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INVITED REVIEW

Points regarding cell transplantation for the treatment of spinal cord injury

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Abstract

Transplantation of somatic cells, including bone marrow stromal cells (BMSCs), bone marrow mononuclear cells (BMNCs), and choroid plexus epithelial cells (CPECs), enhances the outgrowth of regenerating axons and promotes locomotor improvements. They are not integrated into the host spinal cord, but disappear within 2-3 weeks after transplantation. Regenerating axons extend at the spinal cord lesion through the astrocyte-devoid area that is filled with connective tissue matrices. Regenerating axons have characteristics of peripheral nerves: they are associated with Schwann cells, and embedded in connective tissue matrices. It has been suggested that neurotrophic factors secreted from BMSCs and CPECs promote "intrinsic" ability of the spinal cord to regenerate. Transplanted Schwann cells survive long-term, and are integrated into the host spinal cord, serving as an effective scaffold for the outgrowth of regenerating axons in the spinal cord. The disadvantage that axons are blocked to extend through the glial scar at the border of the lesion is overcome. Schwann cells have been approved for clinical applications. Neural stem/progenitor cells (NSPCs) survive long-term, proliferate, and differentiate into glial cells and/or neurons after transplantation. No method is available at present to manipulate and control the behaviors of NPSCs to allow them to appropriately integrate into the host spinal cord. NPSP transplantation is not necessarily effective for locomotor improvement.

Key Words: *nervbone marrow stromal cell; choroid plexus epithelial cell; Schwann cell; neural stem/progenitor cell; axonal regeneration; locomotor improvement; clinical application; intrinsic regeneration ability*

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Introduction

Generally speaking, cell transplantation studies anticipate that transplanted cells survive long-term to be integrated into the host spinal cord tissue, serving as a scaffold for the outgrowth of regenerating axons in spinal cord injury. Schwann cells are one possible cells population. However, this anticipation is not necessarily applicable to some somatic cells: bone marrow stromal cells (BMSCs) did not survive long-term, disappearing from the spinal cord within 2–3 weeks after transplantation in rats. Nevertheless, the transplantation of BMSCs enhanced the outgrowth of regenerating axons and promoted locomotor improvement of rats. This suggests that BMSCs release some trophic factors that are effective for tissue repair and functional recovery in spinal cord injury (SCI). Transplantation of choroid plexus epithelial cells (CPECs) also enhanced the axonal regeneration and locomotor improvement in rats with SCI. However, they disappeared from the spinal cord shortly after transplantation, as in the case of BMSCs.

Immature cells, such as neural stem/progenitor cells (NSPCs), survive long-term, proliferate extensively, and differentiate into glial cells and/or neurons after transplantation. These properties are, although appearing to contribute to tissue repair and the establishment of new neural connections, regarded as deleterious factors from the clinical point of view of safety: no method is available at present to manipulate and control the behaviors of these cells to allow them to appropriately integrate into the host spinal cord.

Cell transplantation studies aiming at the regeneration of tissues or organs have significance only when they are clinically applicable under the condition that the transplants are safe and they promote functional improvements of recipients.

Bone Marrow-Derived Cells

BMSCs are cells that adhere to the dish on culture of a bone marrow perfusate. In an experiment in which BMSCs were transplanted by infusion through the cerebrospinal fluid (CSF) *via* the 4th ventricle, transplanted BMSCs attached to the spinal cord surface, and a few of them homed in on the spinal cord lesions. BMSCs did not survive long-term, disappearing before 3 weeks after infusion. However, locomotor functions were improved, and cavity formation was suppressed. Host spinal cord axons located near the lesions were spared, avoiding secondary degeneration in BMSC-transplanted rats (Ohta et al., 2004). In an experiment in which BMSCs were transplanted directly into the spinal cord lesions of rats with sub-acute SCI, transplanted BMSCs similarly survived short-term (1–2 weeks after transplantation)

as cell assemblies in the lesions, in which no astrocytes were present. Such astrocyte-devoid areas, although appearing empty on immunostaining for glial fibrillary acidic protein (GFAP), were filled with extracellular matrices, through which numerous axons extended (**Figure 1**). These axons were myelinated by Schwann cells, as in the case of peripheral nerves, indicating that they might not be spared but regenerated axons. Features of axons extending through the astrocyte-devoid areas suggest that they are regrowing axons from axotomized fibers, but not sprouts from spared axons. Cavity formation was reduced, and locomotor functions were improved by cell transplantation (Ide et al., 2010). In another experiment, BMSCs were infused through the CSF three times in rats with chronic (4 weeks after injury) SCI. The findings of axonal outgrowth through the astrocyte-devoid areas and reduced cavity formation were the same as those in the preceding studies of acute and subacute SCI.

