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# Interactions between oligodendrocyte precursors control the onset of CNS myelination

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ABSTRACT

The formation of CNS myelin is dependent on the differentiation of oligodendrocyte precursor cells (OPCs) and oligodendrocyte maturation. How the initiation of myelination is regulated is unclear, but it is likely to depend on the development of competence by oligodendrocytes and receptivity by target axons. Here we identify an additional level of control of oligodendrocyte maturation mediated by interactions between the different cellular components of the oligodendrocyte lineage. During development oligodendrocyte precursors mature through a series of stages defined by labeling with monoclonal antibodies A2B5 and O4. Newly differentiated oligodendrocytes begin to express galactocerebroside recognized by O1 antibodies and subsequently mature to myelin basic protein (MBP)-positive cells prior to formation of compact myelin. Using an in vitro brain slice culture system that supports robust myelination, the consequences of ablating cells at different stages of the oligodendrocyte lineage on myelination have been assayed. Elimination of all OPC lineage cells through A2B5+, O4+, and O1+ complement-mediated cell lysis resulted in a delay in development of MBP cells and myelination. Selective elimination of early OPCs (A2B5+) also unexpectedly resulted in delayed MBP expression compared to controls suggesting that early OPCs contribute to the timing of myelination onset. By contrast, elimination of differentiated (O1+) immature oligodendrocytes permanently inhibited the appearance of MBP+ cells suggesting that oligodendrocytes are critical to facilitate the maturation of OPCs. These data illuminate that the presence of intra-lineage feed-forward and feedback cues are important for timely myelination by oligodendrocytes.



## Introduction

The development of oligodendrocytes, the myelinating cells of the vertebrate central nervous system (CNS), has been studied extensively. In vivo oligodendrocyte precursors (OPCs) arise from neural stem cells in distinct regions of the embryonic neural tube as a result of localized signals that modulate the activity of a number of transcription factors such as the *Olig* genes. These OPCs migrate widely through the CNS in response to selected guidance cues and proliferate extensively in response to growth factors such as PDGF (Bogler et al., 1990) prior to differentiating and myelinating adjacent axons in a reproducible pattern. In vitro analyses of OPC development have been facilitated through cell culture and the utilization of antibodies or gene transcripts that distinguish particular stages in OPC development. Each stage is characterized by changes in proliferation, migratory abilities, and morphology (Bansal et al., 1989; Hardy and Reynolds, 1993; Lubetzki et al., 1991). Oligodendrocyte precursors (OPCs) express NG2 and mAb A2B5 (Raff, 1989; Raff et al., 1984a) (Raff et al., 1984b) and proliferate in response to platelet-derived growth factor (PDGF) (Noble et al., 1988; Richardson et al., 1988). Recent evidence suggests OPCs have stem cell-like properties and can generate astrocytes and neurons in addition to oligodendrocytes (Kondo and Raff, 2004). Later in development, labeling with mAb O4 identifies OPCs and immature oligodendrocytes (Bansal et al., 1992). Newly differentiated oligodendrocytes begin to express galactocerebroside recognized by mAb O1 that, with further maturation, expresses myelin basic protein (MBP) followed by the full spectrum of myelin components and elaboration of compact myelin sheaths (Miller, 2002; Rosenberg et al., 2007).

Several mechanisms have been implicated in regulating the progression of OPCs to a myelinating cell. Clonal studies suggested the presence of a cell intrinsic timing mechanism controlling oligodendrocyte differentiation (Barres et al., 1994; Temple and Raff, 1986), a component of which may be the transcription factor GM98 (Emery et al., 2009), while the proliferative capacity of OPCs is mediated in part by $p57^{Kip2}$ (Dugas et al., 2007). The cell intrinsic program of OPC differentiation is subject to significant external

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regulation. For example, in the presence of fibroblast growth Factor (FGF) and PDGF, OPCs continue to proliferate and fail to differentiate (McKinnon et al., 1993; Noble et al., 1988; Raff et al., 1988). Conversely, withdrawal of growth factors stimulates precocious OPC differentiation.

Several lines of evidence implicate interactions between cells of the oligodendrocyte lineage in regulating their behavior. Culture studies demonstrated that the final number of oligodendrocytes that develop is independent of the number of OPCs in the initial culture suggesting the lineage reaches equilibrium (Zhang and Miller, 1996). Similarly over-expression of PDGF in vivo generates more OPCs but no change in the final number of oligodendrocytes (Calver et al., 1998; Richardson et al., 1988). These normalizations of cell number may reflect a density-dependent inhibition of OPC proliferation mediated through control of $P27^{KIP1}$ and Rb phosphorylation (Nakatsuji and Miller, 2001) as well as increases in oligodendrocyte apoptosis (Calver et al., 1998; Richardson et al., 1988). Further evidence of intra-OPC lineage interactions comes from the characterization of the fate of OPCs from distinct sources in the developing forebrain and suggests that competition during normal development between early and late generated OPCs results in the elimination of cohorts of cells (Kessaris et al., 2006).

