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Review

Bone marrow stromal cells for spinal cord repair: A challenge for contemporary neurobiology

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Summary. In the last years, it has been reported that bone marrow stromal cells (BMSC) are able to differentiate towards a neuronal phenotype, *in vitro* as well as *in vivo*, and consequently, the possible use of these cells for the treatment of neurological diseases has acquired enormous importance. The objective of this review is to discuss the experimental findings that suggested the utility of BMSC for the treatment of paraplegia, and the possibilities of its clinical application in patients. For this reason, we revise our previous experimental findings about neuronal transdifferentiation of BMSC, and the utility of local BMSC transplantation in an experimental model of chronic paraplegia. Our current experience supports that a neural transdifferentiation of BMSC is possible after these mesenchymal stem cells are transplanted into injured spinal cord tissue. Furthermore, this cell therapy achieves a clear functional improvement of paraplegic animals, together with morphological evidence of spinal cord regeneration. Although at present our efforts should be guided to obtain a better knowledge of the mechanisms of nervous regeneration induced by bone-marrow derived stem cells, it is obvious that cell therapy for nervous system repair is beginning, and BMSC transplantation offers new hope for the treatment of traumatic paraplegia in humans.

Key words: Neural repair, Stem cells, Spinal cord, Bone marrow stromal cells, Paraplegia

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Introduction

The recent discoveries relating to the biological properties of adult mesenchymal stem cells offer a challenge to accepted biological concepts. After the first descriptions suggesting that adult mesenchymal stem cells obtained from bone marrow can be transformed into neuron-like cells by means of specific chemical agents (Mezey and Chandross, 2000; Sánchez-Ramos et al., 2000; Woodbury et al., 2000; Hung et al., 2002; Dezawa et al., 2004; Hermann et al., 2004; Bossolasco et al., 2005) diverse authors studied the possibility of applying this discovery to the treatment of traumatic paraplegia. As a consequence, in the last years growing evidence has suggested that intramedullary transplantation of bone marrow stromal cells (BMSC) can achieve functional recovery of animals suffering spinal cord injury (SCI) (Chopp et al., 2000; Chopp and Li, 2002; Hofstetter et al., 2002; Lee et al., 2003; Ankeny et al., 2004).

Although the first experimental studies were performed on models of incomplete spinal cord lesions, or by performing intralesional BMSC immediately or one week after trauma, in 2004 we described, for the first time, that this type of cell therapy promotes functional recovery when it is used in animals with complete spinal cord lesion, and in chronic phase (Zurita and Vaquero, 2004). In these experimental conditions, progressive functional motor recovery is achieved in paraplegic adult rats over the course of one year, following the intramedullary administration of BMSC, and this recovery was associated with nervous tissue regeneration in the previously injured spinal cord (Zurita and Vaquero, 2006).

Characteristics of BMSC

Stem cells are undifferentiated cells that retain the ability to divide throughout life and give rise to cells that

can become highly specialized and take the place of cells that die or are lost. In the last years, the use of embryonic stem cells has generated great optimism for therapy of human diseases, but their clinical use remains controversial for ethical and technical reasons, among them the difficulty for their obtaining and the risk of tumor formation. In contrast, adult stem cells have gained importance after the knowledge of the biological properties of BMSC. These cells are multipotent adult stem cells located in bone marrow that have proved capable of differentiation, not only into different mesenchymal cells, such as osteoblasts, condrocytes and myocytes, but also into endothelial and neuroectodermal cells (Dezawa et al., 2004; Kotobuki et al., 2004; Benayahu et al., 2007; Parr et al., 2007). BMSC expresses CD73 and CD105 markers, and also other surface markers which in fact represent molecules of adhesion, such as CD54, CD56, CD90 and CD106. They expand in culture up to sixfold and their biological functions are not altered by ageing.

The controversy about neural transdifferentiation of BMSC

In the last years, several reports have suggested that BMSC could differentiate into neurons and glia *in vitro* and after they are transplanted into the brain and spinal cord (Chopp et al., 2000; Mezey and Chandross, 2000; Sánchez-Ramos et al., 2000; Woodbury et al., 2000; Hofstetter et al, 2002; Hung et al., 2002; Lee et al., 2003; Jendelová et al., 2003; Ankeny et al., 2004; Dezawa et al., 2004; Hermann et al., 2004; Bossolasco et al., 2005; Zurita et al., 2005, 2007a,b, 2008). This finding has been called "transdifferentiation" because it involves the differentiation of a mesenchymal cell towards a cell of different germinal layer, and it represents a new biological concept with possible clinical application. Evidence that BMSC could be rapidly induced to differentiate into neurons by using simple chemical agents was first reported by Woodbury et al. (2000) but questioned by other authors, suggesting that chemical neuronal induction results in cellular stress, leading to the physical contraction of cells into a neuron-like morphology (Liu and Rao, 2003; Lu et al., 2004). Moreover, the possibility that cell fusion originates morphological images, misinterpreted as cell transdifferentiation, has been suggested by diverse authors (Wang et al., 2002; Ying et al., 2002; Alvarez-Dolado et al., 2003; Chen et al., 2006). On the other hand, when neuronal transdifferentiation of BMSC is considered for nervous system repair, manipulation of these cells using chemical agents previously to transplantation procedures outlines diverse questions, such as the possibility of altering the cell genome, or the necessity of maintaining the exposure of BMSC to chemical agents after transplantation. In order to acquire a better knowledge of the neural BMSC trans-differentiation by chemical agents, we differentiated BMSC towards neuron-like cells using the method described by Woodbury et al. (2000), and we studied the effect of the removal of the chemical inducers once the BMSC achieved a neuronal morphology (Zurita et al, 2008). Our results showed that one hour after BMSC were treated by neuronal induction medium containing specific chemical agents, some cells showed a rounded morphology, mixed with cells showing a typical mesenchymal morphology. At 4 hours, most BMSC expressed nestin and a great number of them showed a typical neuronal aspect, with multipolar processes. At 24 hours, most cultured cells showed neuronal aspect (Fig. 1) and the percentage of them showing neuronal markers ranged between 40% and 60%. At 72 hours, practically all BMSC showed neuronal morphology, and the percentage of cells expressing NF-200 was around 80%. Nevertheless, if we replaced the neuronal induction medium by standard alpha-MEM/10% FBS medium, BMSC quickly reversed towards to typical mesenchymal morphology, losing the expression of neuronal markers, and expressing mesenchymal surface markers again, such as CD73 and CD105. These studies confirmed that BMSC can achieve morphological and immunocytochemical features of adult neurons when they are cultured in the presence of specific chemical agents, but the finding that phenotypic modifications of

