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Review

Remyelination after olfactory ensheathing cell transplantation into diverse demyelinating environments

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ABSTRACT

Olfactory ensheathing cells (OECs) can remyelinate demyelinated spinal cord axons when transplanted into chemically induced demyelinated lesions. Cell transplantation is typically performed within a few days after lesion induction, i.e. during active demyelination when myelin debris, cytokine level increases and macrophage/microglia activation is extensive. Inflammatory signaling has been suggested to facilitate remyelination in cell transplant studies. In this review we discuss the migration and remyelination properties of OECs transplanted into various demyelinating lesion environments including conditions when inflammation is active and when it is largely subsided. While sharing many common properties, comparisons of the in vivo fate between OECs and SCs suggest unique properties of OECs as compared to SCs. A complicating factor in the assessment of experimental remyelination by transplantation of myelin-forming cells in general is the rapidity of endogenous myelin repair in most rodent models of demyelination. Alternative persistent demyelination models are discussed as potential tools to study both the competency of chronic demyelinated axons for remyelination and the remyelination potential of cells such as human progenitors that require longer times to mobilize and remyelinate axons. This article is part of a Special Issue entitled: Understanding olfactory ensheathing glia and their prospect for nervous system repair.

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Contents

Introduction

Olfactory ensheathing cells (OECs) and Schwann cells (SCs) share a number of molecular and morphological markers, but have different embryonic origins. OECs are from the olfactory placode and SCs are derived from the neural crest. OECs have both a spindle and a flattened morphology; however, SCs typically are spindle shaped cells in culture. Both cell types express p75, S100, GFAP, and cell adhesion molecules such as L1 and N-CAM (Bartsch, 2003, review; Pellitteri et al., 2010, review). Moreover, both express extracellular matrix molecules such as laminin and fibronectin (Wewetzer et al., 2002, review). OECs and SCs are a rich source of trophic factors including NGF, BDNF, GDNF, CNTF, FGF and VEGF, but the expression of these

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factors may vary depending on culture conditions or the particular in vivo microenvironment (Ramón-Cueto and Avila, 1998; Chuah and West, 2002; Au and Roskams, 2003; Oudega and Xu, 2006). Thus, while of different embryonic origin both cell types have remarkable similarity in terms of their molecular markers. Yet, several unique properties of OECs have been described (Boyd et al., 2006; Franssen et al., 2008; Lankford et al., 2008) suggesting that their in vivo behavior may differ in some conditions from SCs (Kocsis et al., 2009).

Transplantation of myelin-forming cells such as OECs, SCs and oligodendrocyte precursor cells (OPCs) can remyelinate acute demyelinating lesions induced by chemical agents such as lysolecithin or ethidium bromide (EB) in the rat spinal cord (Blakemore and Crang, 1985; Franklin et al., 1996; Honmou et al., 1996, Imaizumi et al., 1998, Kato et al., 2000; Akiyama et al., 2004; Sasaki et al., 2006a; Tetzlaff et al., 2010 review). Chemically induced demyelination in rodent spinal cord is followed by relatively rapid (within a few weeks) and robust remyelination by either endogenous OPCs or SCs (Black et al., 2006). This robust endogenous remyelination potential in the rodent is problematic for cell transplantation studies to study the myelinating potential of a particular cell type because the transplanted cells will compete with endogenous progenitor cells.

Blakemore and Crang (1985) developed a novel demyelination model to provide for a more persistent demyelinating lesion in the rat. In this model several centimeters of rat spinal cord are X-irradiated and a few days later a focal injection of EB is applied to the dorsal funiculus (Honmou et al., 1996; Sasaki et al., 2006a). The irradiation kills $NG2^+$ OPCs (Blakemore and Patterson, 1978) and the EB chelates nucleic acids and kills both oligodendrocytes and astrocytes within the injection site. An aglial demyelinating lesion is formed and persists for 6–8 weeks. This X-EB lesion model is useful for studying the fate of transplanted myelin-forming cells because the competition with endogenous cells is eliminated for up to a couple of months rather days or weeks. With time OPCs or SCs outside of the X-irradiation field will repopulate the lesion site and remyelinate the axons.

Talbott et al. (2006) grafted OPCs into the X-EB lesioned spinal cord; the OPCs differentiated into Schwann cell-like peripheral myelinating cells. However, when OPCs were grafted along with astrocytes, a natural source of noggin that inhibits bone morphogenetic protein (BMP) signaling in vivo, $P0^+$ myelin was not found. Some grafted cells at the edge of the lesion differentiated into central myelinating MBP^+ cells. Talbott et al. (2006) further demonstrated that inhibition of bone morphogenetic protein (BMP) signaling through noggin overexpression by engrafted adult OPCs is sufficient to block SC-like differentiation within this lesion and concluded that SC-like differentiation of OPCs derived from adult spinal cord is BMP dependent.

The literature regarding the survival, remyelinating potential and functional outcome of acutely transplanted OECs in the injured spinal cord is controversial. There are many claims of axonal regeneration that cannot be confirmed independently by others (Tetzlaff et al., 2010). As Tetzlaff et al. (2010) discussed, the reasons for these discrepancies are not fully understood, although experimental bias, variability of the cell sources and culture conditions, and animal or injury model systems are all likely contributing factors. Boyd et al. (2004) reported the failure of fetal-derived OECs to myelinate axons in vivo. They did not find LacZ-expressing cells after transplantation of embryonic day 18-derived rat Lax Z-expressing OECs into a spinal cord compression injury model, but only LacZ-expressing fibroblast-like cells. They concluded that only fibroblast-like cells are derived from the OEC transplantation and that the SC-like cells are derived exclusively from invading SCs, suggesting contaminating SCs may be responsible for the remyelination reported by others. These investigators also claim that OECs, but not SCs, express the muscle fiber actin binding protein calponin (Boyd et al., 2006; Rizek and Kawaja. 2006; Jahed et al., 2007), and that most cells in OEC culture preparations are $p75^+$ and $calponin^-$, thus suggesting a contamination in OEC cultures with Schwann cells. Recent counter studies by Franklin and colleagues, however, clearly demonstrated that calponin was present in the olfactory fibroblast meningeal cells, but not in adult OECs in rodent (Ibanez et al., 2007) and canine (Ito et al., 2008), thus strengthening the argument that remyelination following OEC transplantation was not the result of SC contamination. We observed remyelination by eGFP-OECs transplanted into a dorsal transection (Sasaki et al., 2004), a contusive SCI lesion (Sasaki et al., 2007), and into transected peripheral nerve where they remyelinated regenerated sciatic nerve axons (Dombrowski et al., 2006; Radtke et al., 2009). Thus, OECs have the ability to remyelinate axons in a number of chemically and trauma-induced models of axonal injury. Moreover we observed recovery of locomotor motor function after several weeks of acute OEC transplantation into dorsal transection model (Sasaki et al., 2004).

