

Early development and dispersal of oligodendrocyte precursors in the embryonic chick spinal cord

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SUMMARY

Oligodendrocytes, the myelinating cells of the vertebrate CNS, originally develop from cells of the neuroepithelium. Recent studies suggest that spinal cord oligodendrocyte precursors are initially localized in the region of the ventral ventricular zone and subsequently disperse throughout the spinal cord. The characteristics of these early oligodendrocyte precursors and their subsequent migration has been difficult to assay directly in the rodent spinal cord due to a lack of appropriate reagents. In the developing chick spinal cord, we show that oligodendrocyte precursors can be specifically identified by labeling with O4 monoclonal antibody. In contrast to rodent oligodendrocyte precursors, which express O4 immunoreactivity only during the later stages of maturation, in the chick O4 immunoreactivity appears very early and its expression is retained through cellular maturation. In embryos older than stage 35, O4+ cells represent the most immature, self-renewing, cells of

the chick spinal cord oligodendrocyte lineage. In the intact chick spinal cord, the earliest O4+ cells are located at the ventral ventricular zone where they actually contribute to the ventricular lining of the central canal. The subsequent migration of O4+ cells into the dorsal region of the spinal cord temporally correlates with the capacity of isolated dorsal spinal cord to generate oligodendrocytes in vitro. Biochemical analysis suggests O4 labels a POA-like antigen on the surface of chick spinal cord oligodendrocyte precursors. These studies provide direct evidence for the ventral ventricular origin of spinal cord oligodendrocytes, and suggest that this focal source of oligodendrocytes is a general characteristic of vertebrate development.

Key words: oligodendrocyte, spinal cord, O4 antibody, migration, chick

INTRODUCTION

Oligodendrocytes, the myelinating cells of the vertebrate CNS (Bunge, 1968), develop from cells of the neuroepithelium. Recent studies suggest that the earliest oligodendrocyte precursors arise only in specific regions of the developing CNS (Small et al., 1987; Warf et al., 1991). In the rodent spinal cord, two independent lines of evidence suggest that oligodendrocyte precursors are initially located at the ventral ventricular zone. First, injection of the thymidine analog 5-bromodeoxyuridine (BrdU), into pregnant rats between E16.5 and E18 labels a distinct population of proliferating glial precursors at the ventral ventricular zone in embryonic animals (Noll and Miller, 1993). Cultures derived from these embryos indicate that approximately half the BrdU-labeled cells subsequently differentiate into oligodendrocytes (Noll and Miller, 1993). Second, in situ hybridization with mRNA directed against the receptor for the alpha subunit of Platelet Derived Growth Factor (PDGF r) demonstrates a focus of labeled cells at the ventral ventricular zone (Pringle and Richardson, 1993). Since in older animals, the expression of the alpha receptor for PDGF appears to be restricted to oligodendrocytes precursors or O2A

progenitors (Pringle et al., 1992), this early localization was suggested to reflect a focal origin of oligodendrocytes. More recently, in situ hybridization with probes against the myelin gene 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) have revealed a similar early ventral ventricular localization (Yu et al., 1994).

During maturation, rat oligodendrocyte precursors go through a series of defined stages characterized by the expression of distinct cell surface antigens and mitogenic responses (Gard and Pfeiffer, 1989, 1990). The earliest clearly identifiable cells in the rodent oligodendrocyte lineage label with the A2B5 antibody (Eisenbarth et al., 1979) and proliferate in response to PDGF and FGF (Noble et al., 1988; McKinnon et al., 1990; Bogler et al., 1990; Fok-Seang and Miller, 1994). As maturation proceeds, rat oligodendrocyte precursors begin to express O4 (Sommer and Schachner, 1981) immunoreactivity and lose responsiveness to PDGF (Fok-Seang and Miller, 1994). The initial binding of O4 antibody to oligodendrocyte precursors is mediated through an uncharacterized antigen termed prol oligodendroblast antigen (POA) while, on more mature precursors, O4 recognizes sulfatide (Bansal et al., 1989, 1992). The beginning of oligodendrocyte

differentiation correlates with expression of cell surface galactocerebroside, one of the major glycolipids of myelin (Ranscht et al., 1982; Raff et al., 1978) and a dramatic reduction in cell proliferation.

To determine whether the ventral origin of oligodendrocyte precursors is a general characteristic of vertebrate development, we have analyzed oligodendrocyte development in the chick spinal cord. Although the chick spinal cord has proved an effective model in the analysis of early neuronal development (van Stratten et al., 1985; Leber et al., 1990; Placzek et al., 1990, 1991) relatively little is known about glial development in this region. Early morphological studies suggest that in the embryonic chick spinal cord immature glial cells first appear in the region of the central canal and subsequently migrate to the developing white matter where they differentiate into astrocytes and oligodendrocytes (Fujita, 1965).

In the present study, we demonstrate that the monoclonal antibody O4 identifies chick spinal cord oligodendrocyte precursors at an early stage in their development. *In vivo*, the first O4-immunoreactive cells contribute to the ventral ventricular lining of the chick spinal cord and subsequently disperse to dorsal and lateral locations. Biochemical characterization of the antigen recognized by O4 on immature chick oligodendrocyte precursors suggests it is not a characteristic hydroxy or non-hydroxy form of sulfatide but rather a POA-like antigen previously described (Bansal et al., 1989, 1992). These results suggest that induction of oligodendrocyte precursors in the spinal cord ventral ventricular zone reflects a common vertebrate developmental pathway and occurs while the cells are still an integral part of ventricular lining of the central canal.

MATERIALS AND METHODS

Tissue preparation and section staining

Frozen and vibratome sections were prepared from embryonic White Leghorn chicken embryos. Fertilized eggs (Squire Valley View Farms, Cleveland OH) were incubated at 38°C until the desired developmental stage (Hamburger and Hamilton, 1951).

To localize O4+ cells in frozen sections, the thoracic region of the spinal cord and surrounding tissue was fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (PB pH 7.4) for 1-2 hours at 4°C and incubated in PB containing 30% sucrose overnight at 4°C. Tissues were embedded in OCT (Miles) compound and frozen by immersion in an ethanol-dry ice mixture. 14 µm frozen sections were cut on a Microm cryostat and collected on gelatin-coated glass slides. Antibodies O4 (Sommer and Schachner, 1981) and R-mab (Ranscht et al., 1982; Bansal et al., 1989) were used to label sections. R-mab recognizes both GalC and sulfatide as well as structurally related minor lipids by TLC analysis, and labels oligodendrocyte precursors prior to the expression of GalC (Bansal et al., 1992). In the current study, labeling with R-mab has been used to define more mature cells of the oligodendrocyte lineage. Sections were subsequently incubated in 20 mM lysine in PB for 10 minutes, followed by incubation in primary antibody (O4:supernatant neat or R-mab:ascites fluid 1:200) overnight at 4°C. The following day, the sections were washed extensively in PB and incubated in FITC-labeled goat anti-mouse IgM (for O4) or IgG (for R-mab) at a dilution of 1:100 (Cappel) for 1 hour at room temperature. After washing the sections were mounted in glycerol containing 5% n-propyl gallate, and examined with a Nikon Optiphot fluorescence microscope equipped with appropriate rhodamine and fluorescence filters and photographed on TriX film (ASA 400).

