerable problem to the development of new therapeutic strategies, including cell therapy. Numerous hypotheses have been put forward about the etiopathogenesis of ALS, but it seems that the one involving the immune system is gaining ground.

In the 1990s, a mouse model of ALS was set up that expresses a large number of copies of the Cu/Zn-Superoxide Dismutase (SOD) gene. It is known that this mutation is one of the causes of familial ALS in humans. In G93A/SOD1 mice, motor degeneration and a loss of neurons in the anterior horn of the spinal cord are manifestations of the expression of this mutation [35, 36].

Using this model, cell therapy experiments with hUCB cells have been carried out, taking advantage of their immunomodulating activity. An increase in the lifespan of in G93A/SOD1 mice has been shown following intravenous infusion of large quantities of hUCB cells (33.2-34.0 x 10^6). The average lifespan of control mice was 123.5 days, while for the mice that received the megadose it was 162 days. In another experimental group, an even larger dose of cord cells was injected (70.2-73.3 x $10^{6)}$, with one animal surviving 180 days and another 210 days. A pool with various donors was established to obtain these large quantities of cells. The authors found human DNA in those animals that survived the longest, while no human DNA was seen in the organs of those that received transplantations, but died at the same time as controls [37]. This same research group published an experimental study in which three groups of GG93/SOD1 mice were compared [38]. The first group was composed of untreated control animals, the second group was irradiated and wild-type bone marrow cells were implanted (5x 10^6), and the third group was irradiated and human blood cord cells implanted (34.2-35.6 x 10^6). They found that the average age at death was: 127 days for the untreated group; 138 days for those that received bone marrow cells; and 148 days for those that received cord cells. The increased survival of the mice that received cord blood cells could be because the implanted cells provided adequate (non-mutant) superoxide dismutase, thereby producing a delay in the onset of symptoms of the disease and death. Another possibility is that the implanted cells promoted an increase in the reconstitution of the irradiated cells of the mice.

In another study, a small dose of cells (10^6) was implanted in G93A/SOD1 mice. The transplanted cells survived a long time after transplantation (10-12 weeks) and were found widely distributed in the brain, spinal cord and in other organs. Most of these cells were found associated to blood vessels, some migrated to the brain and the spinal cord and expressed markers of immature neural cells (nestin), neurons (TuJ-1), and astrocytes (GFAP) [39]. A large number of these cells were found in the spleen differentiating into immune system cells, indicating that they are involved in the immune response. These facts suggest that the beneficial effect of the implantation of hUCB cells is due to a possible peripheral immunomodulatory activity, as the cord blood cells have an immune regulatory function [40].

A group of Polish and German researchers have described intrathecal implantation of human cord cells into G93A/SOD1 mice by injection into the cisterna magna, rather than by intravenous injection. The number of cells injected (10^5) was much smaller than that for intravenous administration. Ten days after transplantation, cells were found in the subarachnoid space, but only a few around the spinal cord. The survival of the mice with implants was no better than that of controls. The authors suggested that the negative results of their study were probably due to the small number of cells implanted [41].

BRAIN AND SPINAL CORD TRAUMAS

Cranial traumas are characterized not only by focal abnormalities, but also by multifocal ones and even by global dysfunction of the cerebral structure. Something similar occurs in the spinal cord. Initially after the trauma, in both brain lesions and those of the spinal cord, cell death by necrosis occurs, followed by death by apoptosis in the areas next to the lesion, due to multiple events such as ischemia and excitotoxicity. Stem cells could participate in cellular and molecular reconstruction in both brain and spinal cord lesions. However, it has not yet been established which cell type is ideal for transplantation. A retrospective study of 70 clinical cases of cranial trauma or paraplegic injury treated with NSC concluded that this therapy produced functional improvements [42].

In another clinical trial, fetal nervous system cells and cells from hematopoietic tissue were implanted in the subarachnoid space of 15 patients with severe lesions of the cervical and dorsal regions of the spinal cord. Six patients improved their neurological condition with an incomplete recovery of the motor and sensitive functions, another five patients showed some muscle contractility and incomplete sensitive recovery, while the remaining four showed no improvement. These results demonstrate the clinical relevance of cell therapy in cases of severe lesions of the spinal cord [43].