Bone marrow mononuclear cells (BMNCs) were separated by density-gradient centrifugation from the bone marrow perfusate, and used without culture for transplantation to rats with SCI (Yoshihara et al., 2007). The findings were basically the same as those of BMSC transplantation.

The short-term survival with no integration into the host spinal cord of BMSCs and BMNCs suggested that they secrete some trophic factors that promote axonal regeneration and tissue repair, leading to locomotor improvements. The short-term survival of BMSCs and BMNCs, although appearing to be a disadvantage, guarantees the safety of their transplantation on clinical application. Based on these studies, BMSCs and BMNCs were clinically applied by lumbar puncture for patients with SCI. A total of 5 patients received BMSC transplantation up until 2009, and 10 patients received BMNC transplantation up until 2013 (Suzuki et al., 2014). In both clinical applications, there was no adverse effect, and patients showed varying degrees of improvement in motor, light touch, and pin-prick scores.

Choroid Plexus Epithelial Cells

The choroid plexus (CP) forms the ventricular wall of the brain, and consists of epithelial cells and the underlying pia mater. Choroid plexus epithelial cells (CPECs) are a continuation of ventricular ependymal cells, and the site of CSF production. In addition, CPECs have been studied as the primary gate for trafficking of the immune cells from the vascular system to the CSF in injuries and diseases of the CNS. It has been reported that CPECs secrete many kinds of trophic factors, including insulin-like growth factor (IGF), fibroblast growth factor (FGF), and hepatocyte growth factor (HGF), for the maintenance of normal CNS functions.

The first experiment on CP transplantation was performed using minced CP tissues as a transplant in spinal cord lesions. Numerous regenerating axons extended toward the transplanted CP tissues in the spinal cord, suggesting that the CP might be effective as a transplant for SCI. In an *in vitro* experiment, neurons associated with CPECs extended many long, elaborate neurites on the surface of CPECs. In another *in vitro* study, the conditioned medium (CM) of CPEC culture promoted neuronal survival and neurite extension of hippocampal neurons. On the other hand, Matsumoto et al. (2013) studied the effect of the transplantation of cultured CPECs on brain infarction by infusion into the CSF *via* the 4th ventricle, demonstrating that cultured CPECs markedly reduced infarcted lesions caused by carotid artery ligation. No transplanted CPECs entered the site of ischemic injury. This indicated that CPECs did not replace the infarcted tissue, but exerted their effects by releasing diffusible neurotrophic factors that reached the lesion through the CSF. The CPEC transplantation had effects on the functional recovery of infarction-impaired axons.

A recent study on the transplantation of cultured CPECs into spinal cord lesions demonstrated that CPECs enhanced the extension of regenerating axons in the lesions, and promoted locomotor improvements (Kanekiyo et al., 2016). CPECs did not survive long-term, disappearing from the spinal cord 2–3 weeks after transplantation. There were no findings suggesting the proliferation, differentiation, or migration of transplanted CPECs in the host spinal cord lesions. These characteristics of CPECs as transplants are the same as those of BMSCs and BMNCs. It has been suggested that CPECs may, as in the case of BMSCs and BMNCs, secrete some trophic factors effective for the recovery of spinal cord injury by promoting the "intrinsic" regeneration capacity of the spinal cord. This does not mean that neurotrophic factors work to transform intrinsic cells into new neurons or glial cells, but to enhance tissue repair, including axonal regeneration and cavity suppression. It is hoped that some humoral factors effective for spinal cord injury will be identified in the near future.

We conclude that the transplantation of BMSCs, BMNCs and CPECs arouses and enhances the "intrinsic" ability of the spinal cord to regenerate. Transplanted cells do not need to survive in the host spinal cord to serve as a scaffold for tissue repair, including axonal regeneration. Neurotrophic factors released from transplanted cells are responsible for locomotor improvements and tissue repair. This thought is not in line with the generally believed concept of cell transplantation studies for the treatment of SCI. This proposition is important as a new premise for the study of spinal cord regeneration and the treatment of SCI.