To visualize O4+ cells in vibratome sections of embryonic chick spinal cord, tissues were fixed by immersion in 4% paraformaldehyde and 0.05% glutaraldehyde in PB for 1-2 hours at 4°C and stored overnight in 30% sucrose in PB at 4°C. Spinal cords were then dissected from surrounding vertebra and embedded in 5% agar in PB. 50 µm sections were cut on a vibratome (Series 1000), collected free-floating in PB, and treated with 0.3% hydrogen peroxide for 20 minutes to remove endogenous peroxidase activity. Sections were subsequently incubated in 10% normal goat serum (NGS) for 20 minutes, followed by primary antibody O4 (supernatant: 1:5 in PB +10% NGS) for 4 hours at room temperature or overnight at 4°C, washed thoroughly and then incubated in goat anti-mouse IgM conjugated to horseradish peroxidase (HRP; 1:500; Cappel) for 45 minutes at room temperature. Sections were gently agitated during incubation and washing. Binding of the HRP conjugate was visualized by incubation in 0.05% diaminobenzidine (DAB) and 0.015% hydrogen peroxide in Tris buffer. After washing, sections were mounted on gelatin-coated glass slides and examined as above.

Vibratome sections labeled with O4 and immunoperoxidase were also prepared for electron microscopic analysis. In this case, following rinsing, sections were postfixed with 2% aqueous osmium tetroxide for 1 hour. Sections were then dehydrated through a graded series of ethanol and propylene oxide, and embedded in a mixture of LX-112 resin (Ladd Res. Ind., Burlington, VT). Ultrathin sections were cut on a Reichart ultracut microtome and examined with a Jeol 100 CX11 electron microscope without additional staining.

Explant and dissociated cell cultures

Isolated explant cultures were used to examine the distribution of oligodendrocyte precursors in the early developing spinal cord. Chick embryos between stages 23 and 31 (E4-7) were decapitated and immersed in calcium- and magnesium-free Dulbecco's modified minimum essential medium (S-MEM; Gibco). Embryos were incubated with dispase (0.24-0.8 U/ml in DMEM) for 20 minutes at room temperature prior to dissection. The thoracolumbar spinal cord was dissected and the meninges removed. Spinal cords were separated into ventral and dorsal regions at the sulcus limitans and each half chopped into small pieces in DMEM containing 15% fetal bovine serum and 5% chick serum. Spinal cord explants were plated onto coverslips that had been coated with poly-L-lysine (0.1 mg/ml) laminin (20 µg/ml)-fibronectin (20 mg/ml) and cultures maintained *in vitro* for 1-10 days. From each stage embryos, at least 3 independent cultures were prepared and more than 50 explants examined for the presence of oligodendrocytes and their precursors.

To identify oligodendrocytes and their precursors, explants were labeled with O4 (supernatant neat) or R-mab (1:100) antibodies as previously described (Fok-Seang and Miller, 1994). Briefly, explants were incubated in DMEM containing 50% NGS for 5 minutes, followed by incubation in primary antibodies in DMEM containing 50% NGS for 30 minutes followed by appropriate secondary antibody conjugated to either fluorescein or rhodamine (1:50) for 30 minutes. All labeling was carried out at room temperature. After washing, explants were fixed with 5% acetic acid in methanol for 10 minutes at -20°C and observed with epifluorescence illumination. The proportion of explants containing O4+ or R-mab+ cells was counted.

Dissociated cell cultures and complement-mediated cell lysis

To define the antigenic phenotype of chick oligodendrocyte precursors and assay their development *in vitro*, dissociated cell cultures were established from chicks of various ages and labeled with O4 and R-mab antibodies. Dissociated cell cultures were established according to standard procedures (Fok-Seang and Miller, 1994). Briefly, thoracic and lumbar spinal cord tissue was isolated, the meninges removed and the tissue chopped into 1 mm² pieces. Tissue was incubated first in 0.05% trypsin and 0.02% EDTA for 30 minutes at 37°C and subsequently in DMEM with 10% FBS and 0.125 mg/ml

DNase. Tissue was gently dissociated by trituration and passed through a 140 µm mesh filter. Cells were pelleted and resuspended in DMEM with 10% FBS and plated on poly-L-lysine/laminin-coated coverslips at a density of 10^5 cells/12 mm coverslip. The medium was changed to N2 (Bottenstein and Sato, 1979) +5% FBS after 1 day and replenished every other day.

To eliminate O4+ cells selectively, antibody-mediated complement lysis was performed. O4 (supernatant 1:1) was incubated with a spinal cord cell suspension for 30 minutes at 37°C. A 1:5 dilution of rabbit complement (Gibco) was then added and the cells incubated for a further 30 minutes at 37°C. The remaining cells were washed by centrifugation and cultured as above. In all cases, parallel control cultures treated with either O4 antibody but no complement or complement and no O4 antibody were also examined. Cultures were allowed to develop for various times in vitro and the presence of O4 and R-mab+ cells determined by indirect immunofluorescence as described above.

TLC analysis of O4 immunoreactivity in chick spinal cord

To determine the biochemical nature of O4 immunoreactivity in the chick spinal cord, immunostaining on thin-layer chromatography was performed according to previously published procedures (Bansal et al., 1989, 1992). Briefly, total lipids were extracted (Singh and Pfeiffer, 1985) from E8+1DIV ventral chick spinal cord cultures and E9 whole chick spinal cord. Lipids were separated on silica gel plastic-backed TLC plates (Bansal et al., 1992) and purified sulfatide (50 ng) run in a parallel lane. After drying, nonspecific binding was blocked by incubation of the TLC plate in 10% NGS in PBS for 1 hour at 37°C and the plate was then incubated in 1:50 dilution of O4 antibody with 2% NGS in PBS for 1 hour at room temperature. After extensive washing, the binding of O4 was visualized by subsequent 30 minute incubation in 1:300 dilution of biotinylated goat anti-mouse IgM followed by incubation in alkaline phosphatase-conjugated ABC reagent. Color was developed for 15 minutes using a stabilized alkaline phosphatase substrate.

RESULTS

O4 labels oligodendrocyte precursors in embryonic chick spinal cord cultures

Chick oligodendrocyte precursors express O4 immunoreactivity prior to labeling with R-mab. In explant cultures derived from E4 (stage 24) embryo spinal cords and cultured for 1 day in vitro no O4- or R-mab-immunoreactive cells were detected. By contrast, greater than 25% of E4 explants maintained for 2 days in vitro contained O4+ cells (Table 1). Similarly, 23% of explant cultures derived from E5 (stage 27) animals first contained detectable O4+ cells after one day in vitro suggesting that O4 cells initially appear in the chick spinal cord around E6 (Table 1). Consistent with these observations, O4+ cells were first seen in either freshly dissociated cells or sections of spinal cord at E6 (stage 29) (see below). R-mab+ oligodendrocytes were first detectable in explant cultures at the equivalent of E9 (stage 35). For example, in E4 explant derived cultures R-mab+ cells were first seen in approximately 15% of the O4-containing explants after 5 days in vitro, and in 30% one day later (Table 1). In explants derived from E6 or E7 embryos, the appearance of R-mab+ cells appeared to be delayed and significant number of R-mab+ cells did not appear until 4 or 3 days in vitro respectively, that is the equivalent of E10 (Table 1). Likewise, in dissociated cell cultures R-mab+ oligodendrocytes first appeared around E10. In freshly dissociated cells or frozen sections R-mab+ cells were first detected

Table 1. Timing of oligodendrocyte development in vitro

(A) Appearance of O4+ cells		
Age of tissue +days in culture	Proportion of explants containing O4+ cells	
E4+1=E5	1.3% (1/75)	
E4+2=E6	26.5% (22/83)	
E5+1=E6	23.4% (29/123)	
(B) Appearance of R-mab+ cells		
Age of tissue +days in culture	Proportion of explants containing R-mab+ cells	
E4+4=E8	1.2% (1/82)	
E5+3=E8	0% (0/61)	
E4+5=E9	15.8% (9/57)	
E5+4=E9	14.1% (9/64)	
E6+3=E9	0% (0/134)	
E7+2=E9	1.4% (1/71)	
E4+6=E10	34.4% (22/64)	
E5+5=E10	39.0% (23/59)	
E6+4=E10	50.0% (39/78)	
E7+3=E10	27.2% (9/82)	

at E9 in thoracic spinal cord. Thus, the expression of O4 immunoreactivity precedes R-mab immunoreactivity, which appears slightly delayed under certain in vitro conditions.