Although delayed transplantation of OECs into various SCI models including transection (Ramón-Cueto et al., 1998; Lu et al., 2002; López-Vales et al., 2006; López-Vales et al., 2007; Sasaki et al., 2004), contusion (Plant et al., 2003; Sasaki et al., 2007) and electrolytic lesions (Keyvan-Fouladi et al., 2003) elicits axonal regeneration and functional recovery, delayed transplantation of OECs after demyelinating lesion induction is less well studied. Cell transplantation for myelination has been largely carried out in development (Duncan et al., 1997 review) and actively demyelinating lesions (Blakemore and Crang, 1985; Franklin et al., 1996; Honmou et al., 1996, Imaizumi et al., 1998, Kato et al., 2000; Akiyama et al., 2004; Sasaki et al., 2006a; Talbott et al., 2006), environments considered optimal for myelination or remyelination (Foote and Blakemore, 2005).

A key question has been raised as to whether stably demyelinated axons remain competent for remyelination (Setzu et al., 2004; Foote and Blakemore, 2005). Setzu et al. (2004) demonstrated that transplantation of OPCs into the nerve fiber layer of the retina, where axons are normally unmyelinated, are ectopically myelinated by the transplanted OPCs even in older rats, thus suggesting that at least in this system myelination can be achieved on long term unmyelinated axons. Interestingly, myelination of these retinal axons was enhanced by acute inflammation (Setzu et al., 2006; Zhao et al., 2005; Dubois-Dalcq et al., 2008). Foote and Blakemore (2005) found poor myelination after OPC transplantation into the spinal cord of a late onset chronically demyelinating rat mutant (*taiep*), but more extensive remyelination when local inflammation was induced suggesting that inflammation may facilitate cell transplant-induced remyelination. Taken together these experimental studies suggest that inflammatory signaling may assist in remyelination. The role of inflammation in remyelination has critical implications for remyelinating cell transplantation studies. In this review we describe remyelination by OECs as a model myelinating cell type in different pathological environments. The models are well defined experimental models and caution must be exercised in extrapolation to human demyelinating disorders which present with unique pathological environments.

Inflammatory status of the X-EB demyelinating lesion

The X-EB lesion described above displays hallmark signs of inflammation in the acute phase of the lesion (3 days post-EB injection). Semi-thin plastic sections demonstrated an abundance of macrophages/microglia in the injection site and axons in various stages of demyelination (Fig. 1A and B). Hematoxylin and eosin (HE) staining after 3 days of EB injection when we normally transplant cells shows acute inflammatory cells accumulated in the injection site (Fig. 1C and D). These accumulated cells were $ED1^+$ (Fig. 1E and F) and $GFAP^-$ (Fig. 1G). The $ED1^+$ cells were abundant and confined to the lesion zone (Fig. 1E). The expression of TNF-α was extensively localized throughout the lesion (Fig. 1H). The X-EB lesion without cell transplantation is characterized by virtually complete loss of endogenous glial elements

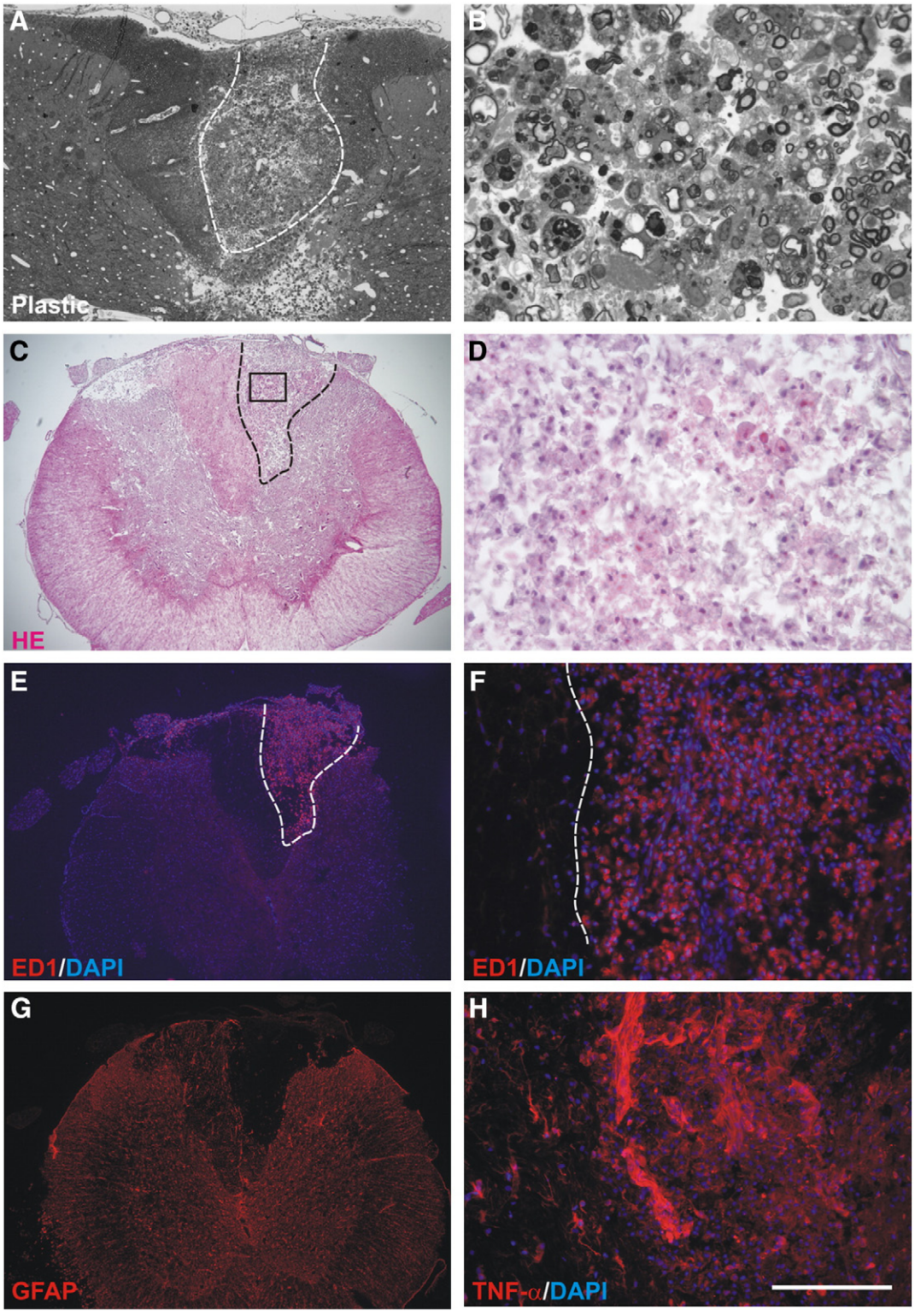


Fig. 1. Acute pathological status of the X-EB lesion. (A and B) Semi-thin plastic sections demonstrate macrophage/microglia and demyelinating axons 3 days after lesion induction. (C and D) Light micrographs of coronal frozen sections with HE staining 3 days after EB injection showing accumulated inflammatory cells in the injection site. Boxed area in (C) is expanded in (D). The accumulated cells were ED1$^+$ (E and F), GFAP$^-$ (G) and TNF-α $^+$ (H) counterstained with DAPI staining for nuclei (E, F, and H). The dashed line demarcates lesion edge. Scale bars = 400 μm (A), 100 μm (D), 40 μm (B), 1 mm (C, E, and G), 20 μm (F and H).

