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Review

Effects of trophic factors on the treatment of spinal cord injury in rats

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Abstract

We studied the effects of the transplantation of bone marrow stromal cells (BMSCs) on spinal cord injury (SCI) in rats. BMSC transplantation promoted distinct locomotor improvements of rats with SCI. However, contrary to expectations, BMSCs disappeared within 2–3 weeks after transplantation. They were not integrated into the host spinal cord tissue. There was no finding indicating that BMSCs supported the outgrowth of regenerating axons as a scaffold in the spinal cord lesion. This finding indicated that BMSCs, instead of physically supporting axonal outgrowth, secreted some trophic factors/molecules effective for tissue repair, including the growth of regenerating axons, in SCI. The same finding was obtained in the study using choroid plexus epithelial cells (CPECs) as a transplant for the treatment of SCI.

These findings are important, as they show that the spinal cord has its own ability to regenerate. This concept is different from the current standpoint of cell transplantation therapy, whereby transplanted cells should be integrated into the host tissue to support the outgrowth of regenerating axons. This generally accepted concept of cell transplantation appears reasonable, but suggests a serious problem. Neural stem cells (NSCs) were integrated into the host tissue, and differentiated into neurons, astrocytes and oligodendrocytes. Neurons that differentiate from NSCs extend numerous axons to distant locations. The problem is that there is no method available to control such unusual axonal extension, differentiation, and migration. There is no way to integrate safely into the host spinal cord tissue. This means that neural stem cell transplantation is too dangerous to be used for clinical application. Regeneration studies have no significance if they have no avenue for clinical application.

In this mini-review, we outline our previous studies, and survey other studies focusing on the use of trophic substances for the treatment of SCI.

Key words: spinal cord injury, cell transplantation, trophic factor, intrinsic ability to regenerate, clinical safety

We have studied the treatment of SCI by the transplantation of somatic cells, including bone marrow-derived cells (Ohta et al., 2004 ; Ide et al., 2010 ; Nakano et al., 2013) and CPECs (Kanekiyo et al., 2016, Ide et al. 2016). These studies revealed that transplanted cells did not survive long-term, but disappeared within 2–3 weeks post-transplantation. In spite of the disappearance of transplanted cells, animals showed locomotor improvements and tissue repair, including axonal regeneration, through the spinal cord lesion. This suggested that transplanted cells did not serve as scaffolds for the growth of regenerating axons, but release some trophic factors that have beneficial effects to promote locomotor improvement and axonal regeneration.

Generally speaking, cell transplantation studies are based on the concept that transplanted cells should be integrated into the host spinal cord, serving as scaffolds for the outgrowth of axons. However, our studies showed that the fate of transplants such as BMSCs and CPECs was different from this generally accepted assumption.

The fact that transplanted cells do not survive but disappear after transplantation may be considered a disadvantage to the recipient in clinical cell transplantation therapies. However, on the contrary, we regarded this property as an advantage for the treatment of spinal cord injury. The disappearance of transplanted cells means that such cell transplantation is safe for patients : as long as transplants continue to survive, there is anxiety and fear concerning the potential for the abnormal proliferation, differentiation, and migration of implants. Neural stem cells (NSCs) that survive long-term to proliferate, differentiate, migrate, and expand in the host spinal cord are associated with serious problems in terms of safety. NSCs will not be applicable for clinical transplantation until a method to control their behaviors in the host spinal cord is developed. On the other hand, somatic stem/progenitor cells, although they no not survive long-term, enhance locomotor improvements after transplantation into rats with SCI. This is important in view of clinical application. Cell transplantation studies are usually based on the premise that transplanted cells survive and differentiate into appropriate cells that can complement the host tissue, contributing to tissue repair and functional recovery. This is too optimistic : there is no method to secure that the transplanted cells function appropriately over a long time period after transplantation. No method is available to control transplanted cell proliferation, differentiation, and integration into the host tissue. The safety of stem cells has so far been discussed from the point of view of tumor development. It should be emphasized that, even if transplants (e. g., neural stem cells) are free from the risk of tumor development, transplants integrated into the host spinal cord tissue will continue to exist as potential sources of abnormal reorganization or function over a long time period (Lu et al., 2014). Even if transplants are apparently differentiated into somatic cells, it is unknown whether they continue to sustain their differentiated normal properties long-term thereafter. Non-self stem cells such as neural stem cells need to be observed carefully after apparently "successful" implantation. On the other hand, somatic stem/progenitor cells are free from such safety problems, because they have only the normal limited range of ability to proliferate, migrate, and differentiate after transplantation (Ide and Kanekiyo, 2016).