O4 and R-mab antibodies label cells of the same lineage. In explant cultures double labeled with O4 and R-mab antibodies, R-mab+ cells were restricted to those explants that also contained O4+ cells. Analysis of individual cells at the equivalent of E10 demonstrated that greater than 98% of the R-mab+ cells also labeled with O4 (Fig. 1). Similarly, in E9-dissociated cell cultures after one day in culture, virtually all the R-mab+ cells were also labeled with O4. It seems likely that O4 labeling is restricted to oligodendrocytes precursors in the chick spinal cord. When chick spinal cord cells were double labeled with O4 and anti-GFAP to identify astrocytes (Bignami et al., 1972) or O4 and anti-neurofilament antibodies to identify neurons, no double labeled cells were seen in either explant or dissociated cell cultures (data not shown). In addition, labeling of treated cultures with anti-GFAP antibodies after O4-mediated complement cell lysis was indistinguishable from non-treated cultures (data not shown).

Chick spinal cord oligodendrocytes require O4+ precursor cells to develop. In a series of O4 antibody-mediated complement cell lysis studies, both O4+ and R-mab+ cells were specifically eliminated from treated cultures. When complement treatments were performed on cells derived from embryos younger than E9 (stage 35), the elimination of O4+ cells was transient. That is, while treated cultures contained no O4+ cells 1-2 days after treatment, when such cultures were maintained until the equivalent of E14, O4+ cells eventually developed (Fig. 2A-D). Significant numbers of R-mab+ oligodendrocytes also developed in such cultures, suggesting that a pre-O4+ cell was competent to repopulate the oligodendrocyte lineage following O4-mediated cell lysis. By contrast, in parallel experiments on cells derived from embryos older than E9 (stage 35), the elimination of O4+ cells was permanent. That is, treated cultures contained no O4+ cells 1-2 days after treatment and when such cultures were maintained until the equivalent of E14 or beyond, no O4+ cells developed (Fig. 2E-H). Likewise, no R-mab+ oligodendrocytes developed in treated cultures. Taken together these data suggest that O4 labels oligodendrocyte precursors in the chick spinal cord and in embryos older than E9 (stage 35), O4+ cells represent the

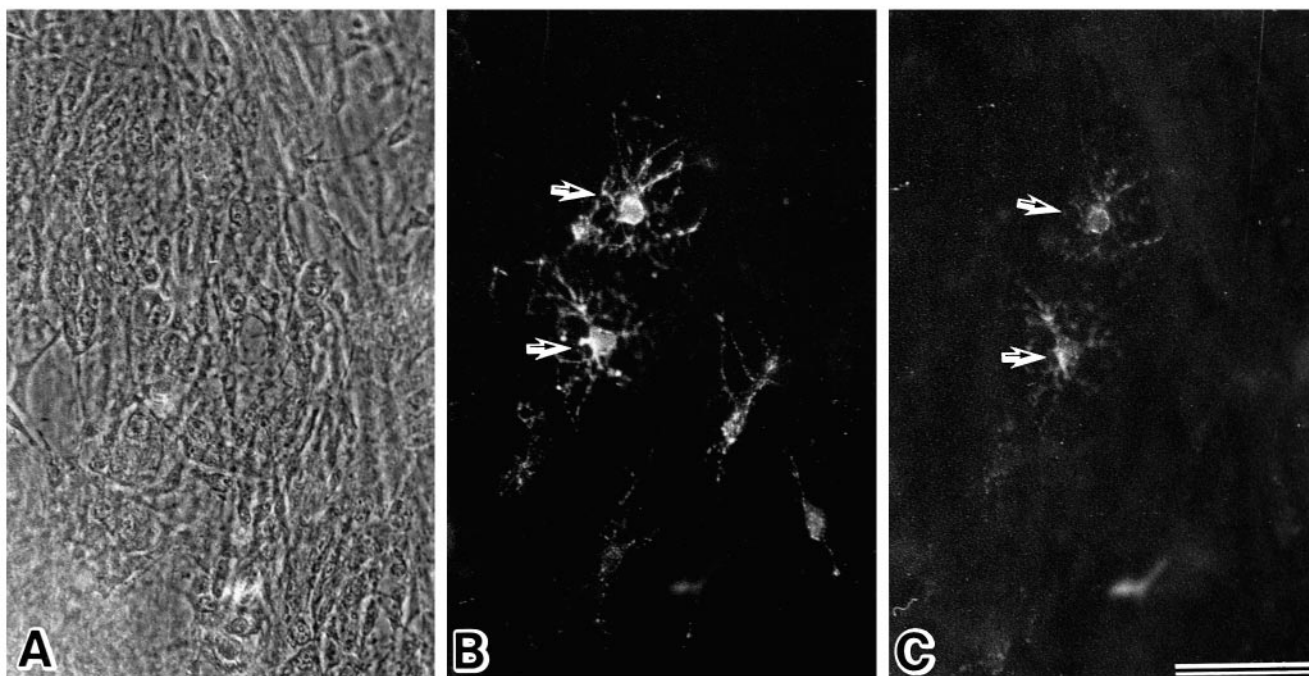


Fig. 1. Chick spinal cord oligodendrocytes develop from O4+ cells. An E4 ventral spinal cord explant culture grown for 6 DIV and double labeled with O4 (B) and R-mab (C) antibodies. (A) Phase-contrast image, (B) several O4+ cells are present of which two have a round cell body, multiple processes (arrows) and also express R-mab immunoreactivity (C). Virtually all R-mab+ cells that develop in these cultures express high levels of O4 immunoreactivity. Bar=100 μ m.

most immature self-renewing progenitor cells of the oligodendrocyte lineage.

Appearance of O4- and R-mab-immunoreactive cells in the developing chick spinal cord

To determine the initial location and stage at which oligodendrocytes and their precursors appeared in the chick spinal cord, transverse sections of thoracic chick spinal cords from embryos between the ages of E5 and E14 were labeled with O4 and R-mab antibodies.

In frozen sections, the earliest detectable O4+ cells in the chick spinal cord appeared at E6 (stage 29). These cells were restricted to the ventral region of the spinal cord. A cluster of O4+ cells were present at the ventral ventricular zone dorsal to the ventral midline (Fig. 3A). O4+ cells were also located in the ventral mantle layer with processes projecting to the ventral marginal zone in the area of the future ventral funiculus. At this stage, O4+ cells were not detected in either the lateral and dorsal marginal zone or the dorsal part of the mantle layer of the spinal cord. In older animals, (E7 stage 31) O4+ cells were present in the ventral spinal cord as well as in the lateral marginal zone in the area of the future lateral funiculus (Fig. 3B). The O4+ cells in the lateral marginal zone were generally radially oriented. Scattered O4+ cells were seen in the dorsal mantle layer while no O4+ cells were detected in the dorsal marginal zone (Fig. 3B). By E9 (stage 35), O4+ cells were detectable in ventral, lateral and dorsal regions of the spinal cord (Fig. 3C). In dorsal regions, O4+ cells were mainly located in the marginal zone in the area of the future dorsal funiculus. Few O4+ cells were seen in the developing dorsal gray matter. By E11 (st37), many intense O4-immunoreactive cells were present throughout the immature spinal cord white

matter while fewer O4+ cells were scattered throughout the developing gray matter (Fig. 3D).