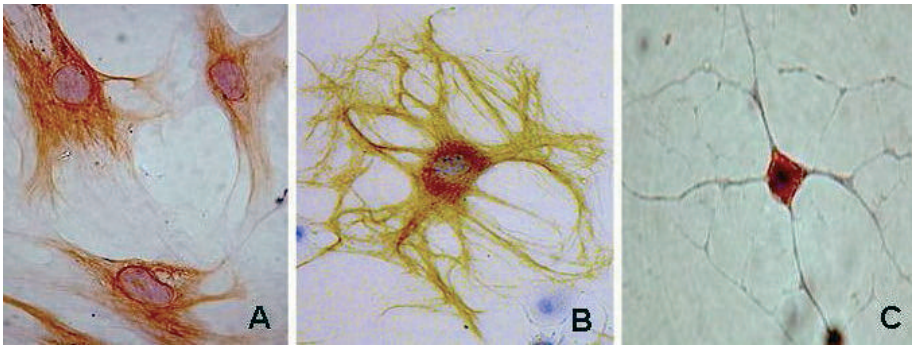

Fig. 1. Progressive transdifferentiation of BMSC to neuron-like cells. **A.** Cultured BMSC showing typical mesenchymal morphology and vimentin expression. **B.** Morphological changes take place 8 hours after BMSC were cultured in the presence of specific induction medium, containing dimethylsulphoxide (DMSO), butylated hydroxy-anisole (BHA), valproic acid, forskolin, hydrocortisone, and insulin. In this image, one BMSC showing morphological changes towards a neuronal phenotype can be seen. **C.** At 24 hours, practically all BMSC showed a typical neuronal morphology. This image shows a transdifferentiated cell. Cells in A were immunostained for demonstration of vimentin (x200). Cells in B and C were immunostained for demonstration of NSE. x 200

BMSC revert after chemical agents are taken out of the culture medium suggested that morphological changes obtained by chemical factors can be the result of incomplete transdifferentiation. Therefore, chemical BMSC transdifferentiation is a reversible phenomenon, doubtfully useful in clinical protocols of cell therapy (Zurita et al., 2008).

In order to obtain a better method of neuronal BMSC transdifferentiation, we studied the effect of co-culturing BMSC and glial cells, demonstrating that BMSC can obtain permanent neuronal features if they were cultured in the presence of Schwann cells (Zurita et al., 2005). This finding suggested that neurotrophic glial factors released by Schwann cells can act on BMSC achieving transdifferentiation. Moreover, Schwann cells have been shown to facilitate axonal regeneration, a process that depends on secreted neurotrophic factors, extracellular matrix and cell adhesion molecules (Bunge, 1991; Yamamoto et al., 1993; Weidner et al., 1999).

Previous studies by us showed that BMSC can be transdifferentiated *in vitro* in the presence of Brain-Derived Neurotrophic Factor (BDNF) or Nervous Growth Factor (NGF) (Zurita et al., 2007a), and bearing in mind that adult Schwann cells and astroglial cells can release these neurotrophic factors (Lin et al., 2006) it is possible to accept that neuronal transdifferentiation of BMSC can be obtained *in vivo* when they are transplanted into nervous tissue, because of the presence of environmental neurotrophic factors. Therefore, when these cells are used for nervous system repair, BMSC transdifferentiation in vitro is not necessary as a first step previous to transplantation.