(astrocytes and oligodendrocytes) with preservation of axons (Fig. 2A) 3 weeks after EB injection. The higher power light micrograph of the lesion (Fig. 2B) demonstrates compact fields of demyelinated axons in close apposition, and clusters of macrophages/microglia (Fig. 2B) which were ED1$^+$ (Fig. 2C) and OX-42$^+$ (Fig. 2D). However, the ED1$^+$ /OX-42$^+$ cells were no longer scattered throughout the lesion as was observed in the acute phase, but were localized in small discrete areas. TNF-α expression was much reduced (Fig. 2F) as compared to the acute lesion (Fig. 1H). While perhaps not completely silent, the inflammatory response was greatly reduced at 3 weeks post-EB injection.

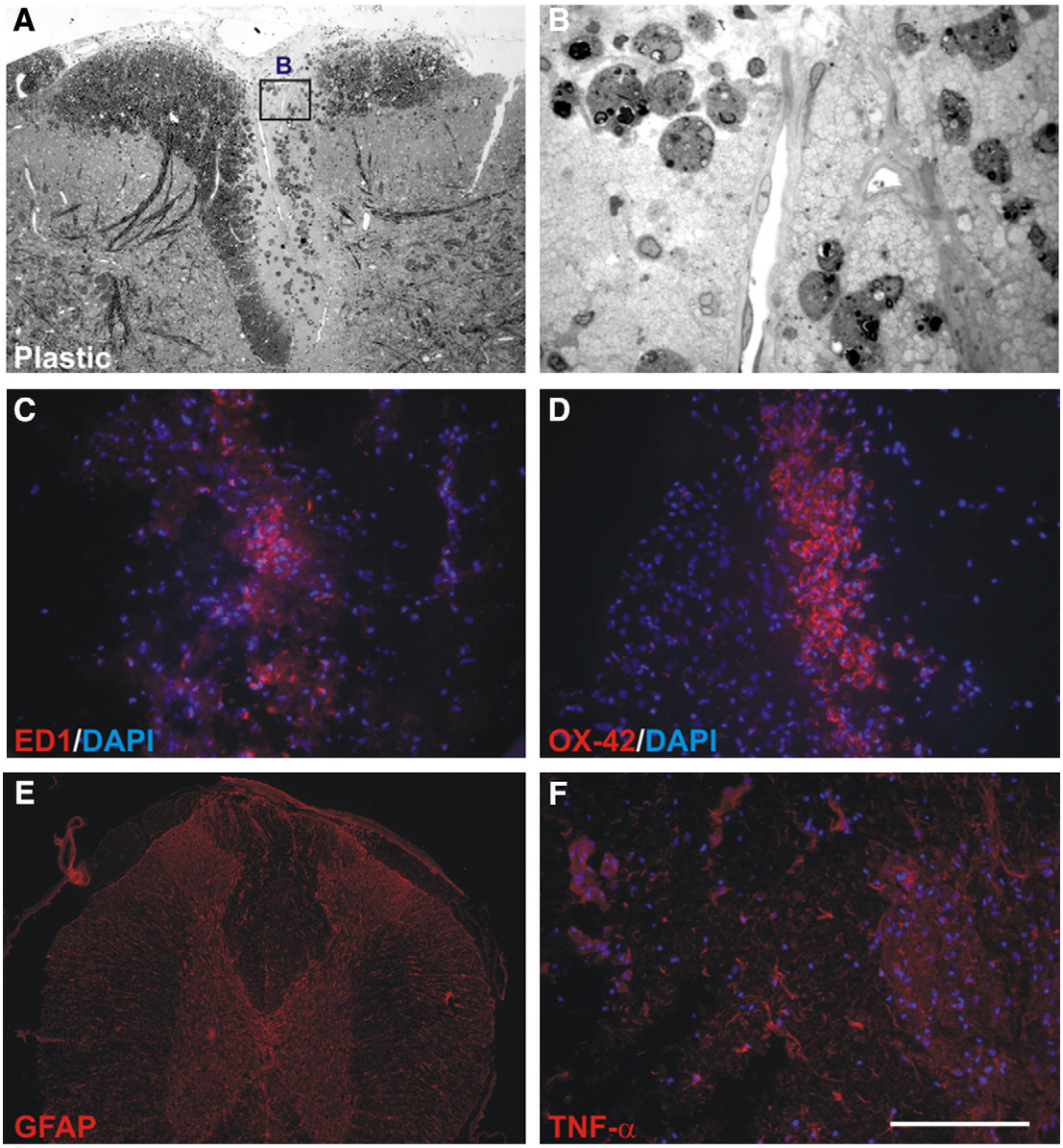


Fig. 2. Subacute status of the X-EB lesion. Light micrographs of semi-thin coronal plastic sections of the dorsal funiculus of the spinal cord showing persistent demyelination (A and B) 3 weeks after EB injection. Examination at higher magnification shows closely packed clusters of demyelinated axons in the dorsal columns (B). Boxed area in (A) is expanded in (B). Frozen coronal section of lesion area showing ED1$^+$ cells (C) and OX-42$^+$ cells (D) counterstained with DAPI staining for nuclei (C, D, and F). Note that the macrophages/microglial are clustered and not distributed throughout the lesion as they were in the acute lesion. TNF-α expression is reduced as compared to an acute lesion (F). Scale bars = 400 μm (A), 20 μm (C, D, and F), 4 μm (B).

Remyelination by transplanted OECs into the actively inflammatory (acute) lesion and after the inflammation has abated (subacute) in the X-EB lesion

In this model system OECs were transplanted in the acute (3 day; Sasaki et al., 2006a) and in the subacute (3 week) phases after lesion induction to determine if the myelinating potential differed depending on inflammation status. The transplanted OECs from donor GFP rats (GFP-OECs) were easily distinguished from endogenous cells in the subacute X-EB lesion 3 weeks after transplantation by their green fluorescence (Fig. 3A1). 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 (Fig. 3A2 and B). Immunostaining for P0, a specific marker of peripheral myelin (Greenfield et al., 1973), was present in the transplant site (Fig. 3C). Higher power images of the transplant site showed that P0 was associated with most of the GFP-OECs and was surrounded by GFP-OEC cytoplasm, indicating that peripheral-type myelin was formed by the transplanted cells within the dorsal column lesion (Fig. 3C). The CNS myelin marker, myelin basic protein (MBP), was minimally expressed (Fig. 3D).

Semi-thin azure II-methylene blue-stained plastic sections demonstrated virtually all axons were remyelinated in the dorsal funiculus 3 weeks after transplantation into the acute lesion (Fig. 4A–C). When cells were transplanted into the subacute lesion (3 weeks post-lesion induction) there was considerable remyelination 3 weeks after transplantation, but it was much less than when the cells were injected in the acute phase (Fig. 4D–F). Note the numerous remaining demyelinating axons in Fig. 4F. These data do not prove, but strongly support the suggestion that inflammation may not only have a role in demyelination, but may also contribute to remyelination. Thus, inflammation may facilitate remyelination in the CNS by the peripheral myelin-forming OECs as well as OPCs.

