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Review

Concepts of cell transplantation for spinal cord injury

—— a short overview ——

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Abstract

In this short overview, I want to focus on two different standpoints in terms of cell transplantation therapy for spinal cord regeneration. In general, cell transplantation is performed with the purpose to supply new tissue components to the lesion. In spinal cord injury, cell transplantation has been performed to supply new neurons or glial cells including Schwann cells into the lesion. During a study on the transplantation of bone marrow stromal cells through the cerebrospinal fluid (CSF) to the spinal cord, we noted that transplanted bone marrow stromal cells (BMSCs) were not integrated into the spinal cord tissue, but disappeared from the spinal cord within 3–4 weeks after transplantation, whereas, the effects of BMSCs were remarkable in terms of the tissue repair as well as behavioral recovery. In vitro studies have shown that BMSCs secrete trophic substances into the culture medium, facilitating the survival of neurons and extension of neurites. It was suggested that transplanted BMSCs exerted their effects by releasing trophic factors into the cerebrospinal fluid, which probably rescued neurons and glial cells in the lesion from degeneration. The volume of cavities in the spinal cord was much smaller, and surviving axonal density was much higher than in the control spinal cord. The behavioral recovery was distinct as compared with the control. These in vivo and in vitro studies indicate that BMSCs exert their effects by releasing some trophic factors that can be conveyed through the cerebrospinal fluid to the injured tissue.

These studies show a new concept of cell transplantation differing from the traditional one which has historically held that the transplanted cells should be incorporated into, and/or differentiate into, the relevant cells within the lesion

Key words: cell transplantation, bone marrow stromal cell, spinal cord regeneration, cavity formation, behavioral assessment

1. Early periods of cell transplantation study

Cajal (1928) extensively studied nerve regeneration using the silver impregnation method, and described important findings in the monograph entitled "Degeneration and Regeneration of the Nervous System". In this monograph, Cajal describes the precise morphology of nerve regeneration in both the peripheral (PNS) and central nervous system (CNS), which forms the basis of contemporary neuroscience including nerve regeneration.

Cajal showed that there is a distinct axonal sprouting in the injured spinal cord, but unfortunately, those sprouts cannot extend further through the lesion into spinal cord tissue distal to the lesion. This finding explains the basic phenomenon concerning failed nerve regeneration in the CNS including the spinal cord. In fact, there is no tissue repair including axonal extension in the lesion, resulting in cavity formation of various sizes in the chronic stages of the CNS lesion. The reason for the failed repair of the CNS lesion has not been clarified, and still remains as a crucial point in terms of achieving successful nerve regeneration in the CNS.

Even in the early days of Cajal, tissue transplantation was being performed in an attempt to make the axonal sprouts grow over a longer distance (Tello, 1911). A short segment of peripheral nerve was used as a transplant. It was known that nerve regeneration occurred in the PNS at that time.

Therefore, it was reasonable to consider that peripheral nerve segments might be good conduits for the growth of regenerating axons even in the CNS. This concept is still valid at the present time, and many studies have been conducted following this concept. Cajal presented the findings that regenerating axons grow through the transplanted peripheral nerve segments (Fig. 1).

After Cajal, however, systematic studies of cell transplantation were not carried out again until the 1980's. As far as I know, one of the most extensive studies on spinal cord regeneration around 1980 was that by Dr Y. Shimizu and colleagues. Dr. Shimizu used cultured fetal cerebellar tissues for transplantation in the dog (Shimizu, 1983). He obtained the finding that, though some dogs could be somewhat improved in locomotion after transplantation, they gradually lost the ability to walk, and were eventually paralyzed in the hind limbs. These changes are almost the same as those reported in the waitress (Clara Nicholas) who was injured by a gun shot to the 7th thoracic vertebra. It seemed that the spinal cord was transected completely. She showed a remarkable recovery with regard to movement and sensation in the lower limbs after 17 months, which continued for another 2 years. However, she became paraplegic 3–4 years after the injury. Dissection showed that her spinal cord had been markedly constricted by connective tissue proliferation at the lesion (Windle, 1954).

