Dorsally-Derived Oligodendrocytes in the Spinal Cord Contribute to Axonal Myelination During Development and Remyelination Following Focal Demyelination

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KEY WORDS
Pax3; transcription factor; cre-lox; cell fate; spinal cord injury

ABSTRACT
In the developing spinal cord, the majority of oligodendrocytes are derived from the ventral ventricular zone. Several recent studies suggested that a small number of oligodendrocyte precursor cells (OPCs) can also be generated in the dorsal spinal cord. However, it is not clear whether these dorsal oligodendrocyte precursor cells participate in myelination and remyelination. To investigate the fate and potential function of these dorsally-derived oligodendrocytes (dOLs) in the adult spinal cord, Cre-lox genetic fate mapping in transgenic mice was employed. We used the Pax3Cre knock-in mouse line, Cre-expressing domains migrate into all regions of spinal cord and subsequently undergo terminal differentiation and axonal myelination. In response to a focal demyelination injury; a large number of newly differentiated oligodendrocytes originated from dOLs, suggesting that dOLs may provide an important source of OPCs for axonal remyelination in multiple sclerosis or spinal cord injury. ©2011 Wiley-Liss, Inc.

INTRODUCTION
Oligodendrocytes (OLs) are macroglial cells responsible for the formation of myelin sheaths around axons in the central nervous system (CNS). In the developing spinal cord, neural progenitor cells in the ventral neural tube first give rise to motor neurons, then switch their cell fate to produce oligodendrocyte precursor cells (OPCs) (Richardson et al., 1997; Rowitch, 2004; Sun et al., 1998). The induction of early OPCs is primarily under the influence of the ventral midline signal Sonic Hedgehog (Shh) (Miller, 2002; Spassky et al., 2000). Soon after their generation, OPCs migrate out of the ventricular zone into the surrounding grey and white matter regions where they undergo proliferation and differentiation. Recent studies in Nkx6−/− and Shh−/− mice revealed a distinct source of oligodendrogenesis in the dorsal spinal cord independent of Shh signaling (Cai et al., 2005). The molecular signaling pathways that specify the fate of these dorsally-derived OPCs (dOPCs) are uncertain at this stage, although it has been suggested that the existence of fibroblast growth factor (FGF) signaling and the progressive reduction of bone morphogenetic protein (BMP) signaling in the dorsal spinal cord may contribute to dorsal oligodendrogenesis (Cai et al., 2005; Vallstedt et al., 2005).

Transgenic studies further verified the existence of dorsally-derived OLs (dOLs) produced from partial dorsal domains such as Dlx1 or Ascl1 (Battiste et al., 2007; Fogarty et al., 2005; Vallstedt et al., 2005; Yue et al., 2006). However, these fate-tracing studies did not include the entire dorsal neuroepithelium and therefore the dorsal contribution to the OL population remains unknown. Moreover, it is not clear whether dOPCs can differentiate into mature OLs and contribute to axonal myelination in the postnatal CNS. Finally, it would be interesting to know whether dOPCs are capable of participating in myelin repair after demyelinating injuries in the adult CNS.

In the present study, we investigated the cell fate and potential function of dOLs in the postnatal spinal cord by labeling all dOLs by crossing the Pax3Cre and Rosa26-lacZ or Z/EG reporter mouse lines. In the developing spinal cord, Pax3 is expressed in the dorsal ventricular zone, spanning the entire dorsal progenitor domains (Goulding et al., 1991, 1993; Lee and Pfaff, 2001). In the PAX3Cre knock-in mouse line, cre expression recapitulates Pax3 protein expression in dorsal neural progenitor domains (dP1–dP6) (Engleka et al., 2005).