Demyelination and remyelination in an inflammatory focal EAE lesion

Experimental autoimmune encephalomyelitis (EAE) is an animal model that shares several clinical and histological similarities with

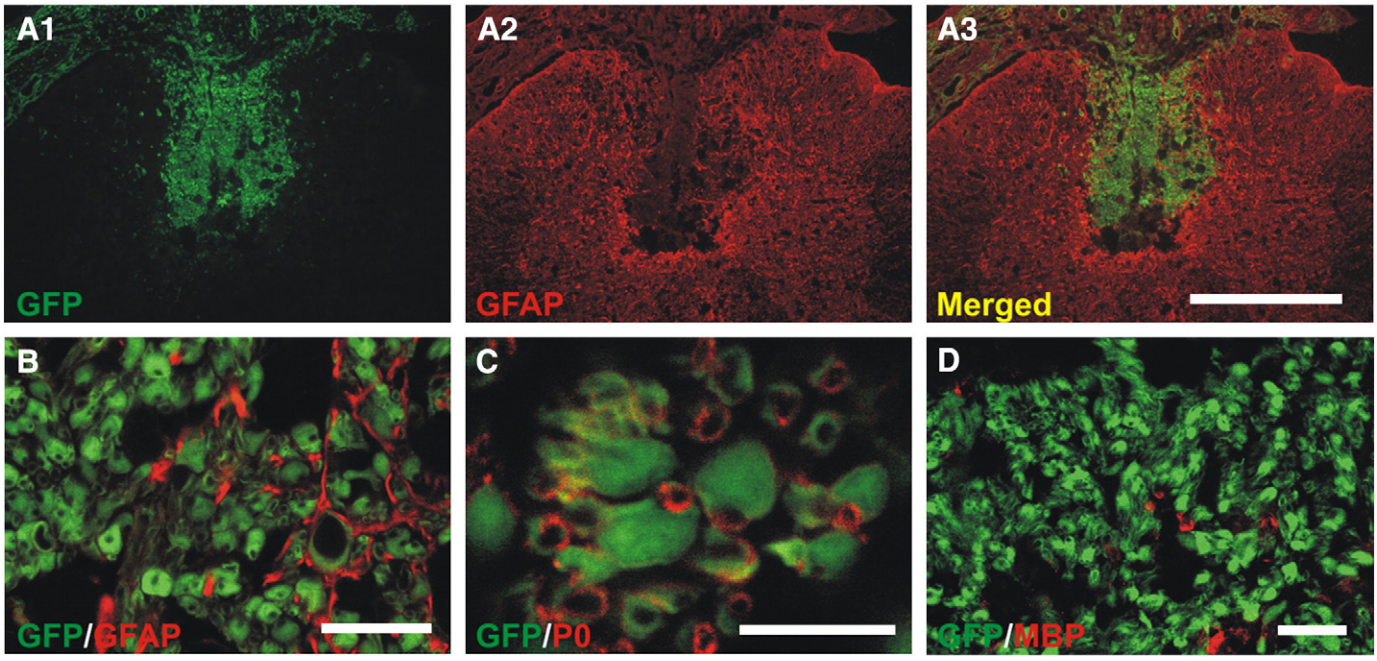


Fig. 3. Remyelination by transplanted GFP-OECs into the subacute lesion. (A1–A3) Low power images showing GFP-OECs and GFAP expression in the transplant site. Higher power images showing GFP-OECs at the margin of the lesion where some GFAP expressing astrocytes are present (B) and the OECs association with peripheral myelin protein P0 rings (C). Little of the central myelin marker MBP was expressed (D). Scale bars = 50 μm (A), 25 μm (B and D), 10 μm (C).

multiple sclerosis (MS) (Gold et al., 2006) and several therapies for MS have led directly from investigations using EAE (Pluchino et al., 2003). The multi-focal nature of demyelination and inflammation in generalized EAE models makes it challenging to precisely target a lesion site and to assess the response to a cell therapy intervention. An important study by Kerschensteiner et al. (2004) reported a novel focal EAE animal model that produced an inflammatory demyelinating lesion in the spinal cord. After inducing subclinical sensitization with MOG, they microinjected a "cocktail" of pro-inflammatory cytokines (IFN-γ, TNF-α) that induced a focal demyelinating lesion similar in pathology to those seen in generalized EAE.

Disruption of the blood–brain barrier (BBB) is a critical event in the pathogenesis of MS. Imaging studies indicate that breakdown of the BBB is observed for both new inflammatory lesion formation and reactivation of older ones (Miller et al., 1988; Kermode et al., 1990). Vascular endothelial growth factor (VEGF) is a well-known mediator of angiogenesis (Carmeliet et al., 1996; Ferrara et al., 1996). However, in the CNS, it is a pro-inflammatory signal that has the ability to transiently and non-traumatically open the BBB (Dobrogowska et al., 1998; Proescholdt et al., 2002). When VEGF is microinjected into the dorsal funiculus of the spinal cord of Lewis rats that have been sensitized with the encephalitogenic antigen, recombinant rat MOG (1–125), a focal inflammatory demyelinating lesion with the histological appearance of EAE is produced (Sasaki et al., 2010). Thus, transient breakdown of the BBB in MOG-sensitized rats is a sufficient condition to induce focal EAE.

In this model system clinical signs were apparent within 72 h and were largely resolved by day 21. The pathology at the injection site consisted of a focal region containing OX-42$^+$ cells, phagocytic cells with debris, extensive demyelination, and some lymphocyte infiltration (Fig. 5A–C). However, similar to chemically demyelinating lesions extensive remyelination was observed within a few weeks (Fig. 5D–F) making it difficult to assess the impact of cell transplantation on remyelination in this inflammatory lesion because of the competition with endogenous progenitors.

It is important to note that a series of studies indicate that pro-inflammatory cytokines IL-1 and TNF-α promote OPC proliferation and remyelination (Mason et al., 2001; Arnett et al., 2003). Kotter et al. (2005) found that depletion of macrophages induced impairment of remyelination and a reduction in OPC responsiveness and altered growth factor expression. The relative role of inflammation in assisting remyelination in EAE is not yet known; however, our preliminary results show that acutely transplanted GFP-OECs derived from GFP-expressing Lewis rat olfactory bulb survived and migrated several millimeters in this model system 6 weeks after transplantation (Fig. 5G–I).

