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Remyelination of the injured spinal cord

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Abstract

Contusive spinal cord injury (SCI) can result in necrosis of the spinal cord, but often long white matter tracts outside of the central necrotic core are demyelinated. One experimental strategy to improve functional outcome following SCI is to transplant myelin-forming cells to remyelinate these axons and improve conduction. This review focuses on transplantation studies using olfactory ensheathing cell (OEC) to improve functional outcome in experimental models of SCI and demyelination. The biology of the OEC, and recent experimental research and clinical studies using OECs as a potential cell therapy candidate are discussed.

Keywords

spinal cord injury; remyelination; olfactory ensheathing cells

Introduction

The clinical pathophysiology of contusive spinal cord injury (SCI) is complex. A combination of hemorrhage, ischemia and/or edema develops resulting in necrosis with tissue loss (Schwab et al., 2006, review). Additionally long tracts of the injured spinal cord may survive, but become demyelinated. Although regeneration of spinal cord axons is an ultimate objective, the presence in many patients with non-penetrating SCI of a population of surviving axons that do not conduct impulse due to demyelination (Keirstead, 2005; Kocsis and Sasaki, 2005, reviews), suggests a cell-based approach for remyelination as a potential strategy for inducing recovery of function in SCI. One approach to this goal capitalizes on recent progress in the transplantation of cells into the injured CNS. Cellular transplantation of appropriate cells into experimental models of SCI can promote axonal regeneration, provide neuroprotective effects by secretion of neurotrophins and remyelinate axons.

One cell of particular interest as a cell therapy candidate to both encourage axonal remyelination and regeneration is a specialized glial cell, the olfactory ensheathing cell (OEC). Adult olfactory receptor neurons continually undergo turnover from an endogenous progenitor pool, and their nascent axons grow through the olfactory nerves and cross the PNS–CNS interface, where they form new synaptic connections in the olfactory bulb (Graziadei et al.,

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1978). OECs associate with olfactory receptor neurons from their peripheral origin to their central projection in the outer nerve layer of the olfactory bulb (Doucette, 1991). This putative support role of OECs in axonal growth within the adult CNS has spawned extensive research to study the potential of OEC transplants encouraging axonal regeneration and functional recovery in SCI models (Li et al., 1997, 1998; Ramon-Cueto et al., 1998, 2000; Imaizumi et al., 2000a, b).

Transplantation of OECs after SCI is associated with functional improvement even when transplantation is delayed by weeks (Lu et al., 2002; Keyvan-Fouladi et al., 2003). While the precise mechanisms of the functional recovery after OEC transplantation are not fully understood, several mechanisms including elongative axonal regeneration, axonal sparing, sprouting and plasticity associated with novel polysynaptic pathways, recruitment of endogenous SCs and remyelination have been proposed (Raisman, 2001; Bareyre et al., 2004; Sasaki et al., 2004; Keyvan-Fouladi et al., 2002). In animals with SCI and OEC transplants, myelinated axons spanning the lesion site display a characteristic peripheral pattern of myelination similar to that of Schwann cell (SC) myelination (Franklin et al., 1996; Li et al., 1997, 1998; Imaizumi et al., 1998, 2000b; Sasaki et al., 2004).

Remyelination by transplantation may be of particular importance because contusive SCI often results in loss of function from demyelination induced by spinal cord trauma. OECs can form myelin when transplanted into demyelinated spinal cord (Franklin et al., 1996; Imaizumi et al., 1998; Sasaki et al., 2004). Moreover, human OECs can form myelin in the immunosuppressed rat (Barnett et al., 2000; Kato et al., 2000) and OECs transplanted into the non-human primate can remyelinate spinal cord axons (Radtke et al., 2004).