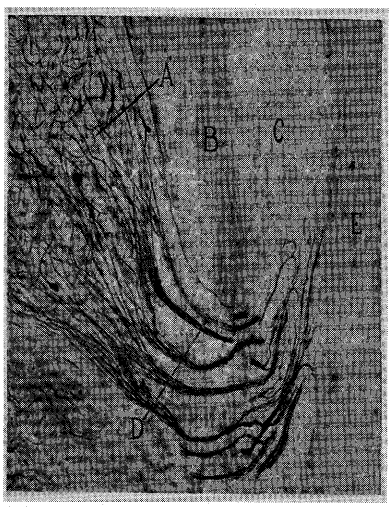

Fig. 1 Transplantation of a sciatic nerve by Tello. A sciatic nerve segment was transplanted into the cerebral cortex of an adult rabbit. This micrograph shows nerve regeneration 14 days after surgery. A: white matter of the cerebrum. B: border of the cerebrum and grafted sciatic nerve. C: sciatic nerve. D: bundles of nerve fibers crossing the border into the sciatic nerve. E: part of the perineurial sheath. (from Cajal, 1928)

Though this connective tissue proliferation might be considered as one of the major causes for the failed recovery, it is difficult to precisely explain the findings of the study by Dr. Shimizu and the clinical processes observed in this case. However, it is conceivable that in both the dog and human cases, there might primarily have occurred nerve regeneration in the lesion, and that these regenerated axons were secondarily degenerated due to their constriction by connective tissue proliferation.

2. Transplantation of peripheral nerves and Schwann cells

One of the studies that hailed a new era in nerve regeneration in the CNS is considered to be that by Aguayo and colleagues (David and Aguayo, 1981). They bridged the spinal cord and medulla oblongata by grafting a peripheral nerve segment, and observed numerous regenerating axons whose cell bodies were within the spinal cord and medulla oblongata extending through the graft from the spinal cord and medulla oblongata, respectively. This showed that neurons in the CNS could extend regenerating axons through the PNS segment, showing the important fact that sprouts from CNS neurons can grow extensively if in an appropriate environment.

Following this study, Schwann cell transplantation has been studied in detail (Guest et al. 1997). Since peripheral nerve axons are ensheathed with Schwann cells, and that Schwann cells can serve as good conduits for regenerating axons in the PNS, it is reasonable to use Schwann cells as transplants for nerve regeneration in the CNS. Unfortunately, however, it has been recognized that Schwann cell transplantation has a critical problem in the CNS: though regenerating axons from the proximal side can grow through the transplants of Schwann cells, they cannot enter the distal side of host spinal cord tissue after traversing the transplant (Xu et al. 1997). The main obstacle for this failed extension would be the glial scar including the basal lamina formed at the border of the lesion. The glial scar is formed by astrocytic process prolif-

eration at the border between the CNS environment and the exterior tissue including the connective tissue. The basal lamina is formed on the astrocytic processes facing the connective tissue. In addition, chondroitin sulfate is secreted from astrocytes along the border. Regenerating axons cannot penetrate the basal lamina. Therefore, both the basal lamina and chondroitin sulfate serve as barriers to the extension of regenerating axons.

In this respect, Schwann cell transplantation needs to involve another step to overcome these barriers. Recent studies show that the application of neurotrophins (BDNF and NT-3) (Bamber et al. 2001) and chondoritinase ABC (Chau et al. 2004) promotes re-entry of regenerating axons into the distal host spinal cord after extension through Schwann cell transplants.