On the other hand, in our co-cultures, cell fusion cannot be excluded as cause of apparent BMSC transdifferentiation (Wang et al., 2002; Ying et al., 2002; Alvarez-Dolado et al., 2003; Chen et al., 2006), and for this reason, in a second step we used a simple method of co-culturing BMSC and Schwann cells using transwell culture dishes with polycarbonate membrane (Zurita et al., 2007b). These culture dishes (Nalgene Nunc., International Corp., Rochester, USA) are commonly used to produce a cell culture environment that closely resembles the in vivo state. The porous membrane of the insert is optically opaque, and we used membranes with a 0.4 µm pore size. In these experimental conditions, the small pore size, relative to the size of the BMSC body (approximately 15-20 µm), inhibits the migration of BMSC into the lower chamber. Schwann cells were cultured in the well, and BMSC were cultured on the permeable membrane support, so that both types of cells were exposed to the same culture media conditions, without cell contact. In the course of the following two weeks, morphology and immunocytochemistry of the cultured BMSC were observed at different time points. In these experimental conditions, the percentages of BMSC showing neuronal differentiation, at different times of co-culture, were similar to those obtained when BMSC and Schwann cells were in contact. Thus, the possibility that cell fusion can originate an apparent neuronal transdifferentiation should not be considered (Figs. 2, 3). In any case, our co-cultures confirmed that neurotrophic factors provided by glial cells induce neuronal BMSC transdifferentiation without the necessity for BMSC to be treated with exogenous chemical agents.

Furthermore, with the purpose of discovering whether soluble neurotrophic factors can induce permanent BMSC transdifferentiation, we studied the effect of eliminating Schwann cells in our co-cultures

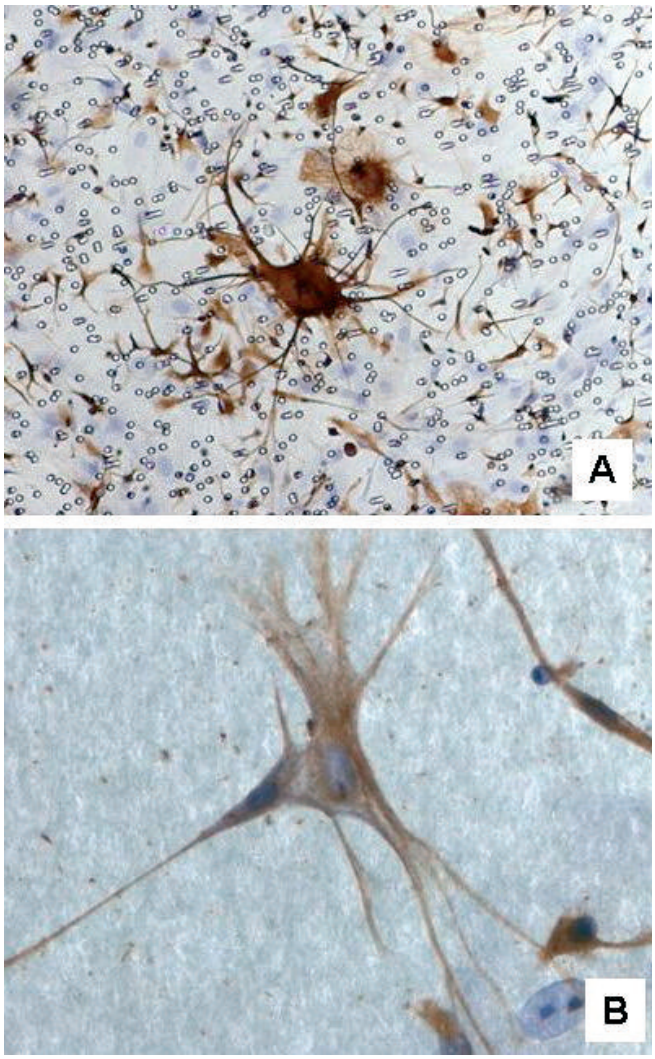

Fig. 2. BMSC co-cultured with Schwann cells, using transwell culture dishes with polycarbonate membranes. Schwann cells were cultured in the well, and BMSCs were cultured on the permeable membrane support, so that both types of cells were exposed to the same culture media conditions, without cell contact. **A and B.** BMSC transdifferentiating to neuron-like cells and expressing ß-III-tubulin. The dirty aspect of the microscopical fields is due to the polycarbonate membranes. In **A**, the pores of the membrane sized 8 µm. In **B**, the pores sized 0.4 µm, preventing contact between BMSC and Schwann cells. x 200

after BMSC showed clear morphological and immunocytochemical evidence of neural trans-differentiation. Consequently, at two weeks of co-culture, the porous polycarbonate membranes separating BMSC and Schwann cells were removed, and BMSC were transferred to Petri dishes containing standard alpha-MEM/10% FBS medium. After this, BMSC maintained a typical neuronal morphology, and the expression of neuronal proteins can be confirmed by RT-PCR analysis. Therefore, as opposed to what happens in chemical transdifferentiation, the BMSC trans-differentiation induced by glial neurotrophic factors seems to represent a biological phenomenon that remains stable after it has been reached. These findings supported the possibility that neuronal differentiation of BMSC can be obtained *in vivo* because of the presence of environmental factors, suggesting that in cell therapy using BMSC for nervous system repair, protocols to obtain neural differentiation of these cells prior to the transplantation procedures are not required.

Intramedullary transplantation of bone marrow stromal cells for spinal cord repair

In the last years, diverse experimental studies have reported the effectiveness of intralesional BMSC transplantation for spinal cord repair, and simultaneously, data suggesting that BMSC transplantation reduces functional deficits after stroke or traumatic injury in adult rats have been reported (Chen et al., 2001; Chopp and Li, 2002; Mahmood et al., 2001, 2002, 2004; Urdzíková et al., 2006).