Remyelination in demyelinated spinal cord

Three types of SCI models are commonly used in experimental studies in rodents: transection, compression and contusion (Rosenzweig and McDonald, 2004, review). However, because of the complex pathophysiological conditions of SCI in these models, the study of remyelination is more complex. Aside from the heterogeneity of the lesion, endogenous remyelination by SCs occurs in these model systems thus making distinction of endogenous remyelination from that of transplanted cells more complex. A model used by several groups including ours to study remyelination by transplanted cells is the X-irradiation/ethidium bromide model (X-EB). Ethidium bromide (EB), a nucleic acid chelator, kills cells in the target zone (dorsal white matter) and focal X-irradiation to blocks mitosis and kills oligodendrocyte progenitors (Blakemore and Crang, 1985). In this model system, endogenous repair in the center of the lesion is delayed by 6–8 weeks thus allowing a stable time window to assess transplanted cells (Honmou et al., 1996). Generally our analyses are carried out within 3 weeks after X-EB lesion induction, which is well within the time at which persistent demyelination is confirmed in a large number of control studies (Blakemore and Crang, 1985; Franklin et al., 1996; Honmou et al., 1996; Kato et al., 2000). The lesion induced by this procedure is characterized by virtually complete loss of endogenous glial elements (astrocytes and oligodendrocytes) with preservation of axons (Fig. 1A). The higher power light micrograph of the lesion shows compact fields of demyelinated axons in close apposition separated by areas of myelin debris and macrophages (Figs. 1A2, A3). The lesion occupies nearly the entire dorso-ventral extent of the dorsal columns for 5–7 mm longitudinally.

A large body of work supports the proposal that transplantation of OECs into various SCI and demyelination models can promote axonal regeneration, remyelination and functional recovery (Franklin et al., 1996; Li et al., 1998; Ramon-Cueto et al., 1998; Imaizumi et al., 2000a, b; Ramon-Cueto et al., 2000; Lu et al., 2002; Keyvan-Fouladi et al., 2003; Plant et al., 2003). Yet, there is an important controversy as to whether the transplanted OECs associate with axons

and form peripheral myelin, as opposed to recruiting endogenous SCs that form myelin (Takami et al., 2002; Boyd et al., 2004). OECs can express a number of trophic factors, transcription factors and extracellular matrix molecules (Ramon-Cueto et al., 1998; Chuah and West, 2002; Au and Roskams, 2003; Ramer et al., 2004), which could facilitate endogenous SC cell invasion, angiogenesis and activation of progenitor cells to facilitate repair.

To address this issue, we used OECs from GFP-expressing rats. After 3 weeks of transplantation into X-EB model, methylene blue/Azure II semithin plastic sections demonstrated extensive remyelination in the demyelinated axons (Fig. 1B). To fully establish that the OECs derived from GFP rats were indeed responsible for the remyelination, immunoelectron microscopy (EM) for GFP was performed. Intense reaction product was observed within the cytoplasm and nuclei of cell profiles surrounding myelinated axons (Fig. 1C). To establish a first approximation of the relative contribution of donor cell to host cell remyelination, quantitative analysis was performed on GFP immuno-EM experiments of two transplanted animals. A total of 136 axons were counted in the lesions, and of these 120 were GFP⁺ cells. Of the GFP⁺ cells, four (3.3%) showed no direct contact with axons. The myelin-forming status of five GFP⁺ cells (4.2%) could not be determined because of poor membrane preservation. Of the remaining 111 GFP⁺ cells, 90 cells (81%; 90 of 111) showed a distinct myelin structure. The remaining 21 GFP⁺ cells (19%; 21 of 111) appeared to be in varying stages of loose wrapping or ensheathing the axons but did not show distinct compact myelin. Within this same region, 16 myelinated axon profiles were detected, which were associated with myelin-forming cells that did not contain DAB reaction product. An example of a non-GFP myelinated axon adjacent to a GFP⁺ myelinating cell is shown in Figs. 1, D1 and D2. Note the presence of a basement membrane surrounding both myelinated axons. In frozen sections, OECs from donor GFP rats (GFP-OEC) exhibited a robust distribution within the X-EB lesioned spinal cord dorsal columns. The GFP-OECs were easily distinguished at this time point by their green fluorescence and were distributed longitudinally along the dorsal columns for ~8 mm (Fig. 2A). GFP-OECs were mostly confined to the lesion zone of the dorsal funiculus. GFAP immunostaining indicated a near absence of astrocytes within the lesion site, but intense GFAP staining was observed at the outer boundary of the lesion zone (Figs. 2B, C). In contrast to GFAP immunolabeling, immunostaining for P0, a specific marker of peripheral myelin (Greenfield et al., 1973), was primarily localized within the lesion and transplantation site (Figs. 2D, E). Ringlet-like P0 immunostaining was associated with GFP-OECs and was surrounded by GFP⁺-OEC cytoplasm, indicating the transplanted cells within the dorsal column lesion formed that peripheral-type myelin. Immunostaining for P0 and NF in coronal section reveals a central NF⁺ axonal core surrounded by P0-identified myelin, which is wrapped by cytoplasm of a GFP-OEC (Fig. 2E), indicating that transplanted OECs remyelinate the demyelinated axons.