3. Transplantation of olfactory ensheathing cells

Olfactory ensheathing cells are those covering the olfactory nerves running from the olfactory epithelium to the olfactory bulb. These cells have properties similar to both Schwann cells and astrocytes. The olfactory nerves are unique in that neurons in the olfactory epithelium can regenerate from the basal cells of the epithelium. New neurons extend axons through the cell columns of ensheathing cells remaining after Waller degeneration of old axons to the olfactory bulb. This phenomenon is meaningful in that regenerating axons from the peripheral nerve can enter the CNS environment through the transitional zone. This property is another unique aspect of olfactory nerves. Generally, regenerating axons of peripheral nerves that are ensheathed by Schwann cells cannot enter the CNS, for example, regenerating axons of the dorsal roots cannot enter the spinal cord because they cannot penetrate through the astrocyte barrier formed at the transition zone to the spinal cord. Unlike other peripheral nerves, regenerating axons of the olfactory nerves have no barrier on entering the olfactory bulb. The reason for this is assumed to be that the ensheathing cells have the dual characters of Schwann cells and astrocytes, as described above. Ramon-Cueto and colleagues utilized this characteristic property of ensheathing cells for cell transplantation in the spinal cord (Ramon-Cueto et al. 1994). Many reports following this study show that olfactory ensheathing cells have facilitating effects on nerve regeneration by supporting axonal growth in the spinal cord (Plant et al. 2003). Neurotrophin (NT-3) expression promotes the spinal cord sparing effects of transplantation of olfactory ensheathing cells (Ruitenberg et al. 2005). This study indicates that trophic factors such as neurotrophins can play a role in rescuing degenerating tissues in the spinal cord. One study suggests that transplanted ensheathing cells become either Schwann cells and /or perineurial cells in the spinal cord (Li and Raisman, 1997). This means that the olfactory ensheathing cells provide the PNS environment within the spinal cord. However, there are many discussions as to the fate of the transplanted ensheathing cells. Therefore, it has yet to be determined what kinds of cells ensheathing cells differentiate into after being transplanted in the spinal cord.

4. Transplantation of neural stem cells

Neural stem cells were once considered to be the most promising cells for use in transplantation for spinal cord injury. Early studies using neural stem cells as transplants were accepted with great expectation as a breakthrough in the history of spinal cord regeneration studies. However, the morphological data of these studies were not sufficient (McDonald et al. 1999). There was no evidence that transplanted cells supported the extension of regenerating axons within the lesion.

It is expected that, in the transplantation of neural stem cells, transplanted cells can differentiate into neurons and glial cells. Newly differentiated neurons might extend axons and serve as interneurons, and new glial cells might support the growth of regenerating axons. Only a few cells have been shown to differentiate into neurons and glial cells within the spinal cord lesion (Ogawa et al. 2002). There has been no sufficient morphological data showing that the repair of the lesion including the growth of regenerating axons is promoted by transplanted neural stem cells. Moreover, it is not known in what conditions neural stem cells can differentiate into neurons and glial cells following transplantation.

Our studies showed that neural stem cells

were, whether being transplanted directly into the lesion or through the cerebrospinal fluid (CSF), incorporated into the lesion and migrated extensively into the host spinal cord tissue (Wu et al. 2001; Wu et al. 2002a, b). Neural stem cells injected into the CSF attached to the pial surface of the spinal cord, and proliferated extensively with time (Bai et al. 2003) (Fig. 2). This means that control of the proliferation of neural stem cells is a critical problem.

There are another two critical problems with neural stem cells: neural stem cells are usually obtained from fetuses, and transplantation of neural stem cells is allogeneic, i. e., there is an immunological reaction following cell transplantation (Cummings et al. 2005). The first problem is the most critical, and the current severe limitations of the use of fetuses renders neural stem cells practically meaningless in terms of cell transplantation therapy for spinal cord injury. The second problem is also a great drawback with regard to the use of neural stem cells in patients. Our study showed that neural stem cells can be obtained from the forebrain of deceased rats (Xu et al. 2003). It is desirable that the transplant is autogenic, and a sufficient amount of cells can be obtained readily from the source of patients themselves.

5. Transplantation of macrophages

Macrophages appear in the lesion following injury to the nerve fibers. These macrophages remove the degraded myelin sheaths of nerve fibers under Waller degeneration.

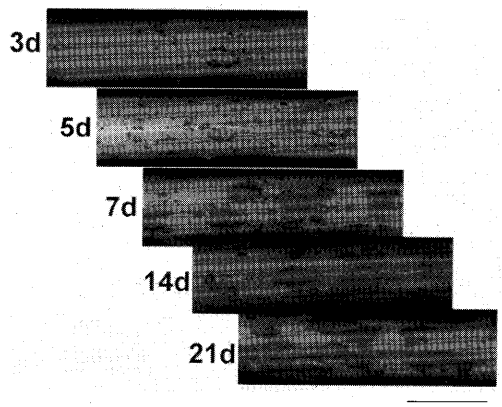

Fig. 2 Neural stem cells injected into the cerebrospinal fluid proliferate on the surface of the spinal cord. Green fluorescent protein (GFP)-labeled neural stem cells were stained by DAB (arrows) after converting into HRP-labeling. DAB-stained neural stem cells proliferated with time from 3 days (3d) to 21 days (21d). Scale bar: 2 mm

It has been observed that the appearance of macrophages in the CNS lesion including spinal cord injury is much delayed as compared to the case of PNS injury. The removal of myelin sheaths by macrophages is so retarded that myelin sheaths of degenerating axons persist for a long time after injury. Along with this retardation of myelin sheath removal, Waller degeneration is very slow. In some cases, normal-looking axons remained even 1 week after axotomy in the spinal cord.