We have used somatic cells such as BMSCs and CPECs as transplants for the treatment of SCI in rats. As described above, these cells disappeared within 2–3 weeks post-transplantation from the host spinal cord. This indicates that some trophic factors released from transplants should have effects on tissue repair and functional improvements. This suggests that the use of appropriate trophic factors might be more desirable for the treatment of spinal cord injury. We examined the effect of conditioned medium of BMSCs on SCI. There was a marked effect on the locomotor recovery of rats with SCI. The fact that trophic factor application is effective for the treatment of spinal cord injury indicates that the spinal cord has an intrinsic ability to regenerate. This is an important concept in the treatment of SCI. Stimulation of this intrinsic ability of the spinal cord by trophic factors is considered to be the safest and most desirable treatment of the spinal cord.

In this short review, we focus on the use of trophic factors for the treatment of SCI. Since the treatment of spinal cord injury requires multiple kinds of molecules for tissue repair and locomotor improvements, the application of only a single trophic factor might be insufficient for the treatment of SCI. The conditioned medium that comprises all components released from cultured cells may be an appropriate agent for the treatment of SCI.

The following is an outline of our studies on the treatment of SCI by cell transplantation.

In our cell transplantation studies, BMSCs for

transplantation were cultured from bone marrow tissues of adult Spraque-Dawley (SD) rats. The spinal cord at T8–9 was exposed with the dura mater on the surface and contusion-injured by letting a metal rod weighing 10 g drop from a height of 5–7.5 cm. BMSC transplantation was performed immediately (acute phase) or 1–4 weeks (subacute and chronic phase) after spinal cord injury. BMSCs were injected directly into the spinal cord lesion, or indirectly through the cerebrospinal fluid (CSF) via the 3rd or 4th ventricle. The locomotor behaviors were evaluated using by BBB (Basso, Beattie, and Bresnahan) score (Basso et al., 1995), and axonal outgrowth and tissue repair in the lesion were examined by immunohistochemistry for axons and astrocytes. The localization of collagen type 1 in the spinal cord lesion was examined by immunohistochemistry as a representative molecule of extracellular matrices.

We performed our first study of BMSC transplantation into rats with SCI in 2002 (Ohta et al., 2004). BMSCs were transplanted through the 4th ventricle immediately after injury. This study showed the significant improvement of the locomotor function. However, unexpectedly, BMSCs did not survive, but disappeared from the host spinal cord 2–3 weeks after transplantation. We concluded that the transplanted BMSCs did not serve as a scaffold for the growth of regenerating axons, but secreted some trophic factors effective for locomotor improvements.

A few years later, a more systematic study was carried out to examine the fate of transplanted cells and their effects on functional recovery (Ide et al., 2010). In this study, extensive axonal regeneration was noted through the astrocyte-devoid areas in the spinal cord lesion. In an immunohistochemical preparation for GFAP, astrocyte-devoid areas looked like cavities formed at the epicenter of the lesion. However, immunohistochemistry for neurofilaments showed that there were numerous axons extending longitudinally within such astrocyte-devoid areas. Transplanted BMSCs were no longer found within the astrocyte-devoid areas 2–3 weeks after transplantation. These findings indicate that BMSCs did not serve as the scaffold for the outgrowth of regenerating axons, but probably released some trophic factors effective for the axonal outgrowth though the astrocyte-devoid areas.

BMSC transplantation is effective even for chronic spinal cord injury in rats (Nakano et al., 2013). In this study, BMSCs also did not serve as the scaffold for the outgrowth of axons, but disappeared from the spinal cord within 2–3 weeks after transplantation. Based on these studies, we proceeded to clinical studies of autologous BMSCs in 2005. Meanwhile, we showed the beneficial effects of the transplantation of bone marrow mononuclear cells (BMNCs) on SCI in 2007 (Yoshihara et al., 2007). As in the case of BMSCs, they disappeared from the host spinal cord tissue within 1–2 weeks after transplantation. As the first step for clinical application, we transplanted cultured autologous BMSCs into 5 patients (Saito et al., 2012). Next, BMNCs were transplanted immediately after separating from the bone marrow of patients themselves into 10 patients (Suzuki et al., 2014). These clinical studies revealed that the implantation of BMSCs and BMNCs is safe. Several patients showed some improvements of clinical signs.

These studies clearly indicate that BMSCs secrete some trophic factors effective for the outgrowth of regenerating axons and locomotor improvements of rats with SCI. Nakano studied the components of conditioned medium of BMSCs, demonstrating that the conditioned medium contained IGF2, bFGF, VEGF, and TNFβ (Nakano et al., 2010). It is conceivable that many other kinds of molecules and factors effective for SCI were mixed in the conditioned medium (CM) of BMSCs.