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Crossing Pax3\textsuperscript{Cre}\textsuperscript{+} knock-in mice with a cre-dependent reporter line such as Rosa26-lacZ or Z/EG permanently labels the Pax3-expressing precursor cells and their progeny, even though the endogenous Pax3 is no longer expressed at later stages. Our results demonstrated that the number of dOLs increased progressively and the majority of these cells were located in dorsal and lateral regions of white matter at postnatal stages. These dOPCs underwent delayed differentiation as compared with their ventral counterparts, and comprised less than 20% of the total OL population in the white matter of the adult spinal cord. In addition, dOLs contributed to axonal myelination during normal development and participated in axonal remyelination following focal demyelination.

**MATERIALS AND METHODS**

**Mouse Lines and Genotyping**

Mice were housed under standard laboratory conditions at the animal facility of the University of Louisville. All experimental procedures conformed to National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the University of Louisville.

Pax3\textsuperscript{Cre}\textsuperscript{−/−}, Rosa26-lacZ, and Z/EG mouse lines were described previously (Engleka et al., 2005; Novak et al., 2000; Soriano, 1999).

**X-Gal Staining and Immunohistochemistry**

Spinal cord tissues from Pax3\textsuperscript{Cre}\textsuperscript{−/−};Rosa26-lacZ double transgenic mice were isolated at various developmental stages and fixed in 2% (w/v) paraformaldehyde (PFA) at 4°C. Following fixation, tissues were transferred to 20% sucrose in phosphate-buffered saline (PBS), embedded in OCT media, and then sectioned (20 μm thickness) on a cryostat. Thereafter, the slides were stained in X-gal staining solution overnight in the dark.

Following X-gal staining, slides were washed with PBS, blocked with normal goat serum, and incubated with anti-Olig2 or anti-APC primary antibodies overnight at 4°C. Slides were then washed with phosphate-buffered saline Tween-20 (PBST), incubated with secondary antibody for 1 h at room temperature. After several washes with PBST, slides were developed with the ABC kit (Vector Laboratories) using 3,3’-diaminobenzidine (DAB) as the chromagen.

**Immunofluorescence**

Postnatal Pax3\textsuperscript{Cre}\textsuperscript{−/−};Z/EG mice were fixed by cardiac perfusion with 4% PFA and spinal cord tissues were dissected out and submerged in 4% PFA at 4°C overnight. Following fixation, tissues were transferred to 20% sucrose in PBS overnight, embedded in OCT media and then sectioned on a cryostat. Double immunofluorescent procedures were modified from previous methods (Xu et al., 2000). The antibodies used were as follows: anti-Pax3 (1:10, Developmental Studies Hybridoma Bank, DSHB), anti-GFP (1:500, Millipore), anti-Olig2 (1:6,000, kind gift of Dr. Charles Stiles), anti-APC/CC1 (1:50, Oncogene), anti-Nkx2.2 (1:10, DSHB), anti-GFAP (1:300, Chemicon), anti-S100β (1:1,000, Millipore), anti-Sox10 (1:3,000, kind gift of Dr. Michael Wegner), anti-MBP (1:10, Millipore), anti-Neurofilament M (1:200, Invitrogen), anti-BrDU (1:200, Biodesign), and anti-Laminin (1:1,000, Sigma). Negative controls consisted of non-immune, species-specific IgGs or sera instead of primary antibody.

**Electron Microscopy (EM)**

Pax3\textsuperscript{Cre}\textsuperscript{−/−}/Z/EG double transgenic mice at P14 were transcardially perfused with 4% PFA and 0.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4 (PB). The spinal cord was dissected out, sectioned into slices 50-μm thick with a vibratome, and placed in PB. GFP expression was revealed by incubating sections with a rabbit anti-GFP antibody diluted 1:500, followed by a biotinylated goat anti-rabbit antibody, ABC, and reacted with nickel-intensified DAB for 5 min, with a final wash in PB. Sections were then postfixed in 2% osmium tetroxide, dehydrated in an ethyl alcohol series, and flat embedded in Durcupan resin between two sheets of Aclar plastic (Ladd Research, Williston, VT). Durcupan-embedded sections were first examined with a light microscope to select areas for electron microscopic analysis. Selected areas were mounted on blocks; ultrathin sections (70–80 nm) were cut using a diamond knife and collected on 200 mesh copper grids. Ultrathin sections were stained with uranium acetate and lead citrate, and examined under the electron microscope.