Moreover, we also transplanted highly purified OECs isolated from transgenic pigs expressing the alpha1, 2 fucosyltransferase gene (H-transferase or HT) into a demyelinated lesion of the African green monkey spinal cord (Radtke et al., 2004). Four weeks post-transplantation, robust remyelination was found in 62.5% of the lesion sites, whereas there was virtually no remyelination in the non-transplanted controls. This was the first demonstration that xenotransplantation of characterized OECs into the primate spinal cord results in remyelination. This together with the immunohistochemical demonstration of the grafted cells within the lesioned area confirmed that remyelination was indeed achieved by OECs.

Nodal reconstruction of remyelinated spinal cord axons

The restoration of rapid and secure impulse conduction after demyelination is dependent on the acquisition of myelin sheaths and the clustering of specific molecules within discrete domains of the myelinated axon membrane. In myelinated axons, voltage-gated sodium

(Na_v) channels are aggregated in high density at nodes of Ranvier, whereas *Shaker*-type potassium (K_v1) channels are separated from nodal Na_v channels by septate-like paranodal junctions (Peles and Salzer, 2000; Rasband and Trimmer, 2001; Girault and Peles, 2002). Of the seven Na_v channel isoforms expressed in nervous tissue (Goldin et al., 2000), Na_v1.6 is the predominant one at mature nodes in both the PNS and CNS (Caldwell et al., 2000; Boiko et al., 2001) following a transition from Na_v1.2 (Boiko et al., 2001; Kaplan et al., 2001; Jenkins and Bennett, 2002; Rios et al., 2003). The channel clustering (Vabnick et al., 1997; Rasband et al., 1999) and the transition from Na_v1.2 to Na_v1.6 (Boiko et al., 2001; Rios et al., 2003) are dependent on interaction of the axon with myelinating cells (Kaplan et al., 1997; Eshed et al., 2005). Remyelinated axons display inappropriately short internodal lengths (Gledhill and McDonald, 1977; Weiner et al., 1980; Blakemore and Murray, 1981; Hildebrand et al., 1985), indicating that new nodes are formed. Despite their location at formerly internodal sites, remyelinated PNS axons have been shown to display high densities of Na_v channels at nodes (Novakovic et al., 1996, 1998) and K_v1 aggregations within juxtaparanodal domains (Rasband et al., 1998). The expression and organization of specific isoforms of Na_v and K_v1 channels in remyelinated CNS axons have not been examined. Recently we reported that EM analysis of spinal cords performed at 3 weeks after GFP-OEC transplantation demonstrated distinct nodes of Ranvier (Fig. 3). Large cytoplasmic and nuclear compartments were present in cells associated with the myelin profiles (Fig. 3A). Longitudinal sections of the dorsal columns revealed well formed nodal and paranodal regions; paranodal loops from adjacent myelin-forming cells were readily recognized flanking nodes (Figs. 3B1, B2). Also, in immunohistochemical analysis we observed Na_v1.6 staining at most nodes, whereas detectable Na_v1.2 immunostaining was not apparent at nodes (Fig. 4). In dorsal columns 3 weeks after transplantation, virtually all nodes bounded by GFP-OEC myelin sheaths exhibited Na_v1.6 staining (Figs. 4A–D); similar to control spinal cord axons, Na_v1.2 immunolabeling was not observed at any nodes (Figs. 4E–H). The Na_v1.6 labeling was localized to the nodal domain and was not observed in paranodal or juxtaparanodal regions or beneath the myelin sheath in remyelinated axons, suggesting that the transplanted GFP-OECs are competent to contribute to the specific clustering of Na_v channels at nodes. As an additional determinant of the ability of axons myelinated by GFP-OECs to support the asymmetric organization of ion channels within remyelinated nodal regions, we examined the distribution of K_v1.2 in the juxtaparanodal region. K_v1.2, as well as K_v1.1, form heteromultimers with K_v1.4 and K_vβ2 (Wang et al., 1993; Rasband et al., 1998) and have been shown previously to be aggregated in juxtaparanodal regions of most spinal cord axons (Rasband et al., 1999; Rasband and Trimmer, 2001). At 3 weeks (Figs. 4I–L), K_v1.2 is aggregated within juxtaparanodal regions of the remyelinated axons, with some nodes exhibiting incursion of K_v1.2 channels into adjacent paranodal regions. K_v1.2 was not observed within nodal areas in these axons.