Recently, we performed the transplantation of CPECs into rats with SCI. In this study also, transplanted CPECs survived for only 1–2 weeks after transplantation; however, the locomotor improvement and axonal extension through astrocyte-devoid areas were marked, indicating that trophic factors released from the CPECs promoted the tissue repair and locomotor improvements (Kanekiyo et al., 2016). Recently, we demonstrated that multiple injections of BMNCs were more effective than single injection for the treatment of SCI in rats (Kanekiyo et al., 2016, in press).

Based on these studies, we are now investigating the effects of CM of BMSCs on the treatment of SCI in rats. There was a marked recovery of the locomotor function within 1 week after transplantation in rats. This and other cell transplantation studies indicate that the spinal cord has an intrinsic ability to regenerate after traumatic injury, and that trophic factors and molecules can promote spinal cord recovery from apoptosis or degeneration, re-activate function-suppressed neurons, and enhance the outgrowth of regenerating axons.

We will explain below some of the important experimental studies concerning the trophic effects on spinal cord injury.

Cantineaux et al. (2013) delivered the conditioned medium (CM) obtained from the culture of bone marrow-derived mesenchymal stem cells directly into the spinal cord injury using Alzet mini-pumps. Such a conditioned medium infusion improved recovery after spinal cord injury in rats, indicating new strategies to avoid cell transplantation. They also showed that the CM suppressed the apoptosis of cerebral granular cells in culture and cell death due to TNFα treatment.

The conditioned medium contained high concentrations of NGF, BDNF, and IL-6. Antibody array revealed that the CM contains anti-apoptotic, proinflammatory, angiogenic, and neuromodulatory molecules. BBB scores for the locomotor function were 17 points for CM-treated, and 14 points for control rats. There were also differences in grid navigation between CM treated and control rats. The CM reduced lesion extension and cavity expansion. There was no difference in glial scar formation between the CM-treated and control rats. Unexpectedly, no difference was seen in immunohistochemistry for GAP-43 and neurofilaments between the CM-treated and control rats. Histological and immunohistochemical findings concerning axonal regeneration are not satisfactory. In vitro, cultured rat aorta fragments showed an enlarged diameter due to CM treatment. The CM stimulates macrophages to secrete pro-inflammatory cytokines, IL-1β, IL-6, and TNFα. This study indicates the utility of a cell-free therapeutic approach to spinal cord injury.

The CM is often called the "secretome" containing, in addition to humoral factors, various kinds of particles released from bone marrow stromal cells.

Haider et al. (2015) demonstrated that the secretome of peripheral bone marrow mononuclear cells (BMNCs) attenuates secondary damage following spinal cord injury in rats. The spinal cord was moderately contusion-injured using a 2.5-mm diameter stainless steel tip placed 1 mm above the exposed spinal cord. A preset force of 150 kDyne was applied with instantaneous retraction of the tip, leading to a contusion injury. The BMNC-secretome was administered by intraperitoneal injection. Locomotor assessment using the BBB score was 16 points for MNC-secretome-treated rats, and 13 points for control rats 4 weeks after secretome injection. Cavity formation on the dorsal side of the spinal cord was markedly suppressed by the secretome treatment. Angiogenic properties were evaluated using a rat aortic model. The BMNC-secretome showed the angiogenic potential. Overall, the inflammatory response is suppressed by BMNC-secretome application.

No finding is presented in that study concerning axonal regeneration extending through the spinal cord lesion. It is likely that the contusion injury was too mild to examine the growth of regenerating axons.

The following description in this study is interesting, "Over the years, the focus of the field of cell-based therapy has moved away from 'cell-center' view toward soluble paracrine factors that have observed effects in multiple pre- and clinical studies. Interestingly, either PDGF or VEGF alone had deleterious effects, while the use of both factors together proved efficacious in a contusion injury model."

The concept that paracrine molecules of transplanted BMSCs play main roles in tissue recovery and functional improvement of spinal cord injury was proposed for the first time in our study (Ohta et al., 2003).

Konala et al. (2016) explain the concept that secretome is a new paradigm for cell-free regeneration. This review explains the effects of the BMSC secretome on pathological conditions of various organs, such as cardiovascular, neurological, renal, and immunological systems. The concise abstract of this review is as follows "Mesenchymal cells are derived from bone marrow, adipose tissue, dental pulp, umbilical cord, and so forth. The conditioned medium of BMSCs has the broad repertoire of trophic factors, and is commonly referred to as the BMSC secretome, which includes soluble factors, apoptotic bodies, exosomes, and microvesicles : the latter two are extracellular vesicles that contain many factors of various trophic functions. Secretome has functions of neuroprotection, angiogenesis, anti-fibrosis, anti-apoptosis, anti-oxidation, chemoattraction, proliferation, anti-bacterial function, hematopoietic stem cell support, and immunomodulation. Microvesicles, measuring 100–1000 nm in diameter, are originated from the plasma membrane with surface markers such as integrin and selectin. Exosomes, measuring 40–100 nm in diameter, are derived from the contents of the multi-vesicular endosome that are released into the extracellular space. Surface markers of exosomes are Rab5, CD63, CD9, and CD81. Apoptotic

bodies, measuring more than 1,000 nm in diameter, are vesicles containing degraded organelles."