**Animal Surgery**

The 2.5-month old Pax3\textsuperscript{Cre}\textsuperscript{−/−};Z/EG double transgenic mice were anesthetized with Avertin (Sigma). Following induction of anesthesia, dorsal laminectomies were performed at the T8/T9 vertebral level and the dura was opened transversely at two sites 2-mm apart. Unilateral injection of 1 μL 1% lysolceithin (Sigma) was delivered at stereotactic coordinates (0.4 mm lateral to midline and depths of 1.1 mm) into the ventral funiculus of the thoracic spinal cord using custom-pulled and beveled micropipettes attached to a Parker picospritzer. All surgical intervention and subsequent care and treatment of all animals used in this study were in strict accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996) and University of Louisville Institutional Animal Care and Use Committee guidelines.
BrdU Labeling

BrdU (Sigma, 20 mg mL\(^{-1}\)) in normal saline was prepared and syringe filtered. Animals received 3 days of intraperitoneal injections of 50 mg kg\(^{-1}\) BrdU delivered every 12 h over a 72-h pulse period, and then sacrificed. Immunofluorescent staining for BrdU was performed as described above, except that sections were incubated in 2 N HCl (30 min) and 0.1 M borate buffer (10 min) prior to blocking steps.

Data Collection and Statistical Analyses

In Pax3\(^{cre}\);Rosa26lacZ double transgenic mice, the contribution of dorsally-derived OLs to the entire OL population in different white matter areas (dorsal, lateral, or ventral funiculus), was assessed by counting the number of Olig2+/LacZ+ double positive cells (dorsally-derived OLs) over the number of all Olig2+ cells (entire OL population) on thoracic sections from three mice at various developmental stages. The same method was used to study the contribution of differentiated dOLs (APC+/LacZ+) to the mature OL population (APC+).

Spinal cord injury experiments were done in Pax3\(^{cre}\);Z/EG double transgenic mice. Following injury, cross sections in the tissue 2-mm rostral to the epicenter was obtained from three animals for further immunohistochemical analysis and quantification at different time points. The percentage of dOLs and ventrally-derived OLs (vOLs) within the lesion was measured by counting the number of GFP+/Olig2+ double positive cells (dOLs) or GFP−/Olig2+ cells (vOLs) over the number of Olig2+ cells (dOLs+vOLs).

Statistical significance \(p\) value was assessed by Student’s \(t\) test.

RESULTS

Spatiotemporal Distribution of Dorsally-Derived Oligodendrocytes in Mouse Spinal Cord

To trace the fate of dOLs in postnatal spinal cord, Pax3\(^{cre}\) knock-in mice, in which the Cre recombinase is expressed from the endogenous Pax3 locus in the entire dorsal neuroepithelium, were crossed with the Rosa26-lacZ reporter line (Engleka et al., 2005) (Fig. 1A). In the double transgenic mice, both neurons and glia derived from Pax3+ dorsal neural progenitor cells were permanently labeled. Spinal cord sections were subjected to X-gal staining followed by anti-Olig2 immunohistochemistry. Olig2+ cells expressing lacZ represented the dOL population. To assess the contribution of dOLs to oligodendroglial populations in different regions of white matter, we calculated the percentage of dOLs in the dorsal, lateral, or ventral funiculus, and the entire white matter (Fig. 1E,F). Consistent with our previous findings, some double-positive cells started to appear in the white matter at E15.5 (Fig. 1E,F), whereas ventrally-derived OPCs (vOPCs) could be detected as early as E12.5 (Cai et al., 2005). The dOL population progressively increased over time at postnatal stages (Fig. 1E,F). At P70, dOLs accounted for about 18% of the total olig2-labeled oligodendroglial population in the spinal cord (Fig. 1B–D,F). In agreement with our previous results, dOPCs, like their counterparts derived from ventral spinal cord, migrated throughout the spinal cord and could be found in all regions of the white matter, but mostly in the dorsal and lateral funiculi (Fig. 1B–E). At all stages, only a small percentage of OL population in the white matter were dorsally derived (Fig. 1F).