During development, not all OPCs undergo differentiation into myelinating oligodendrocytes. Early studies identified "adult OPCs" that proliferate more slowly and in response to different mitogens than their embryonic counterparts (Bogler et al., 1990; Nishiyama et al., 1999; Wolswijk and Noble, 1992). Subsequently these adult OPCs have been shown to persist in significant numbers throughout life and their characterization in vivo reveals unique physiological properties implicating them in control of axonal excitability (De Biase et al., 2010; Karadottir et al., 2008; Nishiyama et al., 1999; Wolswijk and Noble, 1992; Woodruff et al., 2004; Ziskin et al., 2007). Whether these cells represent a reservoir of OPCs for remyelination by replacing oligodendrocytes lost during adulthood is unclear. Remyelination can occur in the adult CNS; however, in certain pathological conditions such as chronic demyelinated plaques seen in multiple sclerosis remyelination fails even though these regions contain cells with the characteristics of OPCs (Chang et al., 2000; Rudick and Trapp, 2009). Why these OPCs that are surrounded by naked axons fail to myelinate is currently unclear.

Slice cultures are emerging as an effective model with which to assess the regulation of myelination (Harrer et al., 2009). Using complement-mediated lysis to selectively eliminate distinct stages of the developing OPC population in slice and dissociated cell culture, we show that the timely maturation of oligodendrocytes is dependent on all stages of the lineage. Oligodendrocyte maturation is delayed by selective removal of early OPCs but inhibited by selective removal of newly generated oligodendrocytes. These studies suggest interdependence among the cellular components of the oligodendrocyte lineage and reveal a novel regulation of oligodendrocyte maturation that may have important implications for development of effective myelin repair strategies.

## Materials and methods

### *Preparation of postnatal rat brain slice culture*

All animals were purchased from Harlan Laboratories (Indianapolis, IN) and housed in the ARC facility of Case Western Reserve University School of Medicine. The Case ARC facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All animal procedures were approved by the Institutional Animal Care and Use Committee of Case Western Reserve University. Timed pregnant Sprague–Dawley (SD) rats were purchased and P2 rat brains dissected and kept in ice-cold PBS buffer supplemented with 10% glucose (Sigma, USA) prior to embedding in 1% agar. Slices were taken from a region encompassing 1.1–0.2 relative to Bregma. All images and quantification were obtained from the region of the motor cortex, cingulum, and underlying subcortical white matter. Slice cultures were prepared from 250-μm-thick coronal slices using a Leica vibrating microtome (Leica, VT 1000S, Germany) and immediately placed into cell-culture inserts (0.4 μm, Millicell-CM, Millipore) and cultured in six-well culture dishes containing basal medium eagle (BME) medium supplement with 25% horse serum, 0.5% glucose, 2.5% Hank's solution, and 1% L-glutamine. Slices were grown at 37 °C/5% $CO_2$, and the growth medium changed every second day.

### *Preparation of dissociated cell cultures*

The brain region containing the corpus callosum was isolated from P2 SD rats, chopped and digested in trypsin with 0.25% EDTA in MEM for 20–25 min at 37 °C. Cell suspensions were filtered through a 30-μm nylon mesh to remove large tissue clumps and cells plated on 12-mm glass coverslips coated with poly-L-lysine (Sigma) at a density of $2\times10^5$ cells/coverslip. Cells were grown in medium (DMEM medium supplemented with 1% FBS-c, PDGF and N2) overnight before antibody-complement treatment using a protocol described below.

### *Preparation of slices for electron microscopy*

To assess the extent of myelination 1 μm Epon embedded sections were labeled with Toluidine blue and ultrathin sections were examined on a Joel 100CX electron microscope at 80 KV. Slices were fixed in glutaraldehyde (2%) and paraformaldehyde (4%) followed by 1% OsO4, block stained with saturated uranyl acetate, dehydrated through graded alcohols, and embedded in Epon 812. Sections were cut in a plane parallel to the surface of the slice.

### *Antibodies and complement-mediated cell lysis*

The primary antibodies used in the study include the following: mouse monoclonals A2B5, O4, and O1 IgM hybridoma supernatant as previously described (Gao et al., 2006; Tsai et al., 2006). NG2+ cells were identified by labeling with the anti-NG2 chondroitin sulfate proteoglycan rabbit polyclonal antibody (Millipore, AB5320). A mouse monoclonal antibody against MBP (Covance, Princeton, NJ) was used as a marker of mature oligodendrocytes. Polyclonal rabbit Neurofilament 200 antibody was used to label axons (Sigma, N4142). Anti-iba-1 polyclonal antibody (Wako Chemicals USA, Inc., VA), purified from rabbit antisera with specific reactivity to microglia and macrophages, was used to assess microglial response. Guinea Pig complement was purchased from Sigma (St. Louis, MO), and cells were eliminated using the following protocol: P2 coronal slices were sectioned and incubated at 37 °C incubation overnight. The following day, slices were incubated in A2B5, O4, or O1 antibodies diluted 1:1 in 10% horse serum in BME at 37 °C for 60 min. Guinea pig complement was then added at a dilution of 1:10 and incubated for 2 h at 37 °C. After washing with BME, the slices were maintained in growth medium overnight. To ensure the majority of the selected cell population was depleted, the procedure was repeated the following day. Following antibody-mediated complement cell lysis, slices were maintained at various time points (P2 + 3 DIV, P2 + 7 DIV, and P2 + 14 DIV) prior to immunostaining. Controls included incubation in complement or antibody alone and neither significantly altered the number of oligodendrocyte lineage cells in the slice. To assess the effectiveness of the cell depletion, slices were labeled with the same antibodies 24 h after complement treatment. Primary antibody labeling was visualized by conjugated secondary Alexa antibodies (Invitrogen, USA) and slices mounted in anti-fading fluorescence medium with or without DAPI from Vector Laboratories (Burlingame, CA). The extent of myelination was confirmed by Black-Gold. Black-Gold (Chemicon, Product# AG390) is a novel haloaurophosphate