Schwartz and colleagues transplanted macrophages that had been sensitized by contacting with the degenerating myelin sheaths of axotomized peripheral nerves before application, and showed that axonal regeneration was enhanced in the spinal cord injury (Rapalino et al. 1998). It is said that sensitized macrophages can phagocytose and remove myelin sheaths rapidly. They considered that the transplantation of sensitized macrophages promoted nerve regeneration by the rapid removal of myelin sheaths as well as through some other immunological effects. It is known that myelin sheaths act as inhibits to the growth of regenerating axons, and therefore the persistence of myelin sheaths within the lesion is not desirable for nerve regeneration.

It has so far been demonstrated that some proteins constituting myelin sheathes, such as oligodendrocyte myelin glycoprotein (OMgp), Nogo-A (Liebscheret al. 2005; Fouad et al. 2004), and myelin-associated glycoprotein (MAG) (Torigoe and Lundborg, 1998), are inhibitory to axonal growth in the CNS. Therefore, the removal of myelin sheaths by macrophages provides a favorable environment for the growth of regenerating axons in the CNS.

The group of Schwartz is investigating the contribution of the immune system to nerve regeneration following spinal cord injury (Hauben et al. 2003). It can be said that the macrophage system is a part of the immune system that contributes to the promotion of nerve regeneration in spinal cord injury.

6. Transplantation of choroid plexus ependymal cells

Choroid plexus tissue produces cerebrospinal fluid (CSF) in the cerebral ventricle.

The choroids plexus is composed of two parts: neural and connective tissue parts. The neural part is simple cuboidal epithelium continuing from the ventricular ependymal layer. Therefore, the epithelial layer of the choroid plexus can be called the choroid plexus ependymal layer. There is a distinct basal lamina under the epithelium at the interface of the connective tissue compartment. The connective tissue part is the continuation of the pia mater at the cerebral surface. The connective tissue contains abundant capillaries and sinusoids, supplying a large amount of blood flow needed for CSF production.

Our previous study showed that transplantation of the choroid plexus enhanced nerve regeneration in the spinal cord lesion (Ide et al. 2001). Before experimentation, we supposed that the basal lamina of the choroid plexus ependymal cells (CPECs) might contribute as the scaffold to axonal growth, and that CPECs themselves might play as a neural tissue some roles in supporting regenerating axons. In addition, the endothelial cells of capillaries might facilitate vascular proliferation in the host spinal cord lesion. Histological and electron microscopic observations indicated that CPECs, rather than basal laminae, greatly contributed to the growth of regenerating axons (Fig. 3). In vitro, CPECs enhanced the growth of neurites of the co-cultured neurons (Chakrabortty et al. 2000 ; Kimura et al. 2004). Some CPECs differentiated into astrocytes in the host spinal cord (Kitada et al. 2001). An in vitro study later clarified that CPECs contain neural stem cells differentiating into neurons, astrocytes and oligodendrocytes (Itokazu et al. 2005). Ependymal cells of the 3rd ventricle contain neural stem cells in the adult rat (Xu et al. 2005). Concerning the trophic effects of CPECs, an in vitro study using the transwell system showed that CPECs enhanced the survival as well as neurite extension of co-cultured neurons (Watanabe et al. 2005). This shows that CPECs secrete various trophic factors into the medium. In fact, it has been demonstrated that CPECs secrete many important functional molecules (Matsumoto et al. 2003 ; Matsumoto et al. 2005)

CSF is considered to circulate through the extracellular spaces in the CNS, similarly to the lymphatic fluid in the peripheral tissues. All together, it is reasonable to consider that CSF has critical influences on the maintenance of the functions of neurons and glial cells in the normal CNS. In our previous study on cerebral ischemia, CPECs exerted strong effects on rescuing neurons and glial cells from degeneration in ischemia-affected cerebral tissue. In this study, cultured ependymal cells were injected into the CSF through the 4th