To confirm the identity of these Pax3-derived cells at postnatal stages, we mated Pax3\(^{cre}\) with Z/EG reporter mice, and examined the dOL distribution in P7 spinal cords. In the white matter, most GFP-expressing cells were concentrated in the dorsolateral funiculus (Fig. 2A). Immunolabeling with anti-sox10 (Fig. 2B–C), anti-APC (Fig. 2D–E), anti-Nx2.2 (Fig. 2F) antibody identified them as OPCs in the white matter. It was noteworthy that few dOPCs differentiate into mature OLs at this stage, based on immunostaining results with Nkx2.2 or APC, which labels differentiating or differentiated OLs (Fig. 2D–F).

Dorsally-Derived Oligodendrocyte Precursor Cells Can Differentiate and form Myelin Sheaths Around Axons

We next examined if these dOPCs are capable of differentiating and contributing to myelin formation after birth. Spinal cord sections from Pax3\(^{cre}\);Z/EG double transgenic mouse at P14 were chosen to perform GFP immunostaining in conjunction with anti-APC. The longitudinal sections clearly showed that many dOPCs differentiated into mature OLs (Fig. 3A–C). Confocal images showed that cell bodies and fine processes of GFP-labeled dOLs were immunolabeled for myelin basic protein (MBP) and these processes were aligned with neurofilament-positive axons, suggesting that these dOLs were synthesizing myelin and possibly forming myelin sheaths around axons (Fig. 3D–E\(^{\prime}\)). Indeed, immunofluorescent staining followed by anti-APC immunohistochemistry on Pax3\(^{cre}\);Rosa26-lacZ mouse spinal cord sections revealed that the percentage of differentiated dOLs in the white matter was very low at P7, but increased progressively as development proceeded (see Fig. 4).

Response of Dorsally-Derived Oligodendrocytes to Focal Demyelination

Although the percentage of dOLs in the spinal cord was limited during normal development, their later emergence and differentiation led to the hypothesis that dOLs may functionally differ from their counterparts in...
the ventral spinal cord and could have a distinct behavior under pathologic conditions. To test this hypothesis, we used lyssolecithin to demyelinate the ventral funiculus in adult Pax3<sup>cre</sup>;Z/EG transgenic mice and studied the response of dOLs (Supp. Info. Fig. 1E). Injection of lyssolecithin into the ventrolateral funiculus caused a prominent demyelinating plaque and the demyelinated area extended from the injection point toward the ventral gray matter medially and pia matter peripherally (Supp. Info. Fig. 1A,B). Ultrastructural analysis showed that compared with the contralateral intact side, most axons in the ipsilateral lesion of spinal cord were demyelinated. Importantly, the myelin structure was well preserved in peripheral nervous system (PNS) on both sides (Supp. Info. Fig. 1C,D).

Interestingly, 48 h after lyssolecithin injection, more GFP-labeled cells were found in the injury side of spinal cord (Supp. Info. Fig. 2). A large number of GFP<sup>+</sup> cells appeared in the ipsilateral side at 3-days post-injury (3 DPI) (Fig. 5B). Double immunofluorescence