Consistent with the *in vitro* observations, R-mab immunoreactivity appeared later in development than O4, and also followed a ventral-to-dorsal sequence. The first detectable R-mab+ cells in frozen sections of embryonic thoracic chick spinal cord were seen at E9 (stage 35) (Fig. 3E). A small cluster of R-mab+ cells were frequently seen at the ventral ventricular zone, consistent with the presence of a persistent precursor zone at the ventral. The majority of R-mab+ cells, however, were located in the ventral and lateral marginal zones while the dorsal marginal zone contained few labeled cells. By E11 (stage 37), R-mab+ cells were more broadly distributed throughout the spinal cord. Labeled cells were evident in the dorsal marginal zone as well as the lateral and ventral funiculi (Fig. 3F), while the immature gray matter contained few labeled cells. Comparison of the distribution of O4- and R-mab-labeled cells in consecutive sections from E9 or E11 animals indicated that R-mab+ cells, while fewer in number, were invariably located in the same regions of the spinal cord that contained numerous O4+ cells (compare Fig. 3C,D and E,F). Thus, it seems likely that *in vivo*, as *in vitro*, R-mab+ cells develop from O4+ cells.

The ventral ventricular zone is the site of origin of oligodendrocyte precursors in the chick spinal cord

To analyze the morphology and cellular attachments of O4+ cells in developing chick spinal cord, O4 was visualized by immunoperoxidase labeling on vibratome sections. The additional thickness of vibratome sections allowed the visualization of the entire labeled cells within the section. The overall distribution of O4+ cells was similar in these preparations to

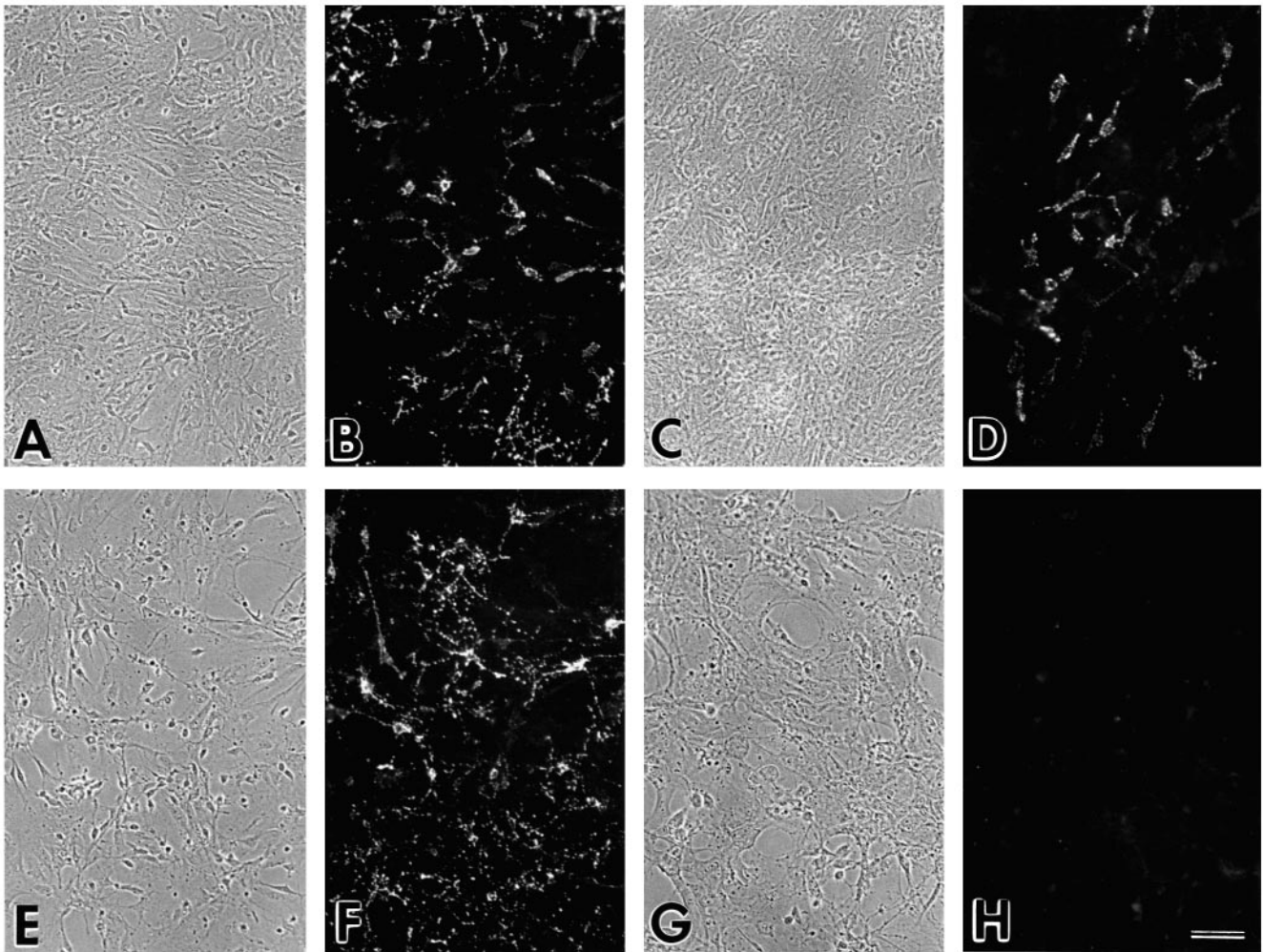


Fig. 2. O4-mediated complement cell lysis permanently eliminates the chick spinal cord oligodendrocyte lineage in embryos after E9 (stage 35). (A-D) E7.5 (stage 33) chick spinal cord cultures grown for 7DIV (to the equivalent of E14.5) and labeled with O4 antibody. (A,B) Control cultures, incubated with O4 antibody alone prior to plating, contain large numbers of O4+ cells. (C,D) Cultures incubated with O4 and complement prior to plating contain fewer but still significant numbers of O4+ cells. (E-H) E9 (stage 35) chick spinal cord cultures grown for 6 DIV (to the equivalent of E15) and labeled with O4 antibody. (E,F) Control cultures incubated with O4 antibody alone prior to plating contain large numbers of O4+ cells. (G,H) Cultures incubated with O4 and complement prior to plating contain no detectable O4+ cells. (A,C,E,F) Phase-contrast micrographs; (B,D,F,H) O4 immunofluorescent micrographs. Bar=50 μ m.

that seen in cryostat sections by immunofluorescence. At E6 (stage 29), a cluster of labeled cells was located at the ventral ventricular zone dorsal to the ventral midline (Fig. 4A,B). Most of the labeled cells in this region appeared to have an apical process closely apposed to the central canal (Fig. 4B-D). The O4+ cells, which were invariably located close to the dorsal aspect of the floor plate, had a neuroepithelial-like morphology and did not extend long processes to the spinal cord white matter (Fig. 4B,C). A subset of the O4+ cells in the ventral ventricular region of the spinal cord did not appear closely apposed to the central canal of the spinal cord (Fig. 4B,D,E). These cells were more elongated and either oriented radially with processes extending into developing gray matter of the spinal cord (Fig. 4D), or dorsoventrally oriented with processes extending dorsally in the region of the spinal cord just lateral to the ventricular zone (Fig. 4E). In both cases, the labeled cells appeared to possess a single predominant process, which terminated in a bulbous enlargement (Fig. 4E).