An experimental study showed that the implantation of hUCB cells via the systemic route to laboratory rats, subjected to a cranial trauma, reduced the neurological deficiencies of these animals. The implanted cells were widely distributed in the brain and peripheral organs. The cells that migrated to the parenchyma of the lesioned brain expressed neuronal (NeuN and MAP2) and astrocytic (GFAP) markers [44]. These cells have also been implanted in animal models of spinal cord compression injury; the implantations were carried out 24 h or 5 days after the injury. Although, in both cases, there was recovery of motor function, the better results were for those undertaken after 5 days. The transplanted cells were detected in the area of the injured spinal cord; In contrast, they were not detected in uninjured animals that also received these cells [45]. Recently, a Japanese group has reported that CD34+ cells from hUCB improved functional recovery, reduced the area of the cystic cavity at the site of injury, increased the volume of residual white matter, and promoted the regeneration of sparing axons in the injured spinal cord. The cells survived in the host spinal cord at least 3 weeks after transplantation but they disappeared by 5 weeks [46].

In an experimental model of moderate spinal cord injury, grafting hUCB cells into the injured area, induced their differentiation into neurons, oligodendrocytes and astrocytes. Colocalization studies prove that oligodendrocytes derived from cord blood cells secrete neurotrophic hormones neurotrofin-3 (NT3) and brain derived neurotrophic factor (BDNF). Cord blood cells aid in the synthesis of myelin basic protein (MBP) and myelin proteolipid protein (PLP) in the injured area, thereby facilitating the process of remyelinization. In addition, the recovery of hind limb locomotor function was enhanced 14 days after grafting [47]. In another study, this research group has reported that after transplantation of hUCB cells in the injured spinal cord, the expression of Fas and caspases on both neurons and oligodendrocytes was efficiently downregulated. They also showed a functional recovery of hind limbs of lesioned rats [48].

These studies clearly show that hUCB cells injected into the circulatory system are able to migrate to lesioned areas, both in the brain and the spinal cord, surviving several weeks after transplantation, and expressing neural markers. The cell migration mechanism is still unclear. A possible explanation could be that the cells enter into the brain through the disruption of the blood-brain barrier (BBB) due to the lesion, or in response to signals emitted by specific cytokines. It may be possible that the microenvironment produced after the lesion induces these cells to express neural phenotypes.

PARKINSON'S, HUNTINGTON'S AND ALZHEIMER'S DISEASES

To our knowledge, only a few experimental studies have been published in which hUCB cells have been used in the treatment of these fatal diseases. In one of these studies, the authors used 32 (6-12 weeks of age) B6CBACa-AW-J/A-Kcnj6^{wv} transgenic mice for Parkinson's disease (Jackson Laboratory) inoculated with a megadose of hUCB cells. In a first group of 10 animals, 5.6 x 10^6 mouse bone marrow cells were implanted by intravenous administration, a second group of 12 mice was inoculated with 100-110 x 10^6 cord cells, while a third group (10 mice) received no implants (control). When 50% of controls had died, only 10% of those treated with bone marrow cells and 16.6% of those implanted with cord cells had died. At the end of the study (200 days) 90% of the controls and 80% of the mice treated with bone marrow cells had died, but only 66% of those treated with cord cells. These results indicate that although there is a beneficial effect on the survival of these mice when using bone marrow cells, there is a greater one when human umbilical cord cells are used [49].

It has recently been reported that amphetamine-induced rotational behavior was partially improved by cells from Wharton's jelly of the umbilical cord transformed into dopaminergic neurons In vitro, and transplanted into the striatum of rats, which had previously been made parkinsonian by unilateral striatal lesion with 6-OHDA. Four months after transplantation these cells were identified by positive tyrosine hydroxylase (TH) staining and they had migrate 1.4 mm rostrally and caudally [50]. Weiss and colleagues have found that undifferentiated cells derived from human umbilical cord Wharton's jelly, also called cord matrix stem cells, and transplanted into brain of hemiparkinsonian rats without immunosuppression, ameliorate apomorphine-induced rotations. These cells implanted into normal rats did not produce brain tumors, rotational behavior or an immune rejection response [51].

In another study, 71-74 x 10^6 and 100-105 x10^6 of hUCB cells were injected into mice with Huntington's disease (B6CBA-TgN (Hdexon1) 62GPb). The average lifespan of the controls was 88 days and that of the treated mice was 97.8 and 103.4 days, respectively. The implant of hUCB cells increased the life expectancy of mice affected with Huntington's disease. Moreover, the greater the dose of cells the higher was the average lifespan [52].