Gu et al. (2010) demonstrated neurotrophic actions of bone marrow stromal cells on primary culture of dorsal root ganglion tissues and neurons. This study suggests that BMSCs trigger endogenous signaling pathways in neurons through their secreted soluble factors, explaining the reason why BMSC transplantation enhances the recovery of the locomotor function within such short a time as 1–2 weeks after transplantation. This study demonstrated that BMSCs enhance endogenous Akt and Erk1/2 pathways, leading to the enhancement of the vitality of DRG neurons. Western blot analysis shows that BMSC-conditioned medium activates Erk1/2 phosphorylation in DRG neurons within 45 min and up to 48 h, and enhances total Akt and phospho-Akt expression in DRG neurons within 45 min and up to 180 min.

Similarly, Gu et al. (2017) examined the effect of the conditioned medium of olfactory ensheathing cells (CM-OEC) to promote functional recovery and axonal regeneration after contusive spinal cord injury. The spinal cord was contusion-injured using a New York University (NYU) weight-drop impactor at the T10 segment of rats, and the CM-OEC was injected intraperitoneally. BBB scores were significantly higher in the CM-OEC- treated group, and a better radiological recovery after SCI was observed in the CM-OEC-treated group. Axonal regeneration was promoted around the injury epicenter.

The anti-apoptotic effects of cell transplantation is also an interesting topic. It is considered to be due to the paracrine effects of humoral factors released from transplants. Dasari et al. (2007) showed that mesenchymal stem cells from rat bone marrow down-regulate the caspase-3-mediated apoptotic pathway after spinal cord injury in rats. In their study, the spinal cord at T10 was injured with a weight drop, and rats were injected 7 days after injury with 5 μL of mononuclear stromal cells (BMNCs) into the contusion site. Caspase-positive cells were markedly reduced in the BMNC-treated spinal cord. Caspase-3 expression in neurons and oligodendrocytes is markedly down-regulated in the BMNC-treated spinal cord. BBB scores show significant improvements 3 weeks after injury.

The effects of specific trophic factors have been reported in several studies. Brock et al. (2010) examined local and remote growth factor effects after primate spinal cord injury. They used autologous fibroblasts genetically modified to secrete BDNF and NT-3 as a transplant into spinal cord injury in monkeys. Axonal regeneration was enhanced and the myelination of regenerated axons by Schwann cells was promoted. In addition, the raphespinal tract (5HT) and cerulospinal tract (TH), but not corticospinal tract, were enhanced. Neurons undergoing atrophic changes due to spinal cord injury were rescued by BDNF and NT-3 administration. They reported as follows, "Injured neural systems retain the ability to respond to growth signals over the extended distance of the primate CNS, promoting local axonal growth and preventing lesion-induced neuronal degeneration at a distance. Remote cortical effects of spinally administered growth factors could prime the neuron to respond to experimental therapies that promote axonal plasticity of regeneration. The use of trophic factors for the treatment of spinal cord injury is based on the premise that the spinal cord has an ability to regenerate. The trophic factors trigger regenerating activity of injured axons, and, in addition, elicit extra-neural reactions such as the production of extracellular matrices and proliferation of non-neural cells such as Schwann cells and fibroblasts." In combination with these reactions, the spinal cord exhibits an intrinsic ability to regenerate after spinal cord injury.

Blesch and Tuszynski (2007) demonstrated that transient growth factor delivery sustains regenerated axons after spinal cord injury. They manipulated genetically modified fibroblasts to secrete BDNF that can be controlled to turn BDNF secretion on or off after transplantation into spinal cord injury. They showed that transient growth factor delivery is sufficient to sustain regenerated axons for prolonged time periods within spinal cord lesion sites. This suggests that axonal regeneration can be triggered by stimulation of appropriate growth factors, and sustained thereafter in the spinal cord lesion.

Jeong et al. (2012) demonstrated that hepatocyte growth factor (HGF) reduces astrocytic scar formation and promotes axonal growth beyond glial scars after spinal cord injury. Mesenchymal stem cells over-expressing HGF (HGF-MSC) were transplanted into hemisection spinal cord lesions at C4. The transplantation of HGF-MSCs markedly reduced TGFβ isoform levels and the extent of astrocyte activation. In addition, HGF-MSCs also significantly decreased neurocan expression and glycosaminoglycan chain deposition around hemisection lesions. There was an increase in axonal growth beyond glial scars and improvement in

forepaw function. Many more 5-HT axons extend in the HGF-MSC-treated spinal cord. However, no report has been published concerning whether such axons extend through the astrocyte-devoid area. Functional improvements (greater grip strength and low error rate on grid walking) were observed.