Ultrastructural analysis of the O4-labeled cells associated

with the ventricular surface demonstrated these cells were an integral component of the lining of the central canal (Fig. 5A). These labeled ventricular cells were elongated mediolaterally and were interdigitated with O4- cells (Fig. 5A,C). The apical surface of these cells directly faced the central canal. Most cells extended microvilli into the lumen of the central canal and some cells also possessed a cilium projecting into the central canal (Fig. 5D). The O4-labeled ventricular cells appeared indistinguishable from their neighboring unlabeled cells, and were connected with neighboring cells by desmosome-like adherens junctions (Fig. 5B). These observations indicate that in the spinal cord ventral ventricular zone individual ventricular cells express distinct characteristics of oligodendrocyte precursors while still participating as integral parts of the ventricular lining of the spinal cord.

Oligodendrocyte precursors migrate dorsally in the spinal cord

To trace the migration of chick spinal cord oligodendrocyte

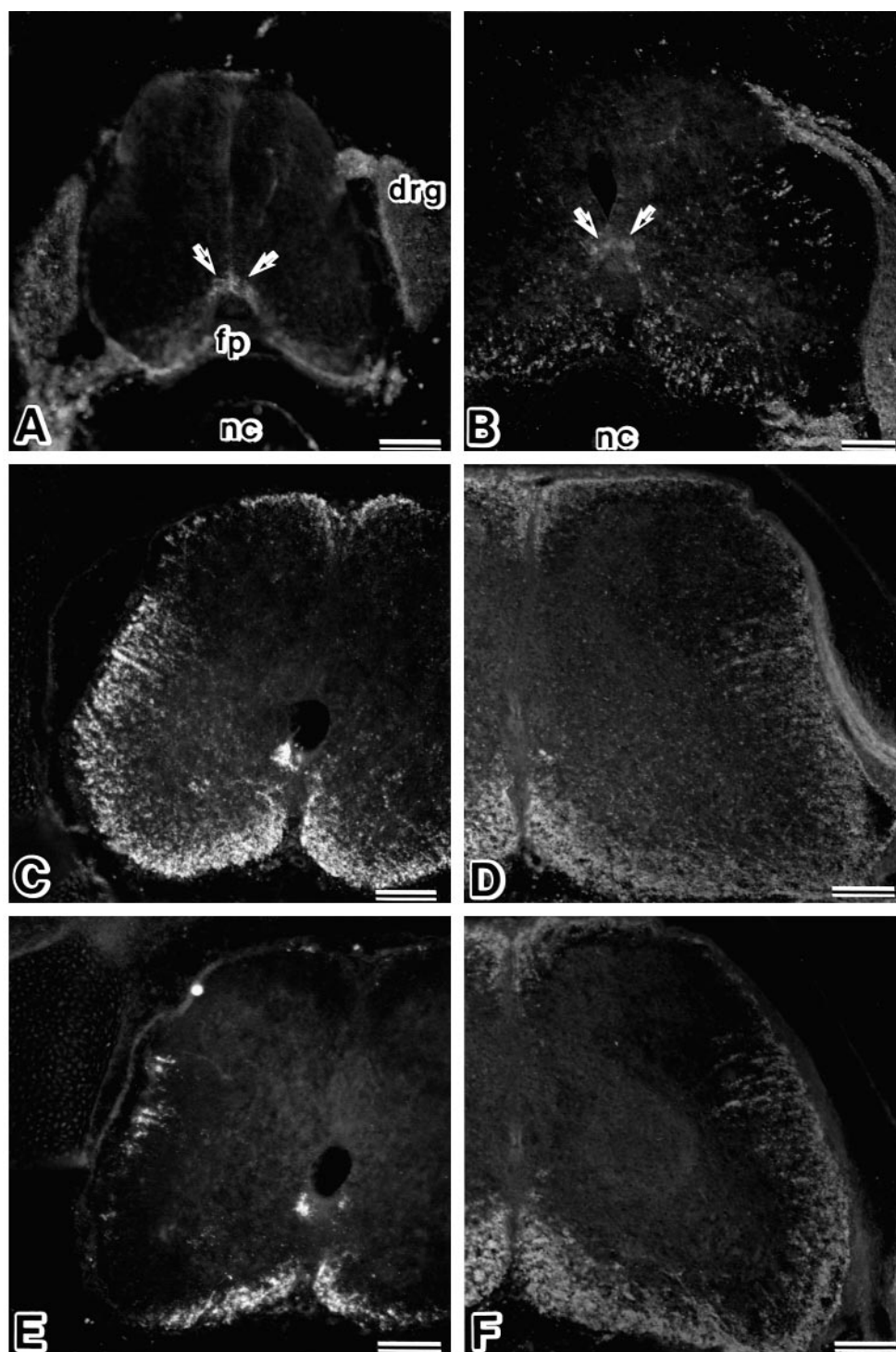


Fig. 3. O4+ cells develop before R-mab+ cells in the embryonic chick spinal cord although both appear in a ventral-to-dorsal sequence. Transverse frozen sections through the thoracic region of embryonic chick spinal cords at different stages of development labeled with O4 antibody (A-D) or R-mab antibody (E-F). (A) At E6 (stage 29), a small cluster of O4+ cells are present bilaterally at the ventral ventricular zone (arrows) just dorsal to the floor plate. A few O4+ cells or processes are also present in the ventral marginal zone but no O4+ cells are present in the dorsal regions of the spinal cord. Outside the spinal cord O4+ cells are present in the ventral roots, dorsal roots and dorsal root ganglion of the PNS. (B) At E7 (stage 32), a cluster of O4+ cells are still present in the ventral ventricular zone (arrows) as well as increased numbers of O4+ cells in the lateral marginal zone and ventral spinal cord. At this stage no O4+ cells are detectable in the dorsal marginal zone. (C) At E9 (stage 35), many O4+ cells are present in all parts of the spinal cord marginal zones, but few O4+ cells are present in the mantle layer. (D) At E11 (stage 37), O4+ cells are densely distributed in the immature spinal cord white matter and scattered in the gray matter. (E) A section adjacent to that in C at (E9: stage 35) labeled with R-mab. At this stage, R-mab+ cells are observed only in the ventral and lateral marginal zones of the spinal cord and no R-mab+ cells are detectable in dorsal spinal cord. (F) A section adjacent to that in D (E11: stage 37) labeled with R-mab. R-mab+ cells are observed in all parts of the marginal zone, while few R-mab+ cells are present in the gray matter. drg, dorsal root ganglion; fp, floor plate; nc, notochord. Bar=100 μ m.

precursors from the ventral ventricular zone, the distribution of O4-labeled cells was assayed in sagittal vibratome sections of E7 (stage 31) and E8 (stage 34) chick spinal cords.