Fig. 1. Spatial and temporal distribution of dOLs. A. Schematic illustration of Pax3 expression in the dorsal neural progenitor domains of the spinal cord and cre-loxP labeling strategy. B–D. Transverse spinal cord sections from P70 Pax3<sup>cre</sup> R26-lacZ mice were subjected to X-gal staining (blue) followed by anti-Olig2 immunohistochemistry (brown). Red arrowheads represent LacZ<sup>+</sup>/Olig2<sup>+</sup> cells (dOLs), the dotted lines delineate the boundary between the grey matter and the white matter, and the inset shows double-positive cells at high magnification. E. The proportion of dOLs in the dorsal, ventral or lateral funiculus increases progressively from E15.5 to P70. F. The percentage of dOLs in the entire white matter increases steadily but is significantly less than that of vOLs (LacZ<sup>+</sup>/Olig2<sup>+</sup>) between E15.5 and P70. Data are shown as mean ± SD (n = 3 mice). ***P < 0.001. Scale bars: 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Fig. 2. Identity of Pax3-derived GFP+ cells. Most GFP-labeled cells are localized in the dorsal spinal cord (A). Transverse spinal cord sections were immunostained with anti-GFP in conjunction with anti-Sox10 (B–C), anti-APC (D,E), anti-Nkx2.2 (F) in P7 Pax3cre;Z/EG double transgenic mice. White arrowheads represent double-positive cells. Some of GFP+ cells in the lateral funiculus (boxed area in A) co-expressed Sox10 (B–C), while few of them co-expressed APC (D,E) or Nkx2.2 (F). WM, white matter; GM, gray matter. Scale bars: A, 100 μm; B, D–F, 50 μm; C–C', 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Fig. 3. Differentiation of and myelination by dOLs. A–F. Double immunostaining of P14 Pax3cre;Z/EG longitudinal spinal cord sections through the central canal with antibodies against GFP and APC (A–C), GFP and MBP (D–D') or GFP and neurofilament M (110 kDa, NF-M) (E–E'). Co-localization of GFP and MBP in cell bodies is represented by purple arrowheads and that in processes is represented by white arrowheads (D–D'). Some cytoplasmic extensions from GFP-labeled cells were overlapped with NF-M+ axons, represented by white arrowheads; cell bodies are indicated by purple arrowheads (E–E'). G. Transverse sections were subjected to anti-GFP immunohistochemistry followed by ultrastructural analysis with transmission EM. DAB-labeled processes from dOLs myelinated two adjacent axons. Scale bars: A–C, 50 μm; D–D', 10 μm; G, 0.5 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Fig. 4. Temporal sequence of dOPC differentiation in postnatal spinal cords. A–C. Transverse spinal cord sections from P70 Pax3<sup>cre</sup>–Rosa26-lacZ mice were subjected to X-gal staining (blue) followed by anti-APC immunohistochemistry (brown). Red arrowheads represent LacZ<sup>1</sup>/APC<sup>1</sup> cells, dotted lines delineate the boundary between the grey matter and the white matter, and the inset shows double-positive cells at high magnification. D. Quantification of differentiated dOLs (LacZ<sup>1</sup>/APC<sup>1</sup>) as a percentage of entire mature OL population (APC<sup>1</sup>) in the dorsal, ventral, or lateral funiculus at different stages. E. Relative ratio of mature dOLs and vOLs in the entire white matter (the sum of dorsal, ventral, and lateral funiculi) of spinal cord of various developmental stages. Data are presented as mean ± SD (n = 3). ***P < 0.001. Scale bars: 100 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Fig. 5. Response of dOLs to ventral demyelination. A–I. Transverse spinal cord sections from Pax3<sup>cre</sup>–Z/EG mice at 3 DPI were immunostained by anti-GFP in conjunction with anti-GFAP (A–C), anti-Olig2 (D–F) or anti-APC (G–I). Compared with the intact side, a large number of GFP<sup>1</sup> cells emerged in the injury side and migrated toward the ipsilateral ventral lesion (outlined by white dotted lines) where GFAP expression and cell density were up-regulated (A–C). GFP<sup>1</sup> cells co-expressed Olig2<sup>1</sup> (D–F) or APC (G–I), represented by white arrowheads. The boxed area in B is shown at higher magnification in D–I. Scale bars: A–C, 100 μm; D–I, 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
staining with antibodies against Olig2 or APC identified those cells as OLs (Fig. 5D–I). At this time point, most GFP-labeled dOLs crowded around the lesion area, indicating they were migrating from dorsal normal tissue toward the ventral lesion but had not reached inside yet. At 14 DPI, many GFP+ cells had infiltrated into the lesion area (Fig. 6A–I). They co-expressed Olig2 or APC (Fig. 6A–F) but not GFAP (Fig. 6G–I). Although Pax3 is also expressed by neural crest cells in the PNS, these GFP-labeled cells in PNS did not express OL markers such as Olig2 and APC (data not shown), indicating that GFP+/Olig2+ or GFP+/APC+ cells observed around or inside the lesion were not derived from PNS. At 28 DPI, GFP+ cells were synthesizing myelin proteins such as MAG (data not shown) and MBP, and some MBP+/GFP+ cells formed myelin sheaths around neurofilament-labeled axons (see Fig. 7). Within the lesion, the percentage of dOLs (GFP+/Olig2+) in the entire OL population (Olig2+) could reach as high as 60% (Fig. 8B). Saline injection in the spinal cord did not cause demyelination or increased proliferation and migration of GFP-labeled cells (data not shown). We also failed to detect Pax3 expression at 5, 24, 48 h, 7 days, 14, or 28 days after injury, excluding the possibility of the local origin of GFP+ cells due to the reactivation of Pax3 by the injury (data not shown).