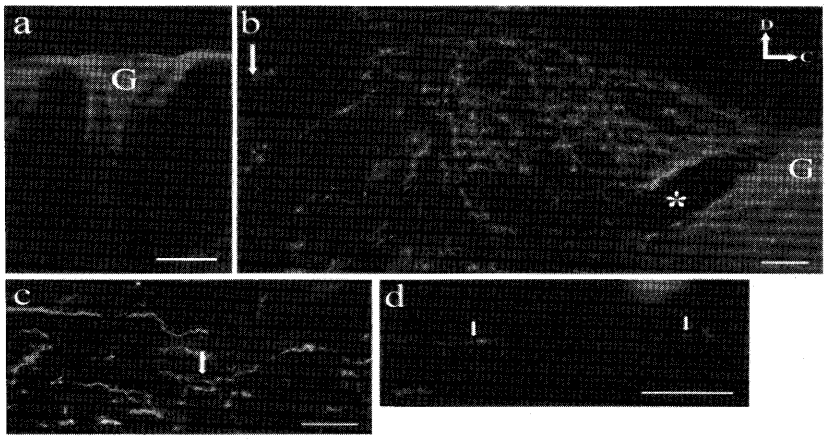

Fig. 3 Grafting of the choroid plexus into the rat spinal cord. Axonal extension was prominent in the lesion 8 months after surgery. Regenerating fibers within the funiculus gracilis were labeled by injecting HRP into the sciatic nerves 2 days before fixation.

(a) Transverse section of the funiculus gracilis (G) at the cervical level. Scale bar : 200 μm
(b) Sagittal section of the lesion. Numerous axons are extending into the lesion from the funiculus gracilis (G). The arrow indicates the rostal part of the lesion. Asterisk : blood vessel. Scale bar : 100 μm
(c) Another example of HRP-labeled axons at the rostal border of the lesion. Scale bar : 100 μm
(d) Few labeled axons extended into the rostal part of the funiculus gracilis of the host spinal cord. Scale bar : 40 μm

ventricle of rats in which the middle cerebral artery had been temporally occluded to cause ischemic injury in the cerebral hemisphere. The volume of tissue degeneration in the infarcted region was greatly decreased in the CPEC-injected rats as compared to the vehicle-injected control rats (Matsumoto et al. 2005).

Our study indicates that CPECs have important functions for the regeneration of CNS tissue that have so far not been suspected. This fact indicates that the choroid plexus must be addressed in detail in the future.

7. Transplantation of bone marrow stromal cells

At present, bone marrow stromal cells (BMSCs) are considered the most promising cells to be used as transplants for spinal cord regeneration. Bone marrow is the blood cell-producing organ composed of hematopoetic and non-hematopoietic supporting tissues. BMSCs are considered to be supporting cells, and are isolated as cells that attach to the culture dish in the culture of bone marrow tissue. BMSCs have been focused on because they have the property of stem cells with the capacity of differentiating into osteocytes, chondrocytes, adipocytes, myocytes, Schwann cells and even neurons (Dezawa et al. 2004). We used BMSCs as transplants for the injured spinal cord. Usually, transplants have been applied directly into the lesion in cell transplantation studies. However, we applied them through the CSF by injecting them into the 4th ventricle (Wu et al 2003). BMSCs flowed through the CSF into the subarachnoidal space of the spinal cord. Some BMSCs invaded the lesion, while many others were attached to the pial surface of the spinal cord, and survived for 3–4 weeks. Three to 4 weeks after cell injection, BMSCs disappeared from the lesion or pial surface of the host spinal cord. However, the behavior of rats was remarkably improved as estimated by the BBB. BBB scores of the cell-transplanted rats recovered to 14–15, whereas those of control rats were 8–10 at 5 weeks after cell transplantation (Fig. 4). Cavity formation at the lesion of the spinal cord was markedly suppressed. As estimated from histological preparations, the volume of cavities of the spinal cord decreased to almost 1/2 of that in the control (Figs. 5, 6). At the same time, neuronal components including axons were well preserved in the vicinity of the cavity, whereas glial cells including astrocytes were, instead of neuronal components, predominant in the spinal cord of control animals (Ohta et al. 2004). These effects are considered to be the results of the suppression of tissue degeneration including neurons and axons that occurs after the injury. Transplanted BMSCs exerted these effects. BMSCs injected into the CSF are considered to effectively rescue degenerating neural tissues in the spinal cord. They are not integrated into the host tissue, but disappear from the spinal cord within 4–5 weeks after injection (Fig. 7). This means that they are not exerting their effects by differentiating into some specific neural