In sections through the most medial region of the E7 spinal cord including the ventricular layer, O4+ cells were restricted to a ribbon of cells several cells thick in the ventral region (Fig. 6A,B). No O4+ cells were detected in the dorsal ventricular zone at any stage of development. In sections through mid-sagittal levels of the spinal cord, the ventral ribbon of O4+ cells was broader and the O4+ cells were predominantly oriented

dorsoventrally (Fig. 6B). By contrast, in ventral white matter many O4+ cells were oriented rostrocaudally (Fig. 6C). While relatively few O4+ cells were seen in the dorsal half of the spinal cord at E7, in similar sections taken one day later (E8) many more O4 cells were located in the dorsal half of the spinal cord and they extended towards the dorsal pial surface (Fig. 6E). These ventrodorsally oriented cells were elongated with a single primary process that terminated in a bulbous expansion. These data suggest that O4+ oligodendrocyte precursors are migrating from ventral to dorsal regions of the spinal cord

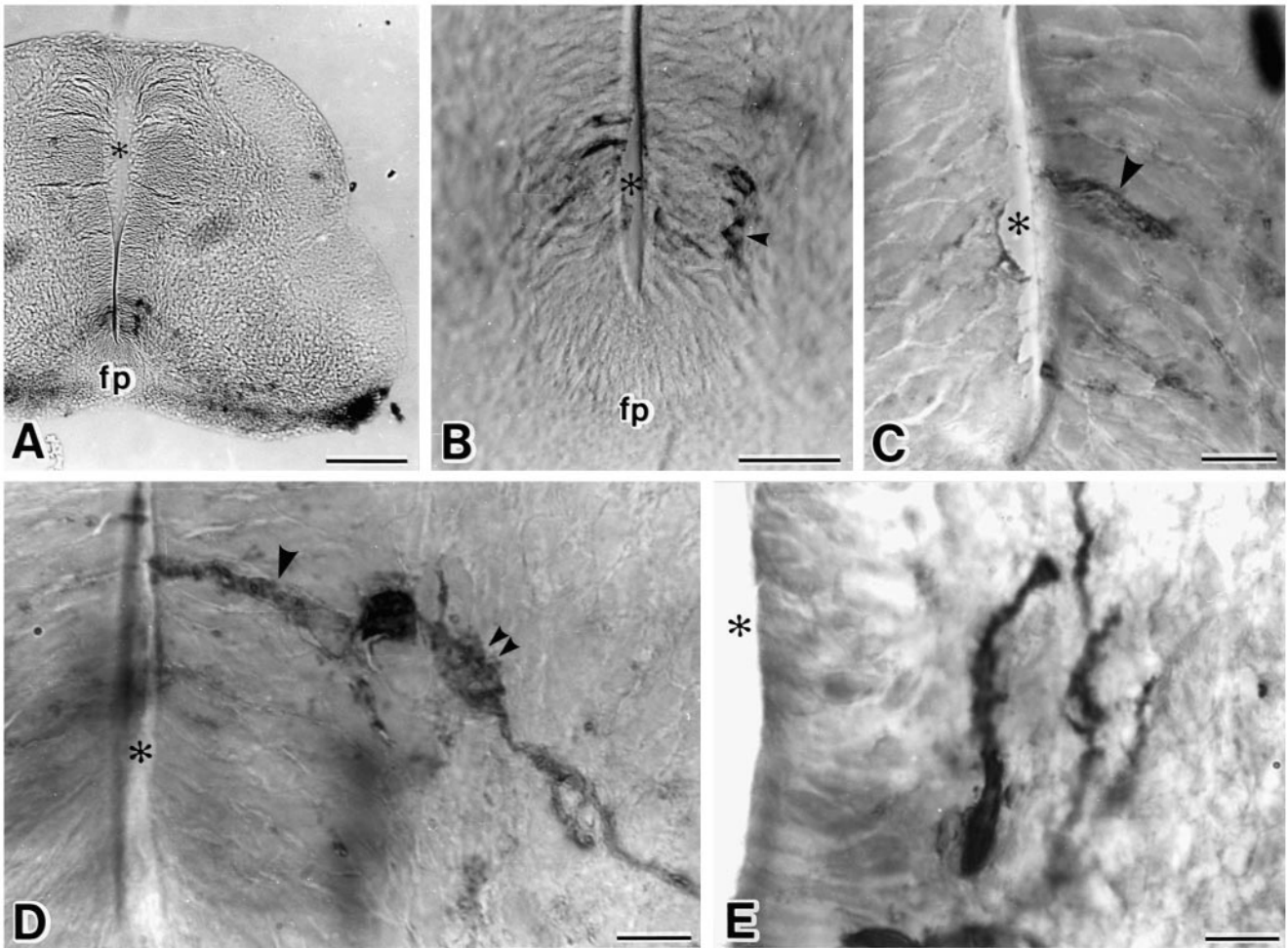


Fig. 4. Oligodendrocyte precursors in the chick spinal cord originate in a discrete region of the ventral ventricular zone dorsal to the floor plate. Transverse vibratome sections through the thoracic regions of an E6 (stage 29; A-D) and E8 (stage 34; E) chick embryo. Sections were labeled with O4 antibody and visualized by immunoperoxidase reaction. (A) Low power micrograph showing the ventral location of the O4+ cells. (B) Intermediate magnification of the ventral ventricular region in A. Several distinct O4+ cells are observed in the ventricular layer oriented mediolaterally. All of these cells are elongated or fusiform in shape and lack long processes. Two additional O4+ cells in present adjacent to the ventricular layer (arrow) and these cells are oriented ventrodorsally. (C,D) Higher magnification of the O4+ ventricular cells in A,B. In C, the apical region of the cells faces the central canal (arrows). At a different depth of focus (D), two O4+ cells are detectable one of which extends an apical process to the central canal (arrowhead) and lacks long processes while the other has a cell body adjacent to the ventricular layer (double arrowhead) and has a long radially oriented peripheral process. (E) High magnification image of an O4+ cell adjacent to the ventral ventricular layer in an E8 chick spinal cord. This cell is oriented ventrodorsally and possesses a single process whose tip ends in a bulbous expansion. Asterisks indicate the central canal and fp = floor plate. Bars, 100 μ m in A, 50 μ m in B and 10 μ m in C-E.

through the developing gray matter of the midsagittal region of the chick spinal between E6 and E8. In sections through the most lateral peripheral white matter regions of the spinal cord, the orientation of the O4+ cells was significantly different. As in ventral white matter, the majority of O4+ cells in lateral white matter were oriented rostrocaudally (Fig. 6C) and most cells possessed a primary process which terminated in a bulbous ending, suggesting that in white matter O4+ cells alter their orientation and become aligned parallel with the longitudinal axons of the spinal cord.

The migration of O4+ cells into the dorsal spinal cord correlates with the capacity to generate oligodendrocytes

To assess whether the migration of O4+ cells into the dorsal spinal cord in vivo correlated with the ability of isolated dorsal

regions of the spinal cord to give rise to oligodendrocytes, in vitro explant analyses were performed.

In dorsal spinal cord explants isolated from embryos between E 4 and E5 (stages 23-27) and grown in vitro until the equivalent of E9 (stage 35) fewer than 10% of the explants contained O4+ cells (Fig. 7) suggesting that up to stage 27 the dorsal spinal cord had yet to acquire the capacity to generate oligodendrocytes. Increasing numbers of dorsal explants derived from older animals grown to the same equivalent age contained O4+ cells. For example, at E6 (stage 29) approximately 20% (42/201) of dorsal explants contained O4+ cells while 2 stages later at E7 (stage 31) greater than 80% (126/157) of dorsal explants contained O4+ cells (Fig. 7). These data suggest that the isolated dorsal region of the chick spinal cord rapidly acquires the capacity to give rise to O4+ oligodendrocyte precursors between stages 29 and 31. The finding that by