Chen et al. (2002) examined the effects of traumatized brain extracts of rats on profiles of various growth factors, such as BDNF, NGF, VEGF, bFGF, and HGF in the culture of human mesenchymal stromal cells (MSCs). MSCs showed time-dependent increases of BDNF, NGF, VEGF, and HGF. This suggests that transplanted hMSCs may provide a therapeutic benefit via the responsive secretion of an array of growth factors that can promote neuroprotection and angiogenesis. The following description is important, "The ability of therapeutically transplanted MSCs to replace injured parenchymal CNS tissue appears limited at best. Tissue replacement, however, is not the only possible compensatory avenue in cell transplantation therapy."

The study of Zacharek et al. (2010) is similar to that by Chen et al. (2002). They compared the effect of treatment of stroke with BMSCs from stroke rats (Isch-BMSC) and normal rats (Nor-BMSC) on the functional outcome. Isch-BMSC and Nor-BMSCs were intravenously injected 24 hours after middle cerebral artery occlusion. Isch-BMSCs significantly promoted functional outcome and enhanced angiogenesis, arterial density, and axonal regeneration compared with Nor-BMSC-treated animals.

Isch-BMSCs are superior to Nor-BMSCs for the neurorestorative treatment of stroke, which may be mediated by the enhanced trophic factors secreted from Isch-BMSC.

This means that BMSCs are affected by brain stroke in terms of their activity to release trophic factors.

Jin et al. (2002) transplanted fibroblasts genetically modified to express BDNF, and showed that axonal regeneration was promoted in chronic spinal cord injury. Fibroblasts genetically modified to express BDNF were transplanted into the chronic (4 weeks) spinal cord lesion of complete ipsilateral hemisection at the cervical spinal cord segment. It was shown that BDNF promoted axonal regeneration from supraspinal (rubrospinal, reticulospinal, and vestibrospinal) neurons with accompanying partial recovery of the locomotor performance.

Zhang et al. (2009) examined the effect of GDNF on axonal regeneration and myelination following SCI.

This study demonstrated the effects of GDNF on axonal regeneration and myelination in spinal cord injury. The problem of whether GDNF exerts its effect on neurons directly or through Schwann cells indirectly is another problem. Using a guideline channel filled with matrigel containing Schwann cells and GDNF, they examined the effect of GDNF on axonal regeneration and myelination in SCI. This study suggested that the combination of GDNF administration and Schwann cell transplantation might represent an effective strategy to promote axonal regeneration and myelin formation after injury of the spinal cord.

Piantino and Benowitz (2006) demonstrated in their studies that hydrogel can be used as the novel way to deliver molecules. Hydrogel was used to deliver NT-3, sustaining the effect over a 2-week period after transplantation. This study indicated that NT-3 promoted axonal growth of major descending pathways, corticospinal and raphespinal tracts, and enhanced functional improvements after spinal cord injury.

Reviews concerning the effects of trophic factors released from transplanted cells in the treatment of spinal cord injury have been published by Harvey et al. (2015) and Teixeira et al. (2013).

Conclusion

It has been demonstrated that trophic factors have an effect on the recovery of an injured spinal cord. The spinal cord has its own intrinsic ability to recover from injury. This ability includes the outgrowth of regenerating axons and formation of extracellular matrices, and stimulation/enhancement of the physiological functions present before injury. The use of the intrinsic ability of the spinal cord to regenerate is the safest and most desirable way to treat SCI. In this sense, the use of trophic factors to stimulate the intrinsic ability of the spinal cord to regenerate is reasonable for clinical application.

On the other hand, cell transplantation has so far been viewed as the most effective treatment for spinal cord injury. It is considered that transplanted cells should be integrated into the host tissue to survive long-term. Somatic stem/progenitor cells have no deleterious effect on structural or functional properties of the host spinal cord, even if they survive long-term after transplantation. However, neural stem cells derived from ES cells or iPS cells are different : they pro-