Nevertheless, the preliminary studies about BMSC transplantation for spinal cord repair were carried out using models of spinal cord injury that do not cause irreversible paraplegia. In addition, intralesional transplantations of BMSC were performed immediately, or one week after trauma (Chopp et al., 2000; Hofstetter et al., 2002).

Bearing in mind that the possible clinical application of cell therapy in humans would imply its use in situations of complete paraplegia, and only when functional deficit could be considered as chronically established, we studied the therapeutic effect of intralesional BMSC transplantation in chronic paraplegic rats. For this, adult female Wistar rats were subjected to severe contusive SCI using a trauma model that causes immediate paraplegia. A necrotic central cord lesion, extending over 1 or 2 spinal cord segments, is always observed one week after injury. Three months after contusion, $5x10^6$ BMSC obtained from adult male donor Wistar rats, in saline solution, for a total volume of 50 µl, were injected into the traumatic central cord cavity of rats with chronic paraplegia, using a microinjection pump, at a rate of 1 µl/min. In control animals, 50 µl of saline solution without BMSC were injected into the central cord cavity (Zurita and Vaquero, 2004, 2006). All the animals underwent daily rehabilitation from the time of injury until they were sacrificed, and behavioral testing was performed weekly in both hindlimbs by an examiner, who was blinded to the group to which each animal belonged, and using the Basso-Beatie-Bresnahan

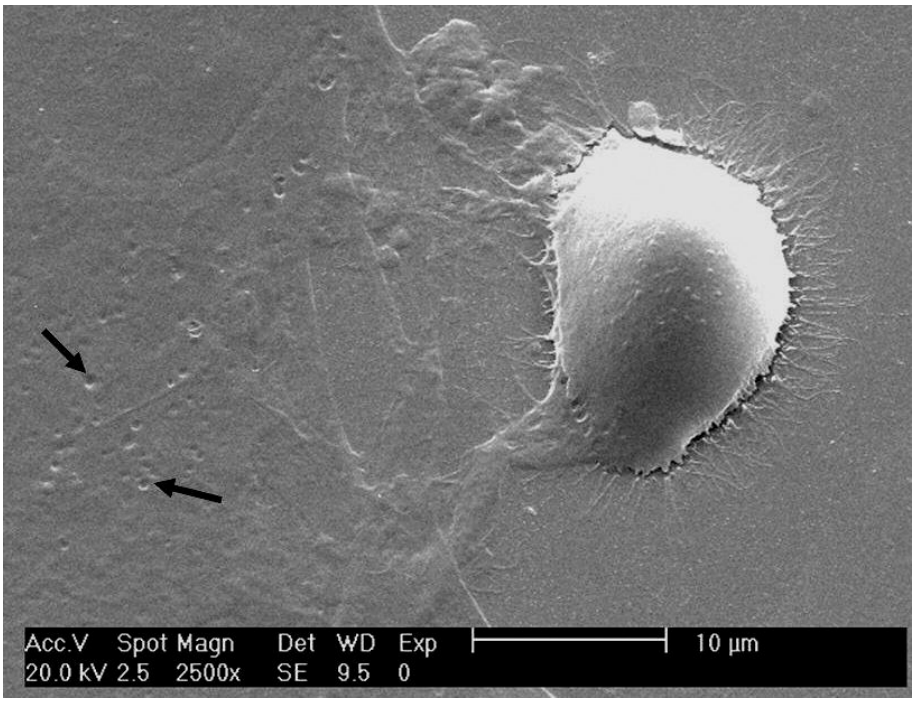

Fig. 3. Image of environmental scanning electron microscopy, showing one BMSC cultured on the permeable membrane support. In this case, a membrane with a 0.4 µm pore size (arrows) was used.

(BBB) scale (Basso et al., 1995). While the animals with intralesional administration of saline alone remained completely paraplegic throughout the entire study, the animals subjected to intralesional injection of BMSC showed an evident and progressive recovery from their paraplegia, starting a few weeks after this procedure. Ten months after treatment, the treated rats had a mean score (± standard deviation) on the BBB scale of 17.1±1.1 (the maximum score in this scale, corresponding to an animal without motor deficits, is 21). From that time on, BBB scores tended to stabilize, and the mean BBB score after 12 months of follow-up was 17.7±1.3 (movies from our original publication can be seen at doi: 10.1016/j.neulet.2006.03.069). This experiment was repeated in different series of animals and the results overlapped completely. In order to determine whether the functional recovery obtained was associated with regeneration of the previously injured spinal cord due to the administration of BMSC, the cells were labeled by retroviral transfection, and for this purpose a retroviral construct encoding ß-galactosidase (Lac-Z) as an expression marker (Retroviral vector pRV LacZ) was used. Two and six months after BMSC administration, animals from the treated group were sacrificed for pathological studies, and the remaining animals were studied morphologically at the end of the follow-up, one year after treatment. Pathological studies showed that two months after intralesional administration of BMSC, tissue bundles partially bridging the central cord cavity could be seen (Fig. 4). These bundles were formed mainly by BMSC, according to the labeling with ß-galactosidase, and cells expressing neuronal and glial markers were identified within the central cord cavity, among NF-positive fibers. Six months after treatment, compact islands of nervous tissue formed by ß-galactosidase-positive cells bridged the central cord cavity, and morphological images supported a transdifferentiation of these cells into neuronal and glial cells. Twelve months after BMSC therapy, the pathological study of spinal cord from previously paraplegic rats showed a regenerated nervous tissue filling the previously well-identified central cord cavity (Fig. 5). In this tissue, ß-galactosidase expression was detected in neurons, astrocytes and oligodendrocytes, and in cells present in the vascular walls. Thus, it is obvious that these cells derived from the previously injected BMSC (Fig. 6). In our material, we used immunohistochemical labeling of axons expressing neurofilament protein (NFP), calcitonin gene-related peptide (CGRP), tyrosine-hydroxylase, serotonin and dopamine beta-hydroxylase, observing that all those