Transected spinal cord

Distribution and myelin formation by transplanted GFP-OECs within the spinal cord

Spinal cord injuries without OEC transplants can show limited SC-like myelination, presumably from invasion of the injury site from endogenous SCs (Brook et al., 1998; Imaizumi et al., 2000a, b; Namiki et al., 2000; Takami et al., 2002) or possibly from precursor cells. The degree to which OECs can integrate into injury sites and survive and whether they form myelin or facilitate endogenous myelin repair mechanisms was controversial. OECs in culture are diverse and exhibit characteristics of astrocytes, SCs and oligodendrocytes. Moreover, they express a number of trophic factors, transcription factors and extracellular matrix molecules (Ramon-Cueto and Avila, 1998; Chuah and West, 2002; Au and Roskams, 2003) that could facilitate endogenous cell invasion, as well as angiogenesis and activation of progenitor cells. A recent study was unable to find evidence of myelination in the compressed spinal cord by the OECs isolated from embryonic day 18 rat, infected with a LacZ-expressing retrovirus, and

suggests that OEC transplantation may facilitate endogenous SC invasion into the lesion site (Boyd et al., 2004).

We transplanted genetically labeled GFP-OECs into a dorsal funiculus transection model in the rat. Five weeks after OEC transplantation, the cells survived and distributed extensively within the transection site and more limitedly beyond the lesion site (Fig. 5A1). High-power micrographs (Fig. 5A2) of AnkyrinG and Caspr staining demonstrate nodal and paranodal regions, respectively, associated with some transplanted GFP-OECs, suggesting forming a new node of Ranvier. Methylene blue/Azure II semithin plastic sections through this region of the lesion 5 weeks after transplantation revealed greater structural detail (Fig. 5B). As reported initially by Li et al. (1997), small groups of myelinated axons were often surrounded by a non-myelinating cell forming tube-like structures around the myelinated axons. This organization is not observed after endogenous repair or after transplantation of SCs (Imaizumi et al., 2000b). These profiles were observed in both dorsal and ventral regions of the lesion zone (Fig. 5B). EM further indicates that the myelinated axons in the lesion zone are surrounded by cytoplasmic extensions of cells forming tunnels (Fig. 5C). These clusters of myelinated axons were variable from animal to animal and confined mostly to and near the lesion zone. Rings of P0 labeling were observed in some regions of the lesion and were often associated with GFP cellular elements. EM examination of anti-GFP immunoperoxidase-reacted sections revealed that many detectable GFP⁺ cells were in direct contact with host axons. Reaction product was clearly evident in the cytoplasm of many cells that formed well-defined multi-laminate structures characteristic of myelin (Fig. 5D). In a longitudinal section of a myelinated axon, intense reaction product can be seen in the cytoplasm of the myelin-forming cell. Large cytoplasmic and nuclear regions as well as the presence of basal lamina and extracellular fibrils (Fig. 5D) indicate a peripheral pattern of myelination.