To assess the proliferative activity of these GFP+/Olig2+ cells, BrdU was injected for 3 consecutive days before sacrificing the animals (Supp. Info. Fig. 1E). Many GFP+ cells incorporated BrdU, indicating they were proliferating (Fig. 9, Supp. Info. Fig. 2). Double immunostaining of longitudinal sections through lesion confirmed that GFP+ cells co-expressed Olig2 and most of these GFP-labeled dOLs bordered along the region of the epicenter (Supp. Info. Fig. 3D–D’’). Increased expression of GFAP and laminin revealed the occurrence of reactive astrogliosis, which results in a glial scar forming around the epicenter (Supp. Info. Fig. 3B,C’’).
**DISCUSSION**

Combining the faithful expression of Cre in this Pax3<sup>cre</sup> knock-in mouse line with lacZ or GFP in reporter line allows spatiotemporal marking of the entire population of dOLs (from dP1 to dP6) in the spinal cord. dOLs share the same molecular characteristics (Olig2, Sox10, APC, myelin proteins) as their ventral counterparts during lineage progression, and are capable of forming myelin sheaths around axons. To our surprise, we found that a large number of dOLs proliferated and became mobilizable and migrate to the lesion, indicating their contribution to remyelination.

**Dorsally-Derived Oligodendrocytes are Predominantly Localized in the Dorsal Spinal Cord During Development**

dOLs are generated relatively late during development independent of Nkx6 regulation and Shh signaling in the spinal cord (Cai et al., 2005; Fogarty et al., 2005; .