OECs, but not SCs, migrate extensively in the X-irradiated spinal cord and establish a unique phagocytic phenotype

Although OECs and SCs share many morphological, antigenic, and proliferative characteristics (Ramón-Cueto and Avila, 1998; Wewetzer et al., 2002), several studies suggest a greater potential for OECs to migration within the CNS (Bartolomei and Greer, 2000; Lakatos and Franklin, 2002; Franklin, 2002; Lu and Ashwell, 2002). OECs distribute more freely with co-cultured astrocytes than SCs (Lakatos et al., 2000, Van den Pol and Santarelli, 2003), are less adhesive in astrocyte monolayers (Fairless et al., 2005), have less of an inductive effect on astrocytic hypertrophy (Lakatos et al., 2000) in vitro, and elicit smaller increases in GFAP and chondroitin sulphate proteoglycan expression after transplantation than SCs (Lakatos et al., 2003; García-Alías et al., 2004; Andrews and Stelzner, 2007). Like SCs, OECs phagocytize cellular debris after injury in vivo (Chuah et al., 1995; Lankford et al., 2008) or in vitro (Wewetzer et al., 2005), but unlike SCs, OECs also exhibit microglia-like cytokine responses to bacterial proteins (Vincent et al., 2007). Together these observations imply that OECs produce a more complex array of responses to local environmental conditions.

Are there differences in migration properties between SCs and OECs in vivo? When transplanted into chemically demyelinated lesions in the spinal cord, both OECs and SCs migrate and myelinate axons within the lesion site (Blakemore, 1977; Harrison, 1980; Honmou et al., 1996; Kato et al., 2000; Franklin, 2003; Akiyama et al., 2004; Sasaki et al., 2006a,b). However, OECs, SCs and OPCs show poor survival and migration after transplantation into normal white matter (Franklin et al., 1996; O'Leary and Blakemore, 1997; Iwashita et al., 2000; Hinks et al., 2001; Lankford et al., 2008). An interesting