Taken together, we conclude that transplanted GFP-OECs integrate into the injury site of a dorsal funiculus transection, distribute and associate longitudinally with axons spanning the lesion site and do form myelin.

Improved hindlimb locomotor function

All OEC-transplanted animals in this model system exhibited a gradual improvement in hindlimb locomotor function during the 5-week recovery period (Fig. 6). The sham injection group recovered to a Basso, Beattie and Bresnahan (BBB) locomotor rating scale (Basso et al., 1995) of ~9 and did not display weight-bearing plantar stepping. However, the OEC transplant group recovered to ~18 on the BBB score and displayed consistent weight-bearing plantar stepping. Statistical analysis indicated that the open-field locomotor scores at 3, 4 and 5 weeks after injury and OEC transplantation was significantly higher than sham injection.

Neuroprotection of dorsal corticospinal tract

A recent study demonstrates that primary motor cortex (M1) pyramidal neurons undergo apoptotic cell death after axotomizing SCI (Hains et al., 2003). Moreover, earlier work has shown that corticospinal neurons become atrophic after spinal cord transection (McBride et al., 1989). To explore the possibility that OECs are neuroprotective for injured corticospinal tract (CST) neurons, we transplanted OECs into the dorsal transected spinal cord (T9) and examined M1 to assess apoptosis and neuronal loss at 1 and 4 weeks post-transplantation. Triple labeling with Hoechst, Fluorogold (FG), and terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate–rhodamine nick end-labeling (TUNEL) identified putatively axotomized pyramidal cells in the SCI group, with and without spinal OEC transplantation. The neuronal subset undergoing apoptosis was readily identified in 1-week tissue. Within these cells, nuclear morphologies of both apoptotic and surrounding normal neurons and glia could be identified (Figs. 7A, B). Hoechst staining was observed in all cells

and uniformly filled the spherical nuclear compartment. In a proportion of cells undergoing apoptosis, Hoechst staining revealed abnormal morphology, including the formation of chromatin condensates within a less-distinct nucleus (Hains et al., 2003). Merging of Hoechst, FG and TUNEL staining (Figs. 7A, B) showed that irregular nuclear fragments identified by Hoechst staining overlap securely with FG- and TUNEL-positive cells, permitting the confident designation of an apoptotic status. The nuclear morphology of TUNEL-positive neurons was identical to that seen in our earlier paper (Hains et al., 2003) (Fig. 7A, insets). Apoptotic (FG- and TUNEL-positive cells) pyramidal neurons were observed both after SCI + FG+DMEM (Fig. 7A, arrowheads) and in the SCI+FG+OEC group (Fig. 7B, arrowheads), but the number of apoptotic neurons was significantly reduced in the SCI+FG+OEC group (Fig. 7C). At 1 week after injury, the number of apoptotic neurons was significantly ($P<0.05$) reduced by 45% (16.4 ± 6.40 vs. 36.1 ± 5.69) in the OEC transplant group compared with the SCI+FG+ DMEM group (Fig. 7C). At 4 weeks, both the SCI+FG+DMEM and SCI+FG+OEC groups showed apoptotic activity comparable with sham controls (0.98 ± 0.81 and 0.75 ± 0.56) (Fig. 7C). We described the supraspinal effects of OEC transplantation on apoptosis and cell survival of CST neurons within the M1 cortex after transection of their axons in the spinal cord. Our results indicate that apoptosis of primary motor cortical neurons is reduced and that cortical neuronal density is increased after OEC transplantation. Enhanced levels of brain-derived neurotrophic factor (BDNF) were observed in the OEC transplanted lesion. Thus, transplantation of OECs into injured spinal cord has a neuroprotective effect on corticospinal neurons. The relative contribution of this effect to the observed functional improvement after OEC transplantation is uncertain, but this data indicates that OEC transplantation results in a larger pool of surviving corticospinal neurons. Thus, OEC transplantation into the injured spinal cord has distant neuroprotective effects on descending cortical projection neurons as well (Sasaki et al., 2006b).