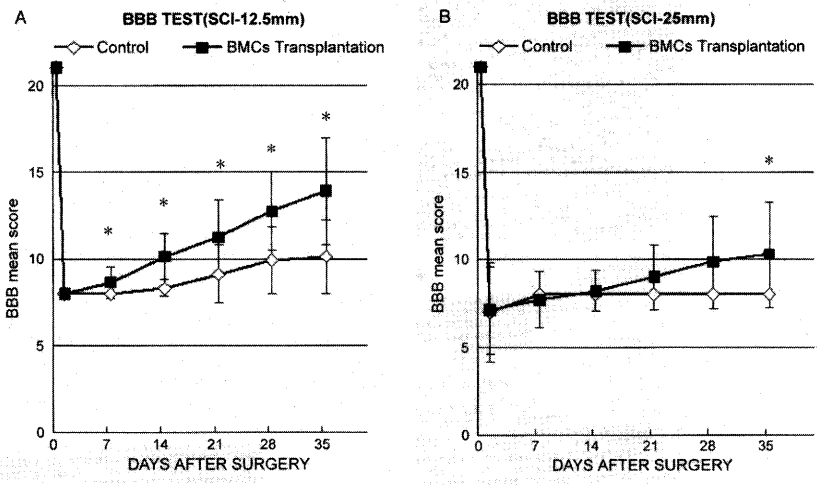

Fig. 4 Locomotion was markedly improved in the cell-transplanted rats. In both mild and severe injury, the level of recovery was significantly higher in the cell-transplanted rats. A : mild injury. B : severe injury

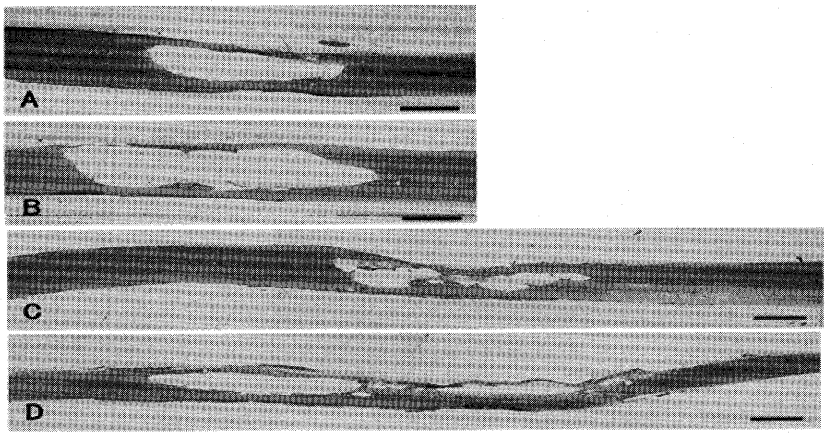

Fig. 5 Cavities were decreased in volume by BMSC transplantation. A and B: mild crush injury by dropping a 10-g weight from a 12.5 mm in height. C and D: Severe crush injury by dropping a 10-g weight from a 25.0 mm in height. Scale bar: 1 mm