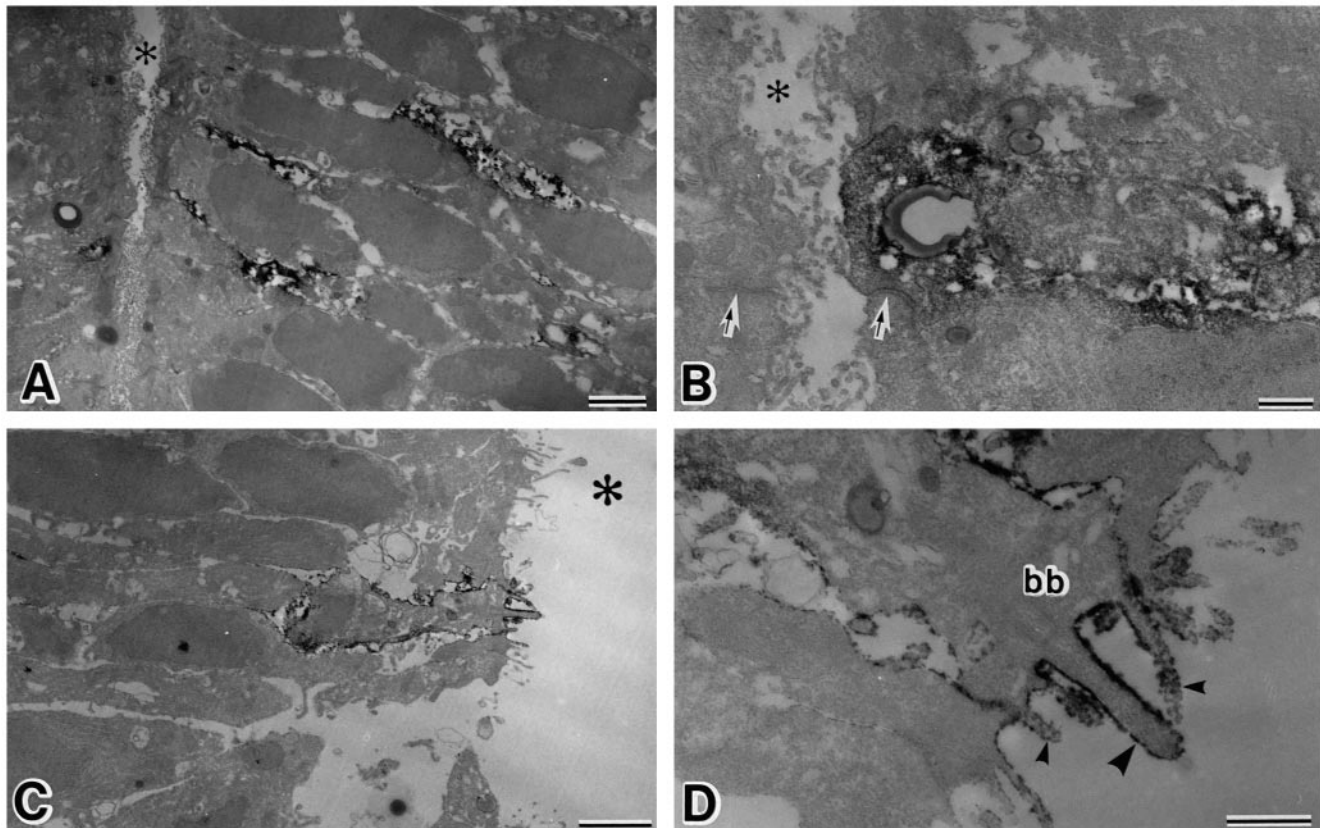


Fig. 5. O4+ oligodendrocyte precursors at the ventral ventricular zone contribute to the lining of the central canal of the spinal cord. Immunoelectron micrographs of O4-labeled cells in the ventral ventricular zone of an E8 (stage 34) chick embryo. (A,C) Low magnification micrographs showing O4-labeled cells surrounded by non-labeled cells. The peroxidase reaction product is primarily restricted to the cell surface and the morphology of the labeled cells is not obviously different from their non-labeled neighbors. (B,D) High magnification of the cells in A and C, respectively. In B, an O4-labeled cell has an apical aspect that faces the central canal. This cell is connected by adherens-like junctions (arrows) to its neighbor. In D, the O4-labeled cell directly abuts the central canal. This cell has many characteristics associated with ventricular cells. It contains a basal body (bb) and extends a cilium (larger arrowhead) as well as microvilli (small arrowheads) into the lumen of the central canal. Bars=2 μ m in A,C and 0.5 μ m in B,D.

E7 (stage 31) the majority of dorsal explants give rise to O4+ oligodendrocyte precursors correlates with the *in vivo* observations that the earliest stage at which significant number of O4+ cells can be identified in the dorsal regions of the spinal cord is E7 (see above). Thus, the ability to detect O4+ cells in a specific region of the developing chick spinal cord correlates directly with the ability of that tissue to give rise to oligodendrocytes *in vitro*.

O4 binds to a novel lipid antigen in chick spinal cord

O4 antibody recognizes at least two distinct antigens. On immature rodent oligodendrocyte precursors O4 recognizes an uncharacterized antigen termed POA while, on GalC+ differentiated oligodendrocytes, it recognizes sulfatide (Bansal et al 1992). To determine if the binding of O4 to immature chick spinal cord oligodendrocytes was mediated through a POA-like or characteristic sulfatide antigen, an immuno-TLC analysis was performed. Both *in vitro* and *in vivo* conditions were selected in which large numbers of O4+ cells were present, but R-mab+ cells had yet to develop in significant numbers: for example, cultures of E8 +1DIV chick ventral spinal cord and E9 lumbar chick spinal cord. Separation of the lipids in each of these samples by TLC and comparison of the

O4-labeling patterns with that of purified sulfatide indicated that, while labeling of the purified sulfatide resolved to the characteristic doublet (Fig. 8 lane 1), the predominant labeling of both cultured chick spinal cord cells and spinal cord samples was a single band which did not co-migrate with either sulfatide band (Fig. 8 lane 2,3). These data suggest that O4 immunoreactivity on early chick spinal cord oligodendrocyte precursors does not reflect the precocious expression of the characteristic forms of hydroxy and non-hydroxy sulfatide on the surface of these cells, but more likely the expression of a POA-like molecule.

DISCUSSION

In this study, we show that embryonic chick spinal cord oligodendrocyte precursors initially appear in the ventral ventricular zone of the spinal cord. These cells or their progeny subsequently migrate both laterally and dorsally to populate the developing white matter of the spinal cord over the next few days.

During development of the oligodendrocyte lineage in chick spinal cord, O4 immunoreactivity is initially detectable on very