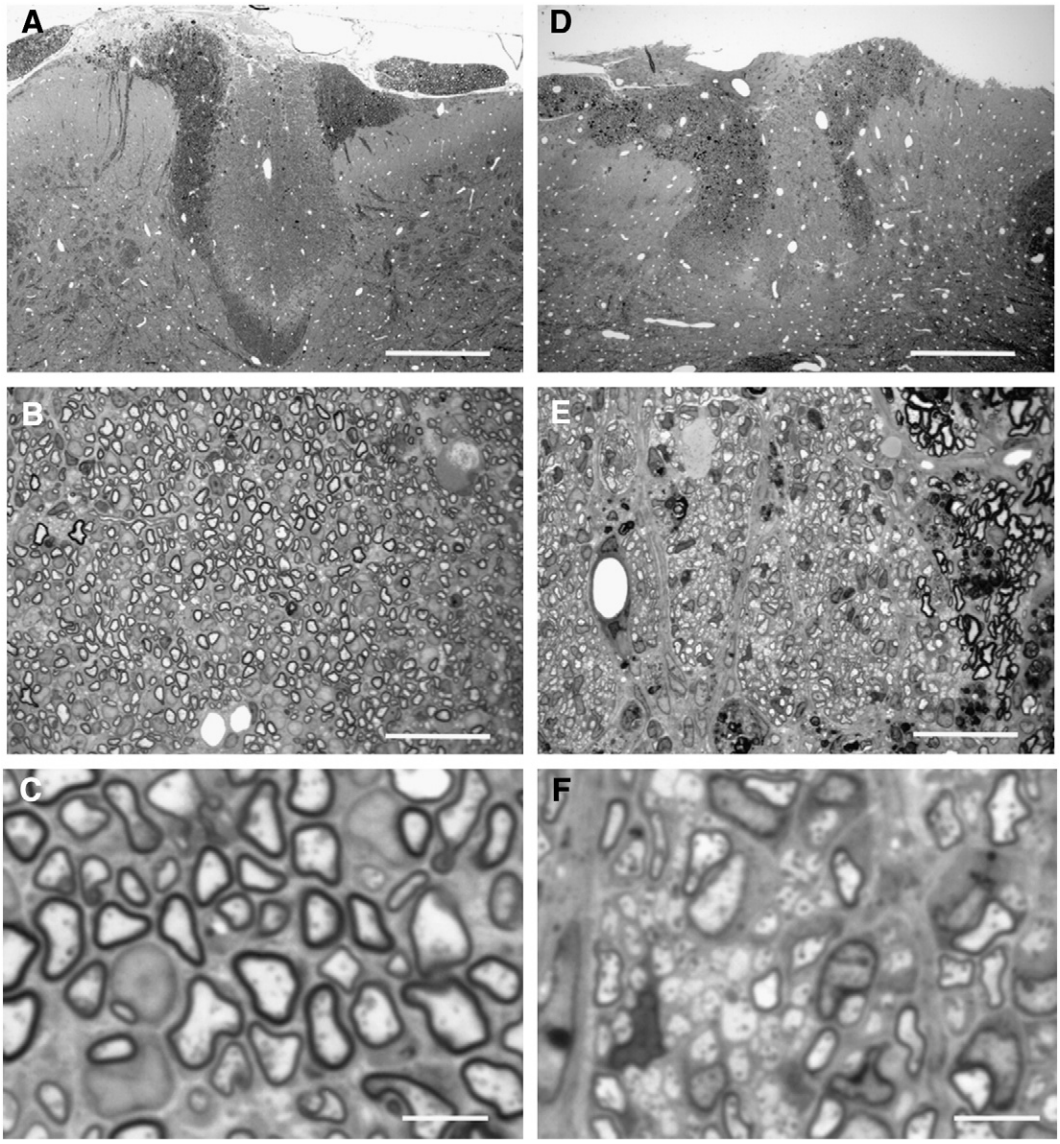


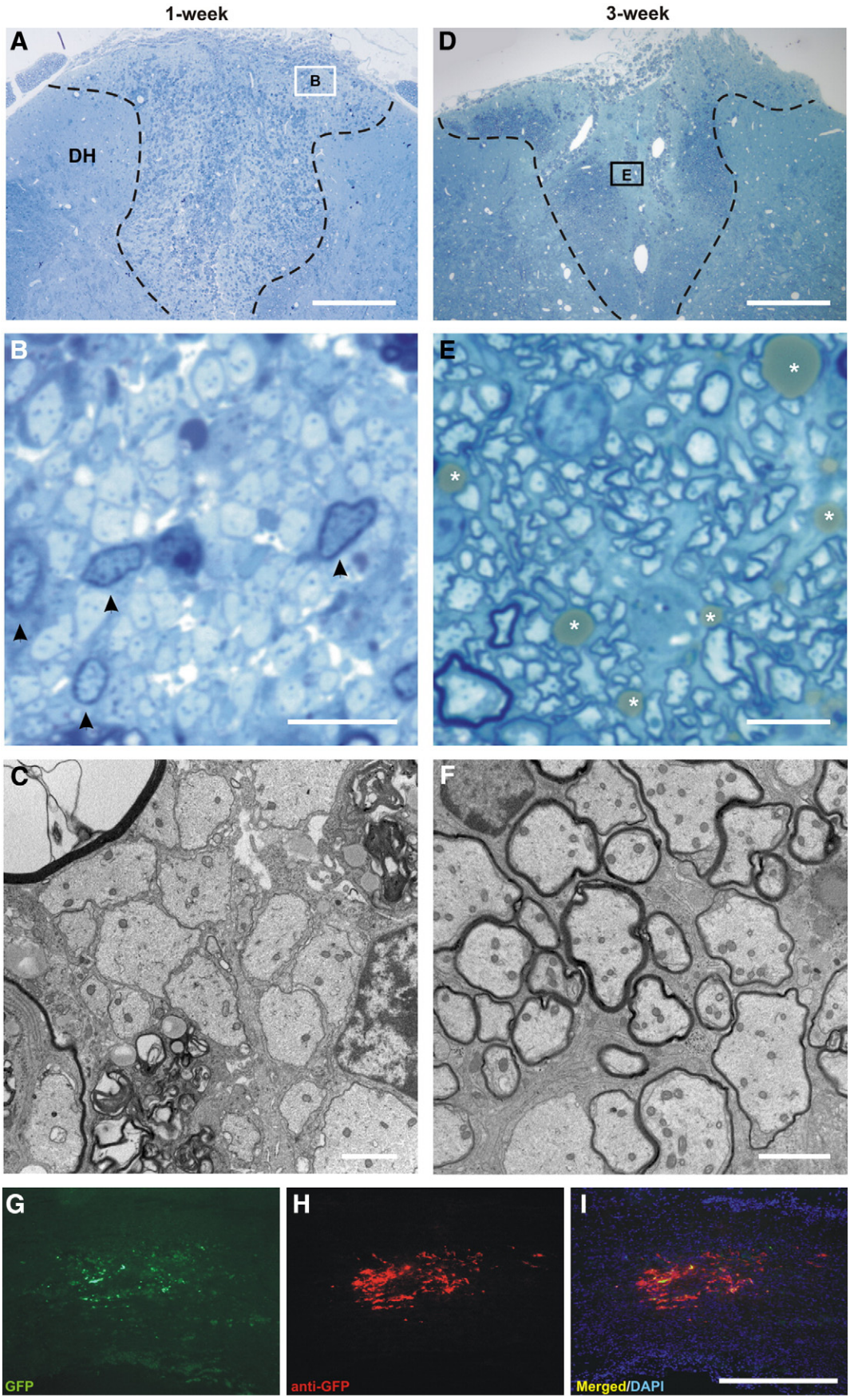
Fig. 4. Remyelination is less when cells are transplanted into a reduced inflammatory environment. Remyelination is extensive 3 weeks after transplantation of OECs into an acute inflammatory demyelinating lesion (A–C), and remyelination is much less 3 weeks after transplantation into a subacute lesion when inflammation is reduced (D–F). Scale bars = 300 μm (A and D), 30 μm (B and E), 5 μm (C and F).

observation was made by Hinks et al. (2001) who demonstrated that focal X-irradiation of the spinal cord a week prior to transplantation facilitates migration of OPCs, but the X-irradiation does not facilitate migration of SCs (Iwashita et al., 2000; Lankford et al., 2008). The OPCs migrated extensively throughout both white and grey matter in the spinal cord. The dosage of X-irradiation used in these studies does not produce obvious gross structural changes in the spinal cord over the course of the study nor are there obvious functional deficits, but $NG2^+$ OPCs are killed in the X-irradiation zone. The precise changes induced by the irradiation that provide for a permissive environment for the enhanced migration of OPCs are not known. Hinks et al. (2001) suggested that killing of endogenous OPCs by the X-irradiation opens structural niches to which the transplanted OPCs can migrate.

While SCs do not migrate in the normal or X-irradiated spinal cord (Iwashita et al., 2000; Lankford et al., 2008), we recently found that OECs migrate extensively in both white and grey matter of the X-irradiated spinal cord similarly to OPCs (Lankford et al., 2008). This behavior indicates a fundamental difference in the in vivo behavior of OECs as compared to SCs. Within the X-irradiated spinal cord, the OECs appeared randomly distributed within their area of dispersion. The cells had relatively small cell bodies (less than 25 μm) and gave rise to variable numbers of branched processes. Although in culture the OECs were $p75^+$, the distributed cells were $p75^-$ suggesting a phenotypic change in vivo. Interestingly the OECs showed weak OX-42 staining on their processes and were phagocytic, and phagocytic debris was present in electron micrographs of identified GFP-expressing OECs that have been transplanted into the X-irradiated spinal cord (Lankford et al., 2008). The OECs might migrate to areas of killed OPCs and phagocytize these cells. This possibility is supported by the observation that there is no clear cell loss other than the OPCs following the irradiation protocol. These results also demonstrate a clear difference between the in vivo properties of OEC and SC populations.

OECs transplanted into the X-irradiated spinal cord can repair subsequently demyelinated axons

Experimental cell transplantation approaches to improve function after SCI or demyelination typically apply cells after lesion induction. We asked the question as to whether OECs could be “pre-loaded” into the X-irradiated spinal cord and if they would respond to subsequent myelin injury (Lankford et al., 2008). The spinal cords were irradiated