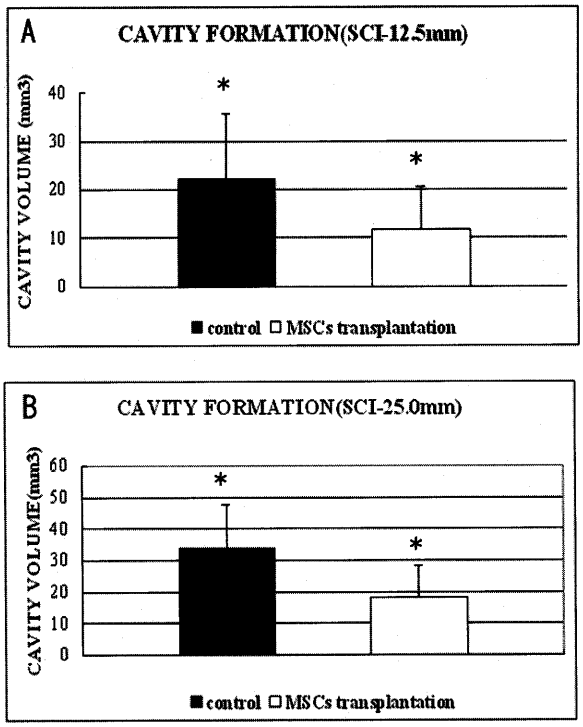

Fig. 6 Measurement of the volume of cavities in the cell-transplanted and control spinal cords. The volume of the cavity in the cell-transplanted rat is almost half of the control ones. A: mild injury. B: severe injury

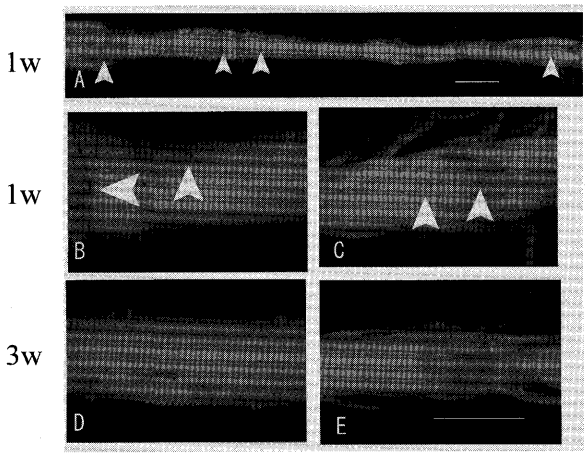

Fig. 7 Bone marrow stromal cells injected into the cerebrospinal fluid attach to the surface of the spinal cord. The attached BMSCs were visualized in the same manner as in Fig. 3. BMSCs (arrows) gradually disappear from the spinal cord. Scale bar: 2 mm

cells in the host tissue, but by releasing some trophic factors into the CSF. Another in vitro study also suggests that BMSCs have strong effects on the survival and neurite extension of neurons in the transwell co-culture system (Ohta et al. in preparation).

There is no finding suggesting that BMSCs might be able to differentiate into other kinds of cells, as cited above in the host spinal cord. This study presents an important problem: transplanted cells are not necessarily integrated into the host tissue. They exert effects by secreting some trophic factors into the CSF. This fact will change the traditional concept that cell transplantation is performed to supply new cells to the lesion to replace lost tissue. However, our study clearly indicates that trophic factors released from the transplanted cells are sufficient to improve the behavioral and histological recovery. BMSCs are appropriate for clinical application, be-

cause they can be obtained from the patients themselves (autograft). The patient's bone marrow is sufficient to provide the source of BMSCs. Accordingly there is no critical ethical problem for the use of BMSCs. It is more desirable if transplants can be obtained directly (i.e., without the step of cell culture) from the bone marrow tissue (Yoshihara et al, in preparation). The possibilities of BMSC application are expanding to other kinds of diseases (Dezawa et al. 2005). These characteristics of BMSCs are superior to other transplant candidates including neural stem cells and olfactory ensheathing cells that are obtained from fetuses, which are inevitably allogeneic in nature. As long as these ethical and immunological problems remain unresolved, neural stem cells and other fetus-derived cells cannot be used for practical transplantation in humans.

We applied for the clinical application of BMSCs to the Medical Ethics Committee of Kansai Medical University in December 2004, and the Committee approved the clinical application on July 1st 2005. We are now at the point of being able to perform clinical application to patients at the Department of Emergency, Kansai Medical University. This application was done in collaboration with another three Departments from Kyoto University and Kansai Medical University : Dr. Y. Suzuki, Department of Plastic and Reconstructive Surgery, Kyoto University, Dr. M. Fukushima, Department of Translational Research, Kyoto University, and Dr. T. Nakatani, Department of Emergency, Kansai Medical University. I would like to thank them for their cooperation in achieving the first step for the clinical application of BMSCs in Japan.

Addendum on proof correction :

The cell transplantation was carried out to a patient with spinal cord injury at C5 in March 2006. This first patient has been under observation with respect to clinical improvement.

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