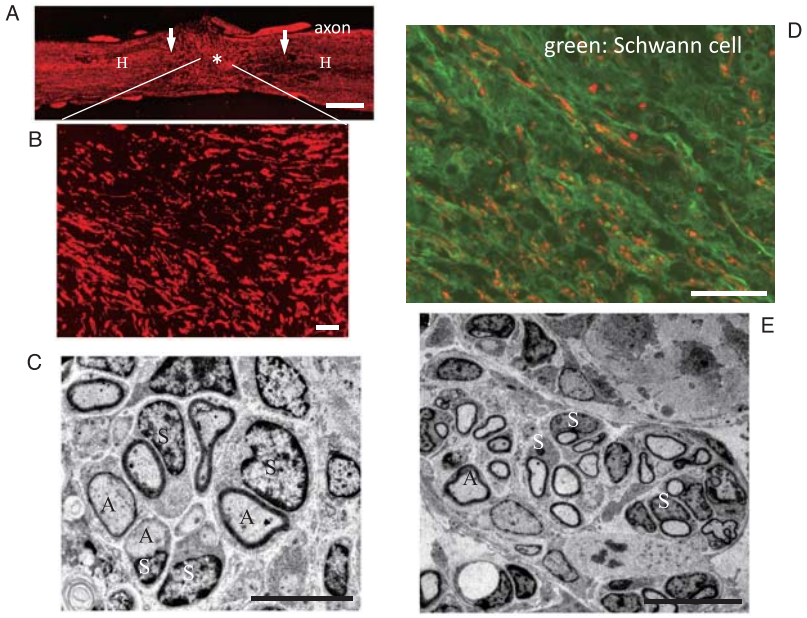

Fig. 1 Bone marrow stromal cell transplantation into SCI rats. Two to 8 weeks after BMSC transplantation or vehicle injection. A. Two weeks after transplantation. Horizontal section. Immunohistochemistry for neurofilaments. Abundant regenerating axons extend longitudinally through the lesion (asterisk). H : intact region of host spinal cord, arrows : border of lesion. B. Enlargement of A. Axons with various diameters extend almost longitudinally. C. Electron microscopy of the part of the lesion as seen in B. Axons (A) are covered by Schwann cells (S). Individual axons are myelinated by Schwann cells. D. Immunohistochemistry for Schwann cells at the epicenter of the spinal cord lesion 8 weeks after transplantation. Axons (red) are associated with Schwann cells (green). E. Eight weeks after transplantation. Electron microscopy of axons extending through the spinal cord lesion. Individual axons (A) are thickly myelinated by Schwann cells (S). From Ide et al. (Brain Res, 2010).
Scale : 500 μm for A, 100 μm for B and D, and 5 μm for C and E

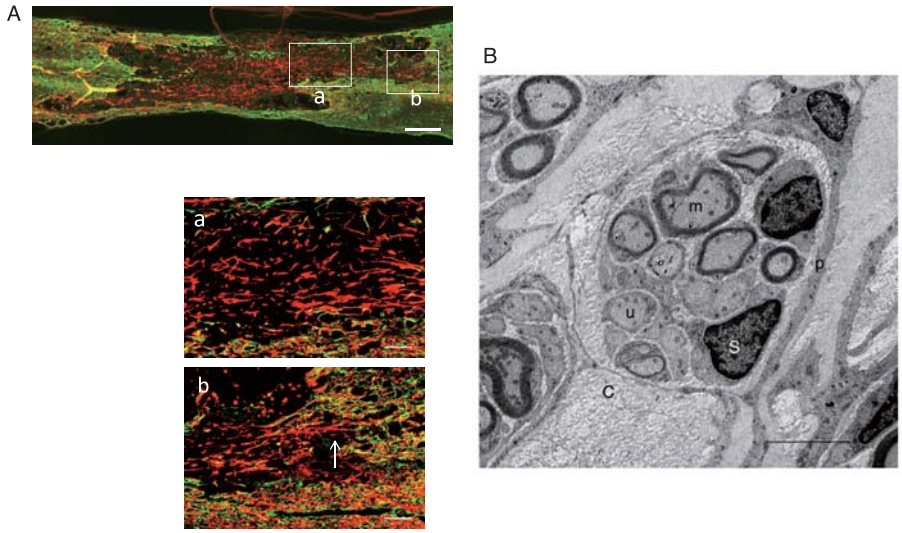

Fig. 2 Choroid plexus epithelial cell transplantation. A. Two weeks post-transplantation. Double-immunostaining of axons (red) and astrocytes (green). Numerous axons extend through the astrocyte-devoid areas in the spinal cord lesion. Transplanted CPECs are not found in this section. The areas enclosed with rectangles were enlarged in panels a and b. Axons in the astrocyte-devoid area extend through the border smoothly (arrow). B. Electron microscopy. Five weeks post-transplantation Axons (m, u) are surrounded and separated by perineurial cells into groups. Collagen matrices (C) are found between perineurial and endoneurial spaces.
Scale : 500 μm for A, 100 μm for a and b, and 5 μm for B.
From Kanekiyo et al. (Restr Neurol Neurosci, 2016).