1-week
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anti-GFP
Merged/DAPI

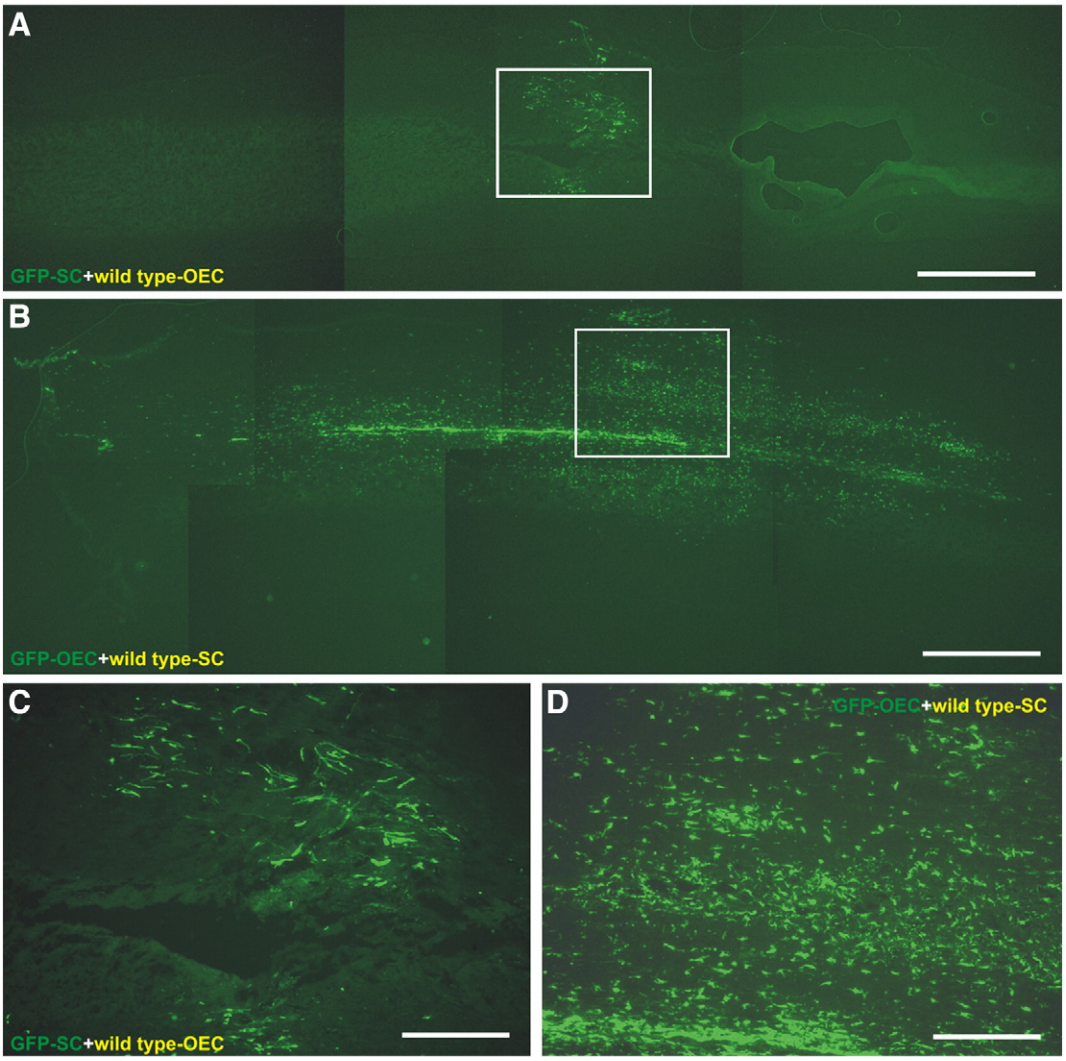

Fig. 6. Migration of GFP-expressing cells following transplantation of mixed SC and OEC populations. Sagittal frozen sections of X-irradiated spinal cord transplanted with a 1:1 mixture of 30,000 cells of GFP-expressing SCs and wild type OECs, $n = 3$ (A), and GFP-expressing OECs and wild type SCs, $n = 3$ (B) 3 weeks after transplantation. Note that GFP-expressing SCs showed limited dispersion in the presence of equal numbers of wild type OECs, but GFP-expressing OECs distributed broadly in the presence of wild type SCs. These data suggest that possible contamination of SCs in OEC preparations would not signal the SCs to increase their migratory potential. Boxed areas in (A) and (B) are enlarged in (C) and (D), respectively. Scale bars = 1 mm (A, B), 300 μm (C and D). Modified from Lankford et al. (2008).

(day 0) and OECs and SCs injected into the dorsal funiculus 1 week later (day 7). While SCs do not migrate in the X-irradiated spinal cord, OECs migrate extensively in both white and grey matter of the X-irradiated spinal cord after 3 weeks of OEC transplantation (day 28) (Lankford et al., 2008). We made a small focal demyelinating lesion by microinjection of EB into the dorsal funiculus (day 28), a time point when the OECs are dispersed throughout white and grey matter ("pre-loaded"). Three weeks after EB injection (day 42), the spinal cords were studied histologically. Interestingly, the GFP identified OECs remyelinated host axons with a peripheral-like pattern of myelin. Thus under these experimental conditions the OECs could be "pre-loaded" into a relatively intact spinal cord, and then mobilize for myelin repair when lesions were induced several weeks later. SCs do not survive well or migrate in the X-irradiated spinal cord. These results revealed a clear difference between the migratory properties of OECs and SCs in the X-irradiated spinal cord and demonstrated that engrafted OECs can participate in repair of subsequent delayed lesions.

In co-transplant experiments in which a mixture (1:1) of GFP-expressing "green" rat SCs was co-transplanted with wild type OECs into X-irradiated spinal cords, we observed minimal dispersion of GFP-expressing "green" SCs 3 weeks post-transplantation (Fig. 6A), compared with a much broader dispersion of "green" OECs co-transplanted with wild type SCs (Fig. 6B). These results indicate the two key points: 1) OECs have unique migratory properties and 2) SC contamination in our cultures cannot account for the migratory and myelination properties of the OECs (Lankford et al., 2008).

Clearly X-irradiating the spinal cord and transplanting cells in anticipation of a subsequent disease such as MS plaques is not feasible. But one might consider such an approach when the spinal cord or brain is therapeutically X-irradiated. Post-radiation necrosis

Fig. 5. Remyelination occurs rapidly in a focal EAE model. (A–C) One week after induction of focal EAE (MOG sensitization and focal VEGF injection into the spinal cord) demyelination and macrophage infiltration are prominent. (D–F) Three weeks later there is extensive endogenous remyelination indicating both remyelination competency of the demyelinated axons in a acute inflammatory lesion and the responsiveness of OPCs immediately after inflammatory-mediated demyelination. (G–I) Sagittal frozen section the spinal cord 6 weeks after acute (3 days after intraspinal VEGF injection) transplantation of OEC derived from GFP-expressing adult Lewis rat olfactory bulb into a focal EAE lesion. Naïve GFP-OEC (G) fluorescence is enhanced by anti-GFP antibody (H; red), merged image with DAPI (I). Scale bars = 300 μm (A and D), 10 μm (B and E), 4 μm (C and F). Panels (A) to (F) are modified from Sasaki et al. (2010).

and myelopathies are potential consequences that can occur weeks or months after radiation therapy. Experimental animal studies to determine if cell transplantation with OECs or other cell types reduces post-radiation pathology will be important.

Myelin competency of chronically demyelinated axons

There are few adult animal model systems to study the myelinating potential of transplanted myelin-forming cells in persistent long term demyelinated spinal cord. The rodent model developed by Blakemore and Crang (1985) and described above does allow for a window of 6–8 weeks of persistent demyelination. However, some human cell types such as OPCs require months to remyelinate after transplantation (Windrem et al., 2004), so even this model system does not have the appropriate temporal range to study efficacy of certain human cell types. In several other chemically or virally induced demyelinated models or EAE models in rodent, robust remyelination occurs within a few weeks after lesion induction. The focal EAE lesion model described above results in extensive acute demyelination in the dorsal funiculus, but endogenous remyelination is prominent at 3 weeks thus limiting the models' usefulness to study transplanted myelin-forming cells because of competition with endogenous cells. Given that clinical studies in SCI patients (Féron et al., 2005; Lima et al., 2006, 2010; Mackay-Sim et al., 2008) have been initiated using human OECs as a potential cell therapy, axo–glial interactions of human OECs in chronically demyelinated axons are important.

Cell transplantation studies performed using human OPCs into the dysmyelinating mutant *Shiverer* did show remarkable chimeric myelination after several months (Windrem et al., 2002; 2004). These animals were transplanted with human OPCs at early neonatal times. In order to study the efficacy of the human OPCs in adult tissue an appropriate persistent model system will need to be developed. One possibility to study the myelinating potential of human OPCs is the nonhuman primate (NHP) spinal cord. We found that EB lesions without X-irradiation in the old world African green monkey remain persistently demyelinated for 6 months (Fig. 7A). This is in contrast to the rat where remyelination was extensive at 3 weeks post-EB injection.