OEC into spinal cord contusion injury

We also evaluated the *in vivo* fate of OECs transplanted into the contused rat spinal cord with the weight-drop device developed at New York University (NYU impactor) (Li et al., 2004). Transplanted GFP-OECs were distributed widely within transplanted spinal cords. In sagittal sections, GFP-OECs were widely distributed along the length of the lesion (Fig. 8A). Immunohistochemistry using an anti-GFP antibody revealed that the identified OECs made direct and extensive contact with host axons (Figs. 8B, C). Transplanted GFP-OECs produced multi-laminate structures surrounding axons in a one-to-one relationship with large amounts of cytoplasm and basal lamina characteristic of peripheral myelin. The typical relationships of transplanted SCs and OECs with host axons were quite different. While the overwhelming majority of GFP positive cells in both transplant conditions were in direct contact with host axons, GFP positive SCs cells were observed almost exclusively in one-to-one associations with host axons, with a high proportion of those contacts associated with myelination, while GFP positive OECs typically wrapped or engulfed several axons with a small percentage of contacts associated with myelination.

Clinical perspectives

Clinical investigations in SCI using OEC transplantation are in progress. A recent report tested the feasibility and safety of transplantation of autologous OECs into the injured spinal cord in human paraplegia in a single blind, Phase I clinical trial (Feron et al., 2005). In this study, 1 year after cell implantation, there were no medical, surgical or other complications to indicate that the procedure is unsafe. They conclude that transplantation of autologous OECs into the injured spinal cord is feasible and is safe up to 1 year post-implantation. Long-term safety studies to compare neurological, functional and psychosocial outcomes are underway. Furthermore, fetal brain tissue has been transplanted into the lesions of more than 400 patients

with SCI in China (Senior, 2002; Huang et al., 2003). Dobkin et al. (2006) compared available reports from China with the experiences and objective findings of patients who received pre-operative and post-operative assessments before and up to 1 year after receiving cellular implants. They concluded that the phenotype and the fate of the transplanted cells, described as OECs in the China study, are unknown. Perioperative morbidity and lack of functional benefit were identified as the most serious clinical shortcomings. The procedures observed did not attempt to meet international standards for either a safety or efficacy trial. In the absence of a valid clinical trial protocol, Dobkin et al. (2006) suggest that physicians should not recommend this procedure to patients.

Concluding remarks

Remyelination of the injured spinal cord is one of the key elements for the functional recovery in SCI. Extensive experimental rodent models of SCI describe the feasibility of OEC transplantation strategy as an approach that may elicit some degree of functional recovery, raising the possibility that this approach might be useful in the treatment of SCI. Experimental work indicates that cell transplantation approaches can facilitate axonal regeneration, remyelination, neuroprotection and possible neovascularization. Importantly, axons remyelinated by transplanted OECs form appropriate nodal sodium channel and conduction is improved. Several clinical studies are ongoing using cell therapy approaches for SCI (Senior, 2002; Dobkin et al., 2006). Future translational studies are highly expected to bridge the gap between basic and clinical research in OEC transplantation for SCI.