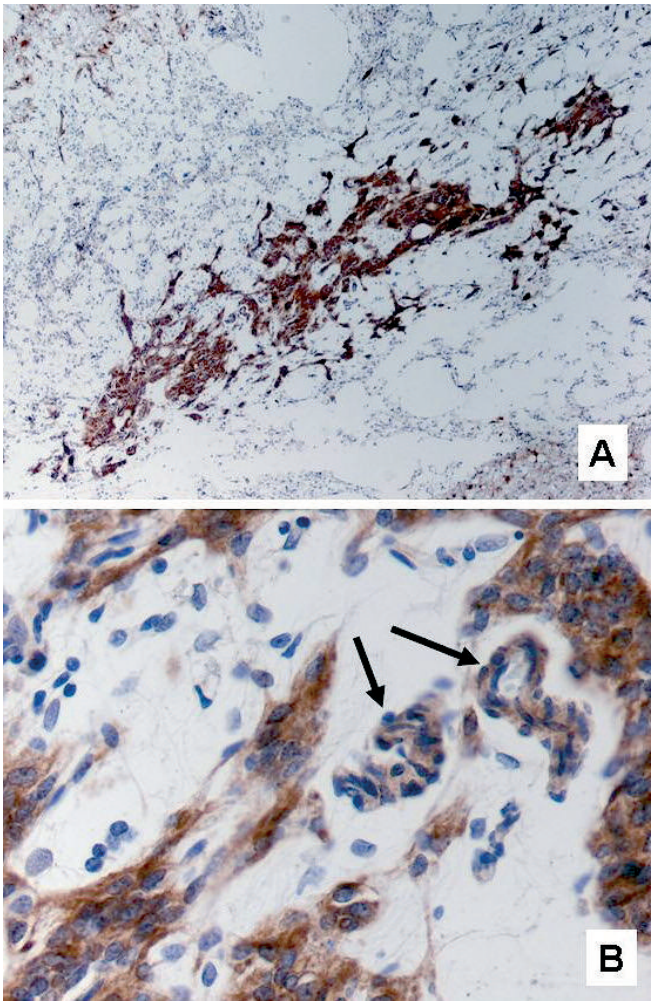

Fig. 4. A, B. Immunohistochemical demonstration of ß-galactosidase. These images show the intramedullary cavity of previously paraplegic rats, two months after local administration of BMSC. At this time, immunostained tissue bundles can be identified in the traumatic central cord cavity. Vessels showing ß-galactosidase expression in their walls can be seen (arrows). A, x 100; B x 200

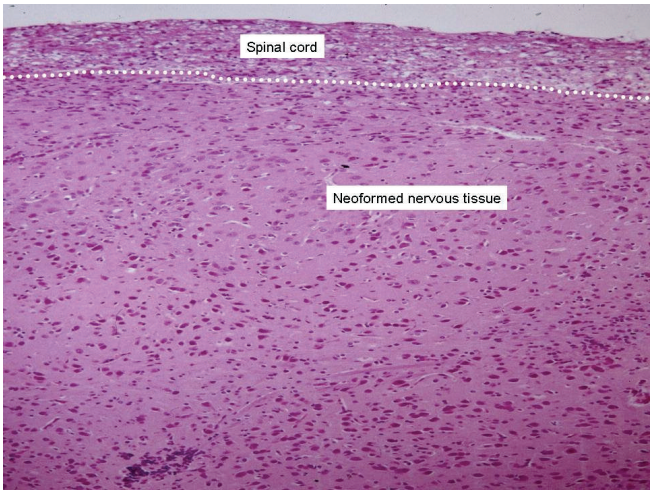

Fig. 5. Histological aspect of the regenerated nervous tissue filling the centromedullary cavity of transplanted rats, one year after the procedure. HE, x 100

axons could be identified in the regenerated spinal cord tissue, among neuronal and glial cells expressing ß-galactosidase. Thus, the newly formed nervous tissue acted as a bridge for the passage of coeruleo-spinal adrenergic descending axons (tyrosine hydroxylase-positive and dopamine beta-hydroxylase-positive), for descending serotoninergic axons of raphe neurons, and for ascending sensory axons (CGRP-positive) (Fig. 7). Furthermore, we injected biotin-dextran-amine (BDA) into the motor cortex of rats that showed a clear recovery of motor function one year after intralesional BMSC administration, and three weeks later, BDA-positive axons were identified in the neoformed nervous tissue and in posterior tracts, in spinal cord segments below the SCI, while in paraplegic animals that had not received BMSC, BDA-positive fibers were identified proximal to the lesion, but in no case in distal segments. These findings confirmed that transplantation of BMSC into injured spinal cord tissue from rats with chronic paraplegia promotes a clear functional recovery starting a few weeks after the procedure, which increases over the following months and stabilizes approximately at one year of follow-up (Zurita and Vaquero, 2006). At this time, the BMSC had regenerated the previously injured spinal cord, forming new nervous tissue, as demonstrated by the presence of neural cells and vessels derived from the injected BMSC, and permitting the passage of descending and ascending axons.