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**Fig. 7.** Remyelination of dOLs in adult Pax3<sup>cre</sup>-Z/EG mice. A–F. Transverse spinal cord sections from Pax3<sup>cre</sup>-Z/EG mice at 28 DPI were immunostained by anti-GFP in conjunction with anti-MBP (A–C) or anti-NF-M (D–F). Many GFP<sup>+</sup> cells in lesion co-expressed MBP (A–C). Insets in A–C show that GFP<sup>+</sup> cells were also co-labeled with another mature OL marker APC. Confocal analysis revealed that GFP-labeled MBP<sup>+</sup> dOLs formed myelin sheaths around axons (indicated by white arrowheads) (D–F). Scale bars: A–C, 10 μm; D–F, 4 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**Fig. 8.** Spatiotemporal distribution of dOLs during remyelination. A. The emergence and spatial distribution of GFP<sup>+</sup> cells over the time in relation to the ventral lesion. In the first 48 h, under the influence of the ventral demyelination, more dOLs (blue) started to be generated in the ipsilateral side of the spinal cord. Beginning at 3 DPI, these dOLs were attracted toward the ventral lesion (red). By 14 DPI, most of the migratory dOLs have infiltrated into the lesion site. B. Quantitative analysis of dOLs in the lesion at 2-mm rostral to the epicenter at 3, 7, 14, and 28 DPI. The number of dOLs (GFP<sup>+</sup>/Olig2<sup>+</sup>) and vOLs (GFP<sup>+</sup>/Olig2<sup>+</sup>) in the lesion on cross sections were calculated from three injured adult Pax3<sup>cre</sup>-Z/EG mice. Data are shown as the mean ± SD. **P < 0.01; ***P < 0.001; n.s., not significant. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
suggestions that dOLs possibly myelinate axon tracts located in dorsal and dorsolateral funiculi such as corticospinal and the reticulospinal tract.

Critical Role of Dorsally-Derived Oligodendrocytes in Spinal Cord Injury

At appropriate concentration and doses, lysolecithin selectively kills oligodendrocytes in the injection site but spares most axons and astrocytes (Arnett et al., 2004; Blakemore and Franklin, 2008; McKay et al., 1998). Thus, it is widely used to create focal demyelinating insults for studying the subsequent responses of OPCs to demyelination injuries.

Our results showed that 48 h after injury, more GFP+ OPCs emerged in the ipsilateral region compared with the contralateral side of the spinal cord, possibly due to increased cell proliferation as evidenced by their BrdU incorporation (Supp. Info. Fig. 2). Starting from 3 DPI, an increasing number of GFP+ dOLs appeared in the dorsal region and migrated toward the ventral lesion (see Fig. 5). By 14 days after injury, many dOLs had infiltrated into the ventral lesion (Fig. 6A–F). Reactive astroglialis is pronounced at this timepoint (Fig. 6A–C,J–L and Supp. Info. Fig. 3C,D′′). Those reactive astrocytes around the lesion could produce chemoattractants to allow OPCs to migrate from resting areas towards the demyelinated axons (Williams et al., 2007). Recruitment of OPCs is a critical first step in remyelination. Thus, in addition to the local proliferation of OPCs, there is a contribution of migratory OPCs from the surrounding areas including the dorsal region. dOPCs are able to undergo terminal differentiation and produce myelin proteins after infiltration into the lesion (Figs. 5–7). Moreover, dOLs could comprise as high as 60% of total OL population within the lesion, indicating the important role of dOLs in remyelination. Those dOLs were mostly crowded around the epicenter 2-weeks post-injury (Supp. Info. Fig. 3D–D′′), consistent with previous reports showing denser distribution of OLs around remyelinating areas than adjacent normal tissue (Blakemore and Keirstead, 1999; Talbott et al., 2005). These results suggest that remyelination requires new OLs and an important source of these new OLs could originate from the dorsal spinal cord.

In conclusion, our findings revealed an important role of dOLs in axonal myelination under normal and pathological conditions. It was previously hypothesized that dOLs may serve as a reservoir for repair (Miller, 2005). Consistent with this hypothesis, dOPCs were highly pro-
liferative and migratory in response to injuries and made up around 60% of OPC population within the ventral lesion site. It is plausible that vOLs mainly function to myelinate axons of neurons during early development, whereas dOLs myelinate axons at late stages and participate in myelin turnover and injury-induced remyelination in adult CNS tissues.

Note added in proof: While this article was in revision, one article came out to demonstrate that dOLs remained mainly in the dorsal and dorsolateral funiculi of the spinal cord through adulthood, consistent with our findings presented in this article. In addition, they showed that dOLs were electrically similar to vOLs but appeared to outcompete them for myelinating dorsal axons during development (Tripathi et al., 2011).

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