Schwann Cells

Since the epoch-making study showing that transplanted peripheral nerve segments provide a favorable environment for the growth of regenerating axons in the CNS, Schwann cells have been regarded as a key cell for providing an appropriate environment for the growth of regenerating axons in the CNS. Schwann cells are obtained from adult peripheral nerves: they are enzymatically dissociated from peripheral nerves, and after treatment with forskolin, cultured for several weeks. Thus, Schwann cells are derived from adult nerves with no genetic manipulation.

Numerous regenerating axons enter the Schwann cell transplants. Schwann cells serve as a scaffold for regenerating axons. However, after growing through the Schwann cell

Table 1 Comparison of transplants

Transplants	Cell fate	Function	Locomotor improvement	Clinical application
BMSC, BMNC, CPEC	Disappear	Neurotrophic factors from transplants promote axonal regeneration	Yes	Yes
Schwann cell	Survive	Scaffold for regenerating axons	Yes	Yes
NSPC	Survive (proliferation, and differentiation)	Supply of new neurons and glial cells Axonal outgrowth	No in some studies	No

CPEC: Choroid plexus epithelial cell; BMNC: bone marrow mononuclear cell; BMSC: bone marrow stromal cell; NSPC: neural stem/progenitor cell.

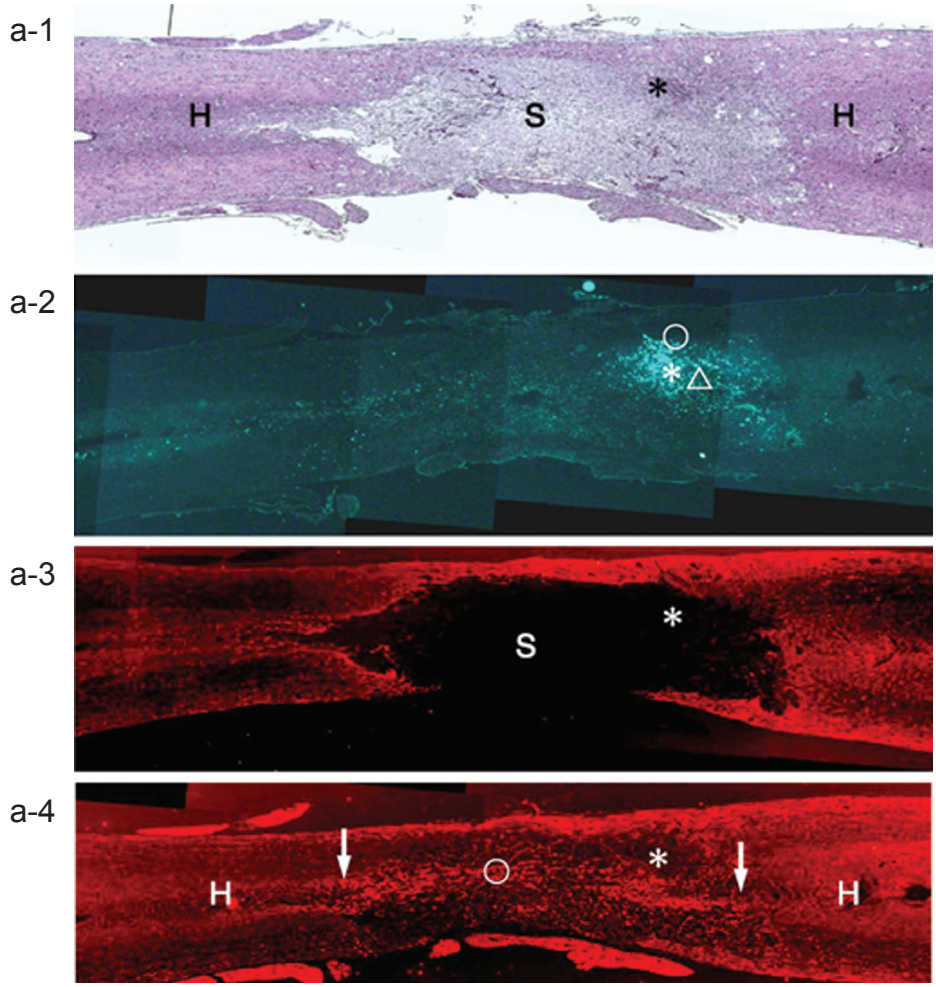

Figure 1 Axonal regeneration through the astrocyte-deroid area.
The spinal cord was contusion-injured at T_{8-9} in adult rats, and bone marrow stromal cells (BMSCs) were transplanted into the spinal cord lesion at 2 weeks post-injury. Rats were fixed at 1 week post-transplantation, and horizontal sections of the spinal cord lesion were observed. Left to right: rostro-caudal direction. (a-1): HE staining. The lesion is filled with tissue (S) different from the spinal cord parenchyma (H). An asterisk indicates the site of engrafted BMSCs shown in a-2. (a-2): The section adjacent to a-1. Engrafted BMSCs are found as cell assemblies (*). (a-3): Glial fibrillary acidic protein (GFAP) immunohistochemistry (red) in the section adjacent to a-2. A large astrocyte-devoid area (S) extends rostro-caudally. Engrafted BMSCs (site indicated by asterisk) are located at the border of the astrocyte-devoid area. (a-4): Immunohistochemistry for neurofilaments in the section adjacent to a-3. Numerous axons (red) extend in a bundle along the total length of the astrocyte-devoid area shown in a-3. There is no finding suggesting the blocking of extension of growing axons at the transition zones (arrows) on the rostral or caudal side. The asterisk indicates the site of engrafted BMSCs. H shows spinal cord parenchyma. From Ide et al. (2010).

assemblies, regenerating axons do not exit the transplants in the spinal cord lesion (Kanno et al., 2015). It is assumed that axons are blocked from extending through the glial scar formed at the border of the lesion. This disadvantage was overcome by the chondroitinase-digestion of chondroitin sulfate, local injection of growth factors (aFGF, GDNF), and/or co-transplantation of Schwann cells with other types of cells such as BMSCs and olfactory ensheathing cells. There is a controversy concerning the significance of glial scar. Recent studies suggest that the astrocyte scar is necessary for axonal regeneration in the spinal cord lesion (Anderson et al., 2016).