Local versus systemic administration

After we were able to confirm the effectiveness of the intramedullary administration of BMSC for functional recovery, and bearing in mind studies reporting that intravenous administration of BMSC achieves functional recovery after traumatic brain injury (Mahmood et al., 2001), we studied whether BMSC can achieve functional recovery of paraplegic rats after systemic administration. As a first step we searched the spinal cord colonization of marked BMSC after

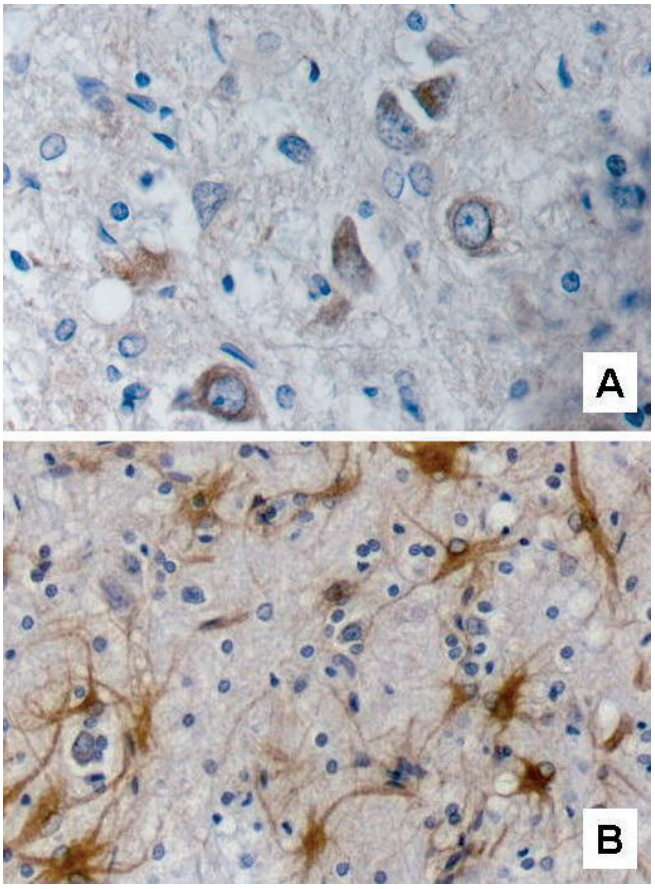

Fig. 6. A. Mature neurons showing positivity to ß-galactosidase can be seen within the neoformed tissue, one year after transplantation. **B.** Astroglial cells showing ß-galactosidase positivity can be seen. x 200

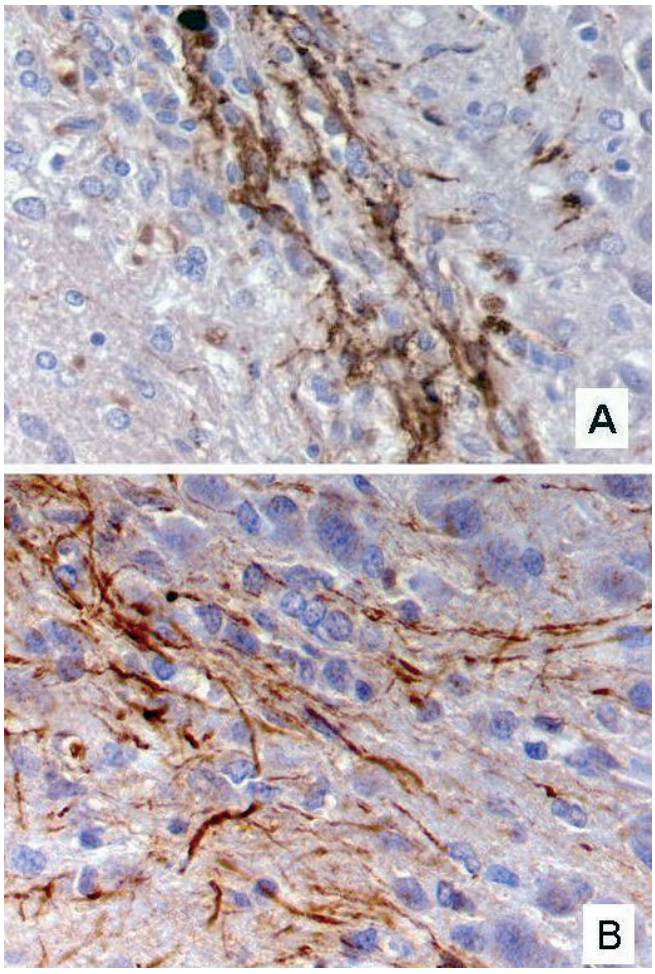

Fig. 7. Ascendent and descendent axons were identified in the regenerated nervous tissue, one year after intralesional administration of BMSC. **A.** CGRP-positive sensory fibers. **B.** Descending tyrosine hydroxylase-positive fibers. x 200