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Abbreviations

BBB	Basso, Bresnahan and Beattie (1995 i.e., and their rating scale for locomotion)
BDNF	brain-derived neurotrophic factor
CNS	central nervous system
CST	corticospinal tract
DMEM	Dulbecco's modified Eagle's medium
EB	ethidium bromide
EM	electron microscopy
FG	Fluorogold
K_v1	

	<i>Shaker</i> -type potassium
M1	primary motor cortex
Na_v	voltage-gated sodium
NYU	New York University
OEC	olfactory ensheathing cells
PNS	peripheral nervous system
SC	Schwann cells
SCI	spinal cord injury
TUNEL	terminal deoxynucleotidyltransferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-rhodamine nick end-labeling

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Fig. 1. Light micrographs of transverse sections of the dorsal spinal cord stained with methylene blue/azure II showing the dorsal funiculus in demyelinated (A) and OEC transplanted (B) rats. Examination at higher magnification show demyelinated (A2, A3) and remyelinated (B2, B3) axons in the dorsal columns. Note the extensive myelination after transplantation of OEC (B). Many myelin-forming cells were similar to peripheral myelin-forming cells, characterized by large nuclear and cytoplasmic regions (B3). A2, B2 and A3, B3 were prepared from the boxed area in A1, B1 and A2, B2 respectively. Immuno-EM for GFP shows numerous GFP⁺ cells remyelinating the demyelinated axons (C, D). Counterstaining for these sections was minimal, and dark staining shows electron dense immunoperoxidase reaction product. Note the electron dense reaction product in the cytoplasm and nuclei of most cells forming myelin (C, D). One cell associated with myelin in this field displays distinct reaction product, whereas an adjacent cell does not have electron dense reaction product in its cytoplasm (D1). D2 is an enlargement of the box in D1. Note the dense reaction product in the cytoplasm of the myelin-forming cell on the right and the basement membrane surrounding the cell. Scale bars = 700 μ m (A1, B1);

70 μm (A2, B2); 1 μm (A3, B3); 5 μm (C); 2 μm (D1); 0.4 (D2). C and D are modified with permission from Sasaki et al. (2006a).

Fig. 2.

Sagittal frozen sections through the lesion site demonstrate the distribution of transplanted GFP-OEC. Transplanted cells are primarily confined to the lesion site. Some cells migrated into the deep white matter. The dashed line demarcates lesion edge (A). Coronal frozen sections in the lesion show the presence of GFP-OEC within a lesion site. Transplanted cells survived primarily in the dorsal funiculus. There was little GFAP staining within the lesion zone (B). GFAP-positive cells were present at the peripheral margin of the lesion. These results indicate that few astrocytes are present in the transplant region, and that there is a preponderance of GFP-OEC in the lesion zone (B, C). P0-immunostaining (red) of the frozen coronal section reveals that most axons remyelinated by the transplanted OECs are surrounded by peripheral

type of myelin. Red-P0 rings are associated with green cellular elements, indicating that transplanted OECs remyelinate the demyelinated axons (D). Expansion of a cell indicated by an arrow (D inset). P0 and neurofilament (NF) staining at lesion boundary (dashed line) and transplant zone (E). Higher magnification showing neurofilament-defined axon cores surrounded by P0 myelin rings enwrapped by GFP-OECs (E2). B–E are coronal. Scale bars: 1 mm (A); 400 μm (B); 30 μm (C); 10 μm (D); 20 μm (A, inset); 10 μm (B, inset); 30 μm (E1); 10 μm (E2); 2.5 μm (E2, inset). Modified with permission from Sasaki et al. (2006a).

Fig. 3.

Nodal structure of remyelinated axons (A, B) at 3 weeks after OEC transplantation. Sagittal section showing a field of myelinated axons interspersed among donor OECs. Note the node structure in the center of the field (A). High-power electron micrograph showing node and paranodal loops (B1). B2 is an enlargement of the boxed area in B1. Scale bars = 5 μm (A); 1 μm (B1); 0.5 μm (B2). Modified with permission from Sasaki et al. (2006a).