It was reported that 60–90% of the transplanted Schwann cells survived over 3 weeks after transplantation. Schwann cell proliferation peaked at 2 weeks, decreased thereafter, and ceased at 12 weeks post-transplantation. Schwann cells show moderate proliferation and migration, but no differentiation after transplantation. Schwann cells are well-integrated into the host spinal cord tissue (Deng et al., 2015). Therefore, they are considered safe as transplants, and available for clinical application. Similarly, olfactory ensheathing cells are regarded as effective and safe transplants for spinal cord injury. The Miami Project to Cure Paralysis at the University of Miami received permission from the FDA to begin a clinical trial of transplanting human autologous Schwann cells to treat patients with SCI in 2012. Schwann cells harvested from the sural nerve of the participant will be autologously transplanted into the epicenter of the participant's spinal cord injury. This clinical trial starts in January 2015, and ends in January 2018.

It was reported that there was a marked infiltration of endogenous Schwann cells into the host spinal cord tissue on Schwann cell transplantation. This finding is in line with our study showing that axons in the astrocyte-devoid areas were surrounded by Schwann cells in BMSC transplantation.

Neural Stem/Progenitor Cells

In the experiment, in which NSPCs were obtained from the fetal CNS, and transplanted directly into the spinal cord lesion or indirectly through the CSF, NSPCs survived, integrated, and migrated extensively. Some of them had differentiated into neurons and astrocytes by 4 weeks post-transplantation. NSPCs infused through the CSF *via* the 4th ventricle attached to the surface of the spinal cord, continued to proliferate, and occupied a large area of the spinal cord 3 weeks after transplantation (Bai et al., 2003). These findings were interesting from the point of view of basic science; however, we wondered at that time whether NSPCs with the potent properties of migration and proliferation were safe as transplants from a clinical point of view. Lu et al. (2014) used human iPSC-derived NSPCs as transplants. The spinal cord was transected at C_5, and NSPCs in fibrin matrices containing a growth factor cocktail were transplanted 2 weeks after transection. Observation was performed 3 months after transplantation. NSPCs filled the lesion cavities, differentiated into neurons, and extended numerous axons rostrally up to the midbrain and even to the cortex. Many axons also extended caudally up to T_6, or even to the T_{12} level. No myelination was noted on regenerated axons extending through the white matter of the host spinal cord. No functional recovery was observed. They suggested that ectopic innervations could potentially be manipulated and shaped with axon-guidance strategies. In addition to ectopic innervations, the proliferation, migration, and differentiation of transplanted cells should be appropriately manipulated in a clinical application. This means that NSPCs cannot be used for clinical purposes until methods are developed by which the behaviors of NSPCs can be appropriately manipulated and controlled.

Regarding functional recovery, NSPC transplantation is not necessarily effective for locomotor improvement, which is the essential parameter for the clinical application of cell transplantation (**Table 1**).

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