intravenous administration. In these studies we used bisbenzimide-labeled-BMSC or ^{111}In-oxine-labeled-BMSC (De Haro et al., 2005). After intravenous administration of ^{111}In-oxine-labeled-BMSC, gammagraphic images showed that the activity distributed all over the organism, but in the spinal cord only scarce activity was identified. When ^{111}In-oxine-labeled-BMSC were injected within the traumatic centromedullary cavity of paraplegic animals, the gammagraphic images showed persistent activity in the lesion zone, without any activity migrating to the rest of the organism, at least during the whole time of the study (10 days after transplantation procedure). Our results demonstrated for the first time the utility of ^{111}In-labeling to find out the permanency and distribution of BMSC after transplantation procedures, a finding with future potential application in humans, and suggested the convenience of the intralesional administration of BMSC, instead of the intravenous administration, when cell therapy is considered for the treatment of chronic traumatic paraplegia.

As a second step, we compared the effect of systemic and local administration of BMSC in adult Wistar rats suffering chronic paraplegia (Vaquero et al., 2006). Adult Wistar rats were subjected to a weight-drop impact causing complete paraplegia, and three months later, when all the animals remained without signs of functional recovery, bisbenzimide-labeled-BMSC were injected intravenously or into traumatic spinal cord cavity. The outcome was evaluated until sacrifice of the animals, six months later, using the BBB score, the cold spray test, and measuring the thigh perimeter. After sacrifice, samples of spinal cord tissue were studied histologically. The results showed that intravenous administration of BMSC achieves some degree of functional recovery when compared to controls, but administration of BMSC into postraumatic spinal cord cavity promotes a clear and progressive functional recovery, significantly superior to the recovery obtained by means of the intravenous administration. When the previously injured spinal cord was histologically studied, paraplegic rats that received BMSC intravenously, or saline without BMSC, and paraplegic rats that received intralesional saline without BMSC, showed the typical histological images associated to severe spinal cord injury, such as the presence of a cystic cavity, occasionally with debris and macrophages. At this level, in some rats that received intravenous BMSC, isolated cells showing a bisbenzimide-labeled nucleus were identified by fluorescence microscopy. On the contrary, in paraplegic rats that received intralesional BMSC, the cystic spinal cord cavity was partially filled and bridged by tissue bundles, in those which NFP-positive fibers were identified. Hematoxylin-eosin staining disclosed a population of cells with rounded or elongated nuclei, distributed throughout the partially filled cavity, and in this zone, fluorescence microscopy showed a variable number of cells containing a bisbenzimide-labeled nucleus, thus supporting the long-term presence of bisbenzimide-marked BMSC after intralesional administration. After these studies, we confirmed that local administration of BMSC into postraumatic spinal cord cavity from paraplegic rats promotes a clear and significant functional recovery, as measured on the BBB

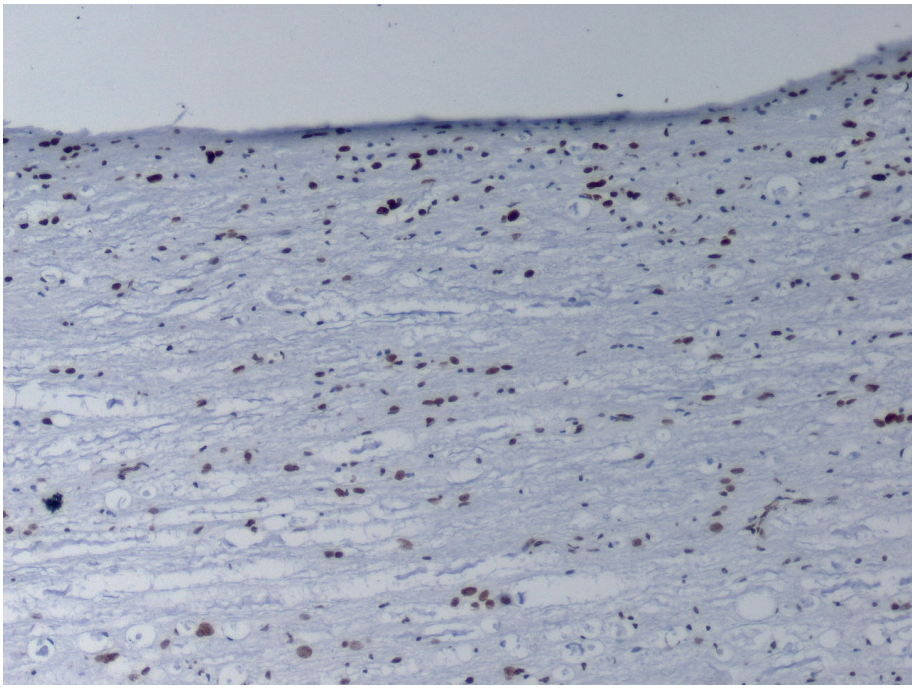

Fig. 8. Immunohistochemical demonstration of bromo-uridine marked BMSC, three months after they were injected into the subarachnoid space of one adult paraplegic pig. Immunostained cells penetrating the spinal cord parenchyma can be seen. x 100