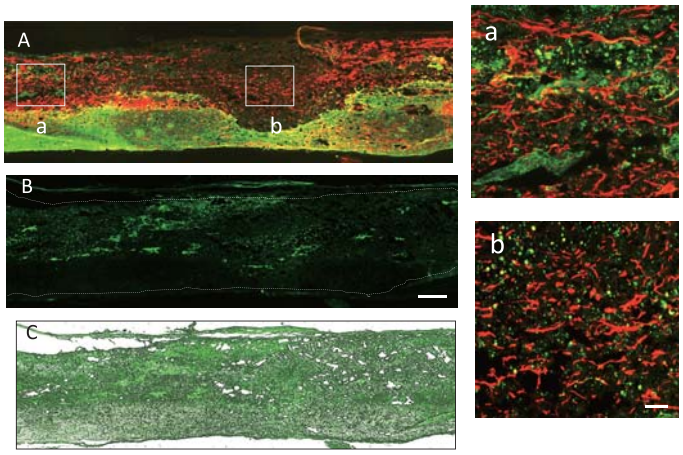

Fig. 3 Choroid plexus epithelial cell transplantation. One week after transplantation.
A : Double-immnostaining for axons (red) and astrocytes (green). The dotted line outlines the spinal cord. Numerous axons extend through the astrocyte-devoid areas. B : A simple fluorescent micrograph taken from a section adjacent to that of panel A. There are various-sized clusters of engrafted CPECs. C : Panel B was merged in the unstained transmitted-light picture to show the localization of engrafted CPECs in the spinal cord tissue. a : Rectangle a in panel A was enlarged. Some CPECs (arrows) can be seen among axons. b : Rectangle b in panel A was enlarged. Many axons extend without an association with engrafted CPECs.
Scale : 500 μm for A-C, and 100 μm for a and b.
From Kanekiyo et al. (Restr Neurol Neurosci, 2016).

liferate, differentiate, and extend numerous axons to extraordinary and more distant places compared with the normal pattern of cell distribution. Such abnormal patterns have, in addition to the possibility of tumor formation, marked effects on the behavior and histology of the host spinal cord. This kind of cell transplantation cannot be applied to patients clinically.

References

Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma, 12, 1–21, 1995.

Blesch A, Tuszynski KM. Transient growth factor delivery sustains regenerated axons after spinal cord injury. J Neurosci 27 : 10535–10545, 2007

Brock JH, Rosenzwig ES, Blesch A, Moseanko R, Havton LA, Edgerton VR. Local and remote growth factor effects after primary spinal cord injury. J Neurosci 30 : 9728–9737, 2010

Cantinieaux D, Quertainmont R, Blacher S, Rossi L, Waner T, Noel A, Brook G, Schenen J, Franzen R. Conditioned medium from bone marrow-derived mesenchymal stem cells improves recovery after spinal cord injury in rats : An original strategy to avoid cell transplantation. PLoS One 8 : e69515. doi : 10.1371/journal. Pone. 0069515, 2013

Chen X, Katakowski M, Li Y, Lu D, Wang L, Zhang L, Chen J, Xu Y, Gautam S, Mahmood A, Chopp M. Human bone marrow stromal cell cultures conditioned by traumatic brain tissue extracts : growth factor production. J Neurosci Res 69 : 687–691, 2002

Dasari, VR, Spomar DG, Cady C, Gujrati M, Rao JS, Dinh DH. Mesenchymal cstem cells from rat bone marrow down regulate Caspase-3 mediated apoptotic pathway after spinal cord injury in rats. Neurochem Res 32 : 2080–2093, 2007

Gu Y, Wang JD, Ding F, Hu N, Wang Y, Gu X. Neurotrophic actions of bone marrow stromal cells on primary culture of dorsal root ganglion tissues and neurons. J Mol Neurosci 40 : 332–341, 2010

Gu M, Gao Z. Lo X, Guo L, Lu T, Lo Y, He X. Conditioned medium of olfactory ensheathing cells promotes the functional trecovery and axonl regeneration after contusive spinal cord injury. Brain Res 1654 : 43–54, 2017

Haider T, Hoftberger R, Ruger B, Mildner M, Blumer R, Mittenbauer A, Buchacher T, Sherif C, Altman P, Redi H, Gabriel C, Gyongyosi M, Fischer MB, Lubec G, Ankersmit HJ. The secretome of apoptotic human peripheral blood mononuclear cells attenuates secondary damage following spinal cord injury in rats. Exp Neurol 267 : 230–242, 2015

Harvey AR, Lovett SJ, Majda BT, Yoon JH, Wheeler LP, Hodgetts SI. Neurotrophic factors for spinal cord repair : Which, where, how and when to apply, and for what period of time? Brain Res 1619 : 36–71, 2015

Ide C, Nakano N, Seo T-B, Yamada Y, Rbdo K, Saito F, Suzuki Y, Fukushima M, Nakatani T. Bone marrow stromal cell transplantation for treatment of sub-acute spinal cord injury in the rat. Brain Res, 1332, 32–47, 2010

Ide C, Kanekiyo K. Points regarding cell transplantation for the treatment of spinal cord injury. Neural Regen Res 11 : 1046–1049, 2016