Fig. 4. $\text{Na}_v1.2$ and $\text{Na}_v1.6$ at GFP-OEC nodes in remyelinated dorsal columns. At 3 weeks after transplantation, $\text{Na}_v1.6$ clustering is displayed at most Caspr-delimited (A–D) nodes formed by GFP-OECs at 3 weeks. In contrast, Caspr-delimited nodes formed by GFP-OECs do not exhibit $\text{Na}_v1.2$ immunostaining (E–H). Merged images of A–C and E–G are shown in D and H, respectively. Juxta-paranodal $\text{K}_v1.2$ immunolabeling at 3 weeks after GFP-OEC transplantation dorsal columns. Paranodes display Caspr staining (I) that is flanked by $\text{K}_v1.2$ aggregations within juxtaparanodal regions. Merged images of I–K are shown in L, respectively. Scale bars = 10 μm . Modified with permission from Sasaki et al. (2006a).

Fig. 5.

Montage image of sagittal frozen section showing distribution of GFP-OECs within and beyond the transection site (A). Semithin plastic sections stained with methylene blue/Azure II through the transection site 5 weeks after transplantation of OECs. Low-power micrograph showing completeness of the transection through the entire dorsal funiculus and beyond (B). EM from the same lesion showing myelinated axons surrounded by a cellular element forming a tunnel (C). EM of anti-GFP immunoperoxidase staining of OEC transplant (D). Reaction product can be seen in cytoplasmic regions of the myelin-forming cell but not in the myelin. A crosscut section of axon showing cytoplasmic reaction product. Enlargement of the boxed area is shown in D2. Note the presence of extracellular fibrous elements in D1. Scale bars = 250 μm (A); 0.75 μm (B); 5 μm (C); 1 μm (D1); 0.25 μm (D2). Modified with permission from Sasaki et al. (2004).

Fig. 6. Open-field locomotor scores for OEC transplant ($n = 20$) and sham injection ($n = 6$) groups tested 1 week before and for 5 weeks after transplantation. Modified with permission from Sasaki et al. (2004).

Fig. 7.

Hoechst 33342, Fluorogold (FG), and TUNEL triple labeling of corticospinal neurons 1 week after injury. Hoechst staining of non-TUNEL-positive (arrows with tails) and TUNEL-positive (arrowheads) neurons with corresponding FG-backfilling are shown. In SCI+FG+OEC animals (B), fewer TUNEL-positive FG-backfilled neurons are observed compared with SCI+FG+DMEM (A). Insets in A show two TUNEL-positive neurons exhibiting nuclear compartmentalization and formation of nucleosomes, hallmarks of apoptosis. Quantification of neurons that are both TUNEL- and FG-positive (C) reveals that OEC transplantation significantly ($*P<0.05$) reduces apoptotic cell death at 1 week. No evidence of death was

observed at any other time-point. Scale bars = 125 μm in A, B; 20 μm in inset in A. Modified with permission from Sasaki et al. (2006b).

Fig. 8.

Distribution of transplanted OECs into a contused rat spinal cord (A). Sagittal section of a segment of the spinal cord ~8 mm rostral and caudal to the injured area showed transplanted OECs concentrated near the center and distributed along the longitudinal axes of the spinal cord at 3 weeks after transplantation (A), arrows indicated transplantation points. A2 and A3 are enlargements of boxed areas in A1. Immuno EM for GFP revealed that many detectable GFP⁺ cells were in direct contact with host axons (Arrows). Reaction product was clearly evident in the cytoplasm of many cells that formed well-defined multi-laminate structures characteristic of myelin at 4 weeks after transplantation (8B, C). Scale bars = 1 mm (A1); 100 μ m (A2, A3); 5 μ m (B); 1 μ m (C).