scale and the cold spray test. The functional recovery already begins two weeks after BMSC administration and it is progressive, at least during the six months of follow-up after the procedure. Furthermore, this procedure promotes a progressive and significant increase in the leg muscle mass, which was previously decreased as consequence of SCI, when compared to controls. On the other hand, the functional recovery obtained after intravenous BMSC administration was poor. It was significant when compared to controls, but smaller than after intralesional administration. Therefore, we concluded that intravenous administration of BMSC should not be considered for spinal cord repair, because this route of administration does not achieves very significant effectiveness, at least when compared to intralesional administration (Vaquero et al., 2006). Nevertheless, these results have been obtained on a model of chronic paraplegia, and the possibility that intravenous BMSC administration can lead to significant positive effects in a phase acute after trauma, at a time when the blood-brain barrier is open, should be considered.

Perspectives for transplantation of bone marrow-derived stem cells in paraplegic patients

At present, the beneficial results obtained in paraplegic rodents after transplantation of bone marrow-derived stem cells seem to be reproducible, and this therapeutic effectiveness has been confirmed in primates (Deng et al., 2005, 2006). Consequently, clinical application of this type of cell therapy in paraplegic patients is beginning, and preliminary studies confirmed that it is safe and promising (Park et al., 2005; Syková et al., 2006; Yoon et al., 2007). Recently, Geffner et al. (unpublished data) presented at 2007 Congress of Neurological Surgeons Annual Meeting in San Diego, CA, a preliminary study with 38 paraplegic patients treated with transplantation of autologous bone marrow-derived stem cells, obtaining clear improvements in sensitivity, motility and sphincters, after one year of follow-up. Based on this evidence, enough justification exists to apply this therapy in humans, but it is obvious that, at present, trials involving a large enough population of patients are needed before further conclusions can be drawn.

At present, it is difficult to find arguments favouring the superiority of BMSC, or bone marrow containing hematopoietic stem cells, for spinal cord repair. Nevertheless, finding out the most useful type of bone marrow-derived stem cell is a question that should be resolved. Although our studies and those of other authors showed a clear effectiveness of BMSC (Chopp et al., 2000; Hofstetter et al., 2002; Bakshi et al., 2004; Ohta et al., 2004; Zurita and Vaquero, 2004, 2006; Himes et al., 2006; Vaquero et al., 2006; Parr et al., 2007) other authors described functional improvement after SCI using a population of bone marrow-derived stem cells, including hematopoietic progenitors (Park et al., 2005; Syková et al., 2006; Urdzíková et al., 2006; Yoon et al., 2007).

Because BMSC represent less than 1% of bone marrow, their use in protocols of cell therapy requires manipulation of these cells *in vitro*, in order to obtain a sufficient number of them for trasplantation procedures. This adds a technical complexity and the need for appropriate installations for the handling of the bone marrow-extracted cells when these protocols are used in patients. On the other hand, the use of BMSC offers the advantage of its low antigenicity (Le Blanc and Ringdén, 2005), an important factor for experimental studies in rodents, due to the impossibility of obtaining a sufficient number of autologous stem cells in these animals, and additionally, this low antigenicity could allow us to allogeneic transplantation in humans. Furthermore, BMSC expand easily in culture, and this property allows a greater number of stem cells to be obtained for transplantation procedures, compared to the use of whole bone marrow containing hematopoietic stem cells. It is an important factor, because our experimental results in chronic paraplegic pigs suggested that the effectiveness of the autologous transplantation procedures using BMSC is dose dependent (unpublished data).

On the other hand, we must still obtain a better knowledge of the mechanisms by which bone marrow stem cells carry out their beneficial effects, because our experimental results showed that functional recovery can be observed in a precocious phase after transplantation, when there is still a lack images showing a complete filler of the centromedullary cavities. Thus, it is obvious that diverse mechanisms of recovery may play a role after transplantation, including expression of neurotrophic factors, or activation of endogenous spinal cord mechanisms able to restore neurological functions previously suppressed.

Lastly, our experimental studies point to the potential advantage that significant numbers of BMSC remain in close proximity to the transplantation site, thus future investigations should focus on strategies to maximize cell survival in this location. Recent experimental studies showed that bone marrow-derived stem cells can arrive at the injured spinal cord tissue after being injected into the subarachnoid space (Bakshi et al., 2004, 2006; Ohta et al., 2004; Sateke et al., 2004; Himes et al., 2006) (Fig. 8), and these observations should be considered in humans, since this strategy could be complementary to the intralesional stem cells administration, increasing its effectiveness.

Conclusions

In the last years, the possibilities of cell therapy using adult stem cells, and the current concepts about the regeneration of the nervous system, offer new hope for the treatment of traumatic paraplegia, and simultaneously, a new challenge for neural repair. Based on reproducible experimental studies, the clinical application of cell therapies is beginning. Nevertheless,

the advances in this field, like in many others of neurobiology, will require a close collaboration among basic and clinical investigators to be able to apply to humans, under strict ethical and methodological controls, the potentiality that adult stem cells can provide. At present, our efforts should be guided to obtain a better knowledge of the mechanisms of nervous regeneration induced by these new techniques, which are already profiled as one of the most spectacular advances in Medicine of the XXI century.

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