Ide C, Nakano N, Kanekiyo K. Cell transplantation for the treatment of spinal cord injury-bone marrow stromal cells and choroid plexus epithelial cells. Neural Regen Res 11 : 1385–1388, 2016

Jeong R, Kwon MJ, Lee HG, Joe EH, Lee JH, Kim SS, Suh-Kim H, Kim BG. Hepatocyte growth factor reduces astrocytic scar formation and promotes axonal growth beyond glial scars after spinal cord injury. Exp Neurol 233 : 312–322, 2012

Jin Y, Fischer I, Tessler A, Houle JD. Transplants of fibroblasts genetically modified to express BDNF promote axonal regeneration from supraspinal neurons following chronic spinal cord injury. Exp Neurol 177 : 265–275, 2002

Kanekiyo K, Nakano N, Noda T, Yamada Y, Suzuki Y, Ohta M, Yokota A, Fukushima M, Ide C. Transplantation of choroid plexus epithelial cells into contusion-injured spinal cord of rats. Restor Neurol Neurosci 34 : 347–366, 2016

Kanekiyo K, Nakano N, Homma T, Yamada Y, Tamachi M, Suzuki Y, Fukushima M, Saito F, Ide C. Effects of multiple-injection of bone marrow mononuclear cells on spinal cord injury of rats. J Neurotrauma, 35 : 521–532, 2018

Konala VHR, Mamidi MK, Bhonde R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome : A new paradigm for cell-free regeneration. Cytotherapy 18 : 13–24, 2016

Lu P, Woodruff G, Wang Y, Graham L, Hunt M, Wu D, Boehle A, Ahmad R, Polawski G, Brock J, Goldstein LSB, Tuszynski MH. Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. Neuron 83 : 786–796, 2014

Nakano N, Nakai Y, Seo T-B, Yamada Y, Ohno T, Yamanaka A, Nagai Y, Fukushima M, Suzuki Y, Nakatani T, Ide C. Characterization of conditioned medium of cultured bone marrow stromal cells. Neurosci Lett, 483 : 57–61, 2010

Nakano N, Nakai Y, Seo T-B, Homma T, Yamada Y, Ohta M, Suzuki Y, Nakatani T, Fukushima M, Hayashibe M, Ide C. Effects of bone marrow stromal cell transplantation through CSF on the subacute and chronic spinal cord injury in rats. PLoS ONE, 8(9) : e73494, 2013

Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, Kitaoka K, Chou H, Ishikawa N, Matsumoto N, Iwashita Y, Mizuta E., Kubo S, Ide C. Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. Exp Neurol, 187 : 266–278, 2004

Piantino J, Burdick JA, Goldberg D, Langer R, Benowitz LI. An injectable, biodegradable hydrogel for trophic factor delivery enhances axonal rewiring and improves performance after spinal cord injury. Exp Neurol, 201 : 359–367, 2006

Saito F, Nakatani T, Iwase I, Maeda M, Murao M, Suzuki Y, Fukushima M, IdeC. Administration of cultured autologous bone marrow stromal cells into cerebrospinal fluid in spinal injury patients : A pilot study. Restor Neurol Neurosci, 30 : 127–136, 2012

Suzuki Y, Ishikawa N, Omae K, Hirai T, Ohnishi K, Nakano N, Nishida H, Nakatani T, Fukushima M, Ide C. Bone marrow-derived mononuclear cell transplantation in spinal cord injury patients by lumbar puncture. Restor Neurol Neurosci, 32, 473–482, 2014

Teixeira FG, Carvalho MM, Sousa N, Salgado AJ. Mesenchymal stem cells secretome : a new paradigm for central nervous system regeneration? Cell Mol Life Sci 70 : 3871–3882, 2013

Yoshihara T, Ohta M, Itokazu Y, Matsumoto N, Dezawa M, Suzuki Y, Taguchi A, Watanabe Y, Adashi Y, IkeharaS, Sugimoto H, Ide C. Neuroprotective effect of bone marrow-derived mononuclear cells promoting functional recovery from spinal cord injury. J Neurotrauma, 24, 1026–1036, 2007

Zacharek A, Shehadah A, Chen J, Cui X, Roberts C, Lu M, Chopp M. Comparison of bone marrow stromal cells derived from stroke and normal rats for stroke treatment. Stroke 41 : 524–530, 2010

Zhang L, Ma Z, Smith GM, Wen X, Pressman Y, Wood PM, Xu XM. GDNF-enhanced axonal regeneration and myelination following spinal cord injury is mediated by primary effects on neurons. Glia 57 : 178–1191, 2009

Zhukareva V, Obrocka M, Houle JD, Fischer I, Neuhuber B. Section profile of human bone marrow stromal cells : donor variability and response to inflammatory stimuli. Cytokine 40 : 317–321, 2010