Finally, these authors carried out a similar experiment using transgenic mice (HuAPP 695.SWE) that overexpress the amyloid precursor protein (APP) of human Alzheimer's disease. Mice were distributed into three groups. A control group, consisting of 9 untreated mice, a second group that received 5.6 x 10^6 of mouse bone marrow cells, and a third group that received 110 x 10^6 of hUCB cells. After 266 days all but two of the control mice had died. Only one of the remaining two animals survived up to 406 days (the end of the experiment). Six of the seven mice that received bone marrow cells died by 168 days, with only one reaching 357 days. However, all the animals treated with cord blood cells were alive after 266 days. One of them died after 357 days, and another at 390 days. The remaining six survived until the end of the experiment (406 days). The survival curves of controls and of the mice treated with bone marrow cells were similar. In contrast, the survival curve for the mice treated with hUCB cells showed a significant difference as compared with controls (P=0.001) [53]. The authors are aware that these results only open the floodgates to a sea of questions and new experiments to try to understand why umbilical cord blood cells are capable of improving the life expectancy of mice with neurodegenerative syndromes. Indeed, they indicate that it is necessary to undertake additional studies to know if these findings have a potential clinical significance.

CONCLUSION

Stem cells with the ability to self-renew and to differentiate into any type of specialized cell have been proposed for cell replace-

ment therapies. This review has focused on umbilical cord blood stem cells with a high tolerance to HLA, which is advantageous in transplantation because it lowers graft rejection, resulting in a increase of pool of donors.

The most important characteristics that any source of stem cells must fulfill for cell replacement therapies are:

1) The stem cell pool has to be easily accessible.
2) A sufficient quantity of stem cells must be available.
3) The transplanted cells must be well-tolerated by the host.
4) The differentiation of the cells and their ability to graft on to damaged tissue must be sufficient to regenerate the lesioned organ.

Human umbilical cord blood cells easily comply with the first requirement, as accessibility is total, as well as rapid and without danger to the mother or the neonate. Cells taken from placenta blood and from that of the umbilical cord after birth can, after undergoing HLA typing, be cryopreserved frozen for many years. We need to keep in mind that these cells were routinely placed into biohazardous waste after birth.

The second requirement, accessibility, is perhaps a critical one for this type of cell, although as pointed-out earlier collection methods are improving. Moreover, it should be stressed that a large number of experiments have been carried out successfully to expand the *in vitro* supply of these cells without losing their stem potential.

One of the biggest challenges to cell therapy is the ability of the host to tolerate the engraftment, while another one is to limit as much as possible the risk of immunological rejection. In this context, umbilical cord blood cells are perhaps the most permissive hematopoietic cells, as being more immature they present less risk of rejection.

The capability of homing and implanting themselves into the tissue is essential for targeting the cells towards the damaged organ. The damaged tissue has to send out specific signals to attract the cells towards the lesioned area. Various cytokines are responsible for this cell traffic. Umbilical cord blood cells are relatively ineffective at expressing these homing cytokines. Various ex vivo manipulations have been used to reverse this disadvantageous transmigratory behavior; microparticles have been used to which cell adhesion molecules have been incorporated, or recombinant human stem cell factor has been added. These strategies have improved the transmigratory behavior of cord cells.

In short, hUCB cells are easily accessible by non-invasive means, In vitro amplifiable, well tolerated by the host and possess primitive molecular characteristics that give them considerable flexibility.

For the possible use of hUCB cells in therapies for CNS regeneration, we should look at the still rather scarce research undertaken, although these cells already show potential for contributing to the repair of damaged organs. As mentioned in this review, cord cells, both In vivo and In vitro, have demonstrated a high degree of plasticity and can differentiate into blood cells, immune cells or even neural ones. Systemic administration of these cells to experimental animals models for different neurological diseases, improves their neurological symptoms and life expectancy. Implanted cord blood cells migrate, live for long periods and induce the expression of various neural markers in the host tissues. Interestingly, it seems that the beneficial effects of these cells derive from their roles as neuroprotectors and/ or releasers of antiinflammatory or trophic factors, rather than having a role in neuronal replacement. One of the aspects not covered in the various studies reviewed here is the possible teratogenic potential of these cells in host tissue, perhaps because the study periods of the experiments have not been long enough as yet. Moreover, the molecular and cellular mechanisms of action of these cells still have to be explained.

The overall conclusion of this review article is that the preliminary studies with umbilical cord blood cells, both *in vivo* and *in vitro*, have opened up an incredible field of research, as well as the possibility of their clinical use in the treatment of neurodegenerative diseases. Therefore, the number of human cord blood cell banks should increase substantially in the near future.

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