Six months after demyelination of the NHP dorsal funiculus, only a few, apparently quiescent phagocytic cells were observed within the demyelinated zone (Fig. 7A), indicating that inflammation was largely abated by this time point. However, these long term demyelinated (6 month) axons remained competent for remyelination since large areas of remyelination were observed within these lesions 6 months

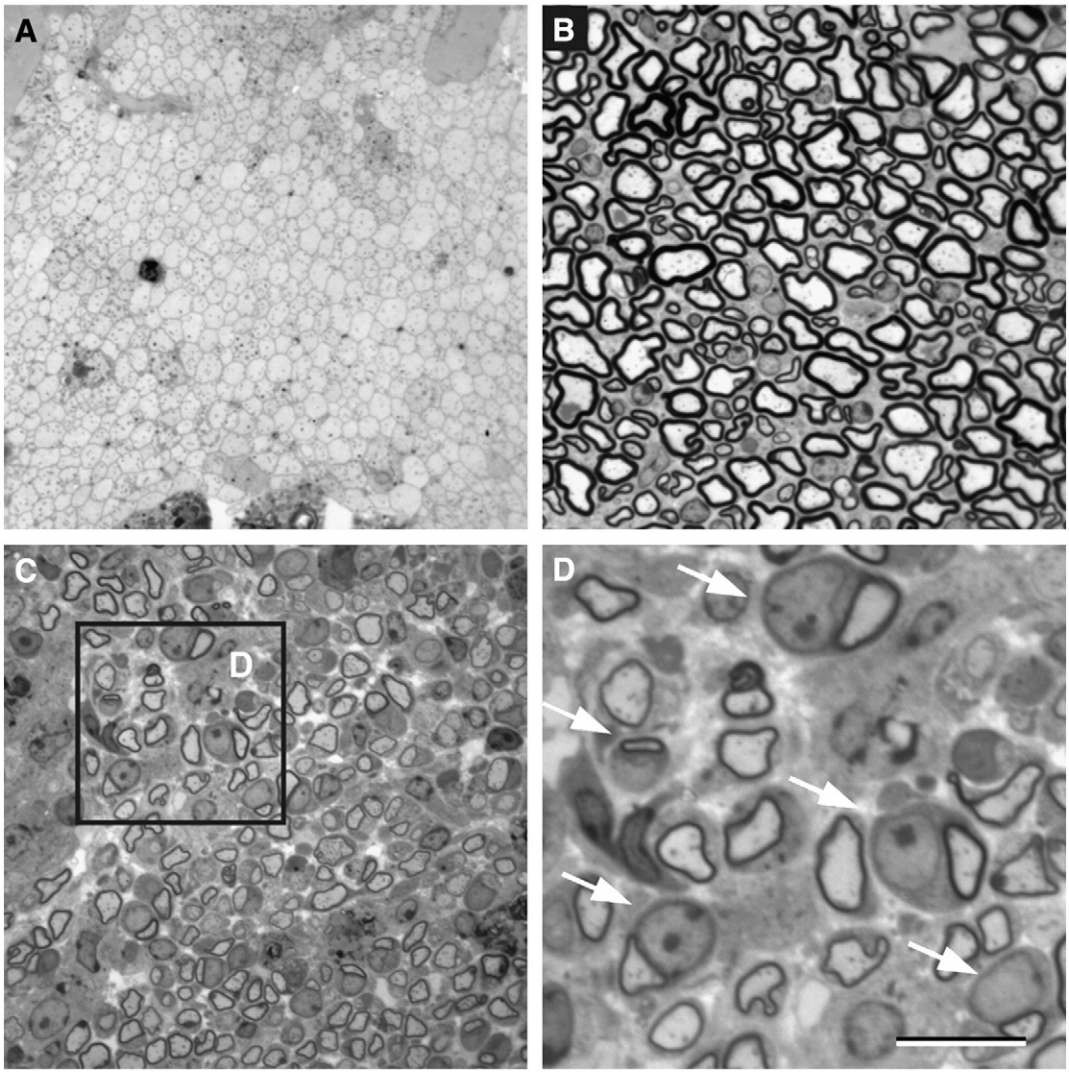

Fig. 7. Transplantation of porcine OECs remyelinate demyelinated nonhuman primate spinal cord. A field of persistently demyelinated spinal cord axons at 6 months after EB injection in the NHP. (B) Without cell transplantation endogenous remyelination is observed at 1 year after lesion induction. (C and D) Transplantation of porcine olfactory bulb-derived OECs 1 week after demyelination induction leads to extensive remyelination 4 weeks later. Note the peripheral pattern of remyelination for the OECs (arrows in D). Scale bars = 20 mm (A–C), 50 mm (D). Panels (C) and (D) are modified from Radtke et al. (2004).

later, at the 1 year post-lesion time point (Fig. 7B). Although some areas of Schwann cell-like remyelination of NHP spinal cord axons were observed at the 1 year time point, the majority of myelinated axons exhibited an oligodendrocyte phenotype. Thus it is likely that a longer time period is required for recruitment of endogenous progenitors in the NHP than in the rodent. Thus, it is likely that a longer time period was required for recruitment of endogenous progenitors in the NHP than in the rodent. While this time delay in the NHP may be a species related characteristic, it is important to note that Sim et al. (2002) found an age-dependent decrease in central remyelination efficiency they attributed to impairment of both OPC recruitment and differentiation. The precise age of the NHP for the data shown in Fig. 7 is not known, but veterinary estimates from body weight and dental structure are that the animal was a young adult. Regardless of species or age as to the accountable factor for the delayed remyelination, the results indicate a model system to study the remyelination potential of cells that require a long time frame to remyelinate.

In this NHP model system, OECs have been shown to remyelinate demyelinated spinal cord when transplanted within a week after lesion induction (Radtke et al., 2004). Fig. 7C–D shows a field of axons remyelinated by porcine olfactory bulb-derived OECs transplanted into the immunosuppressed NHP demyelinated thoracic spinal cord (5 weeks post-transplantation). Note the large number of myelinated axons with a typical peripheral pattern of myelination characteristic of OEC remyelination. The myelinated axons are surrounded by large cytoplastic domains with nuclei. Electron microscopic images of these remyelinated axons indicate that they are surrounded by a basement membrane (Radtke et al., 2004). Because the OEC remyelination was observed at 6 weeks post-lesion induction (5 weeks post-transplantation) and endogenous repair does not occur until 6 months, this indicates that the demyelinated axons are competent for remyelination. The delay for endogenous repair is likely the result of impairment of OPC recruitment and differentiation as suggested by Sim et al. (2002). It will be interesting to compare the remyelinating potential of OECs in the NHP when transplanted several months after lesion induction when inflammation is reduced to determine if efficiency of remyelination by the transplanted cells is more robust when transplanted in an inflammatory environment as has been reported for rodent. We stress that the model systems described here are well structured experimental models to study interactions of OECs in these defined environments, and that caution should be taken to extrapolate to a clinical context.

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