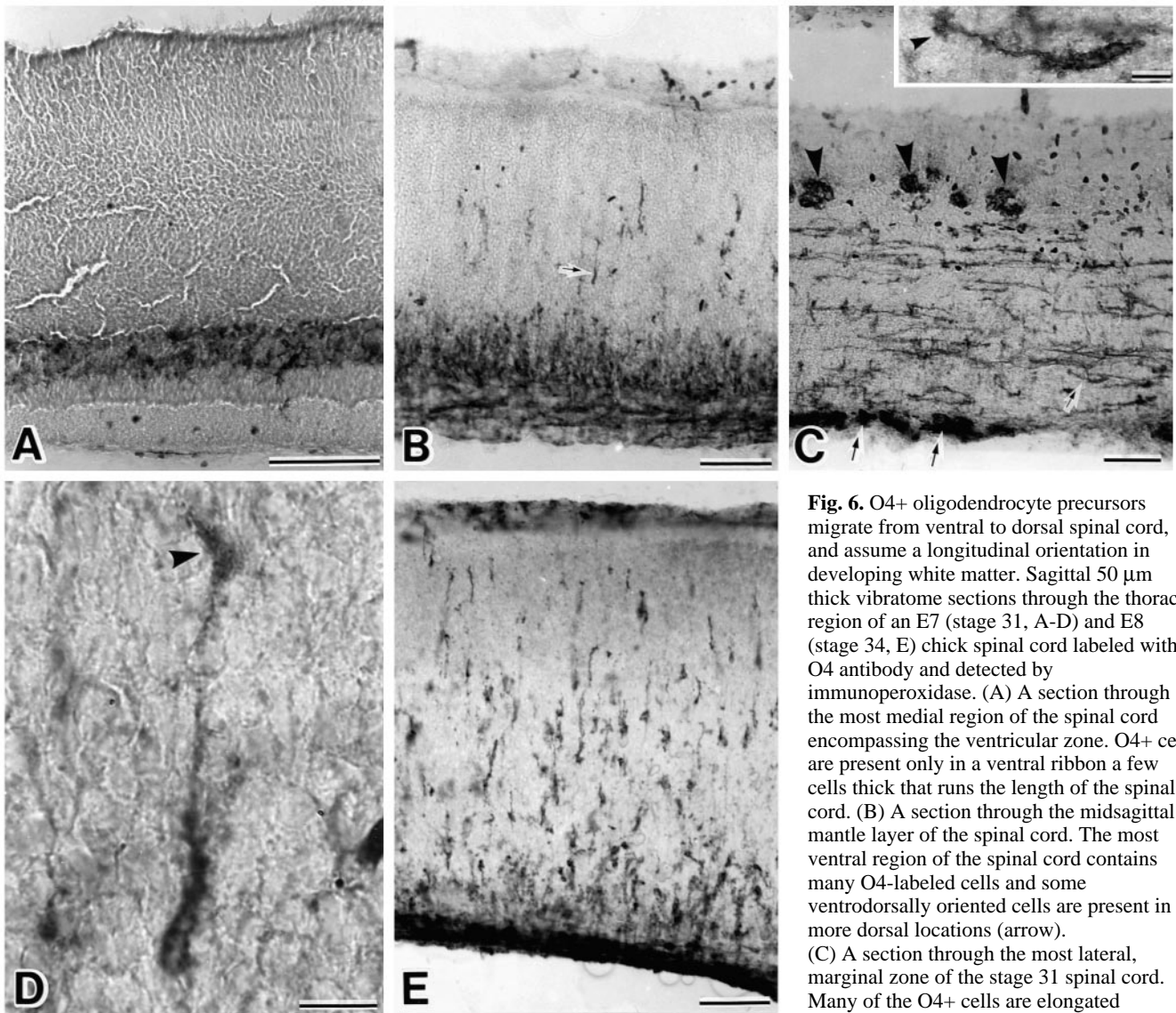


Fig. 6. O4+ oligodendrocyte precursors migrate from ventral to dorsal spinal cord, and assume a longitudinal orientation in developing white matter. Sagittal 50 μ m thick vibratome sections through the thoracic region of an E7 (stage 31, A-D) and E8 (stage 34, E) chick spinal cord labeled with O4 antibody and detected by immunoperoxidase. (A) A section through the most medial region of the spinal cord encompassing the ventricular zone. O4+ cells are present only in a ventral ribbon a few cells thick that runs the length of the spinal cord. (B) A section through the midsagittal mantle layer of the spinal cord. The most ventral region of the spinal cord contains many O4-labeled cells and some ventrodorsally oriented cells are present in more dorsal locations (arrow). (C) A section through the most lateral, marginal zone of the stage 31 spinal cord. Many of the O4+ cells are elongated longitudinally along the spinal cord. The

arrowed cell is shown in higher magnification in the upper right inset where the primary process and growth cone-like terminal are evident (arrowhead). Large arrowhead and smaller arrows indicate the dorsal and ventral roots, respectively. (D) Higher magnification of the O4+ cell arrowed in B. The labeled cell extends a long process in the ventrodorsal plane that terminates in a growth cone-like enlargement (arrowhead). (E) A section through the midsagittal level of an E8 (stage 34) thoracic chick spinal cord. Compared with earlier stages (B), there is a more uniform distribution of O4+ cells throughout the whole spinal cord although they are still at a higher density in the ventral regions. Most of the labeled cells in this region are clearly oriented ventrodorsally. Bars=100 μ m in A,B,C and E and 5 μ m in D and the insert in C.

immature precursors. For example, the earliest detectable O4+ cells *in vivo* contribute to the ventricular lining of the spinal cord consistent with the immature nature of these cells. In addition, O4-mediated complement cell lysis on spinal cord cells derived from E9 (stage 35) animals effectively eliminates the oligodendrocyte lineage in treated cultures. The results of this complement lysis analysis differ significantly from similar studies in cultures of developing rodent CNS. Complement-mediated cell lysis with either A2B5 antibody a marker of O2-A progenitor cells (Raff et al., 1983) or O4 antibody results in only a temporary elimination of the oligodendrocyte lineage cells in treated cultures (Greenspan et al., 1990; Hardy and Reynolds, 1991; Fok-Seang and Miller, 1994). These studies suggest that, while the rodent CNS contains an uncharacterized

persistent self-renewing founder cell of the oligodendrocyte lineage, in embryonic chick spinal cord after stage 35, such cells can be identified by the expression of O4 immunoreactivity. Such O4+ cells may correspond to adult oligodendrocyte progenitors described in the rodent (Wolswijk and Noble, 1989) and human (Armstrong et al., 1992) CNS.

The expression of O4 and R-mab immunoreactivity appears to be more synchronous in oligodendrocyte precursors in the chick spinal cord than in rat. It has therefore been possible to isolate spinal cord cells and tissue that express high levels of O4, but virtually no R-mab immunoreactivity and determine the nature of the antigen recognized by O4 on the surface of immature chick oligodendrocyte precursors. The initial binding of O4 antibody to mammalian oligodendrocyte precursors is

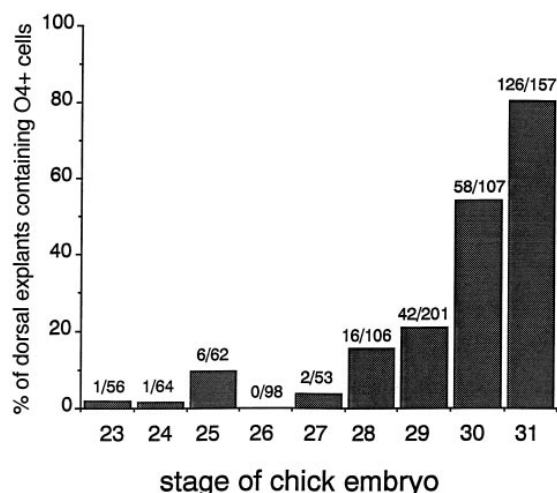


Fig. 7. The proportion of dorsal explants that contain oligodendrocyte precursors increases dramatically between E6 (stage 29) and E7 (stage 31). Isolated dorsal explants were prepared from animals at different stages, grown until the equivalent of E9 and then labeled with O4 antibody by indirect immunofluorescence. The number of explants containing O4+ cells and the total number of explants examined at each stage is shown above each bar.

mediated through the prolignodendroblast antigen or POA while subsequent binding to GalC+ oligodendrocytes is mediated through sulfatide (Bansal et al., 1989, 1992). The biochemical nature of POA on mammalian oligodendrocyte precursors is currently unknown (Bansal et al., 1989, 1992). Biochemical characterization of the antigen recognized by O4 antibody on embryonic chick spinal cord oligodendrocyte precursors suggest that it is a lipid distinct from the characteristic hydroxy and non-hydroxy sulfatide in terms of its mobility on TLC plates. Although the possibility that this antigen may be an unknown form of sulfatide cannot currently be ruled out, it seems more likely that the antigen recognized by O4 on immature chick oligodendrocyte precursors is the equivalent of the POA antigen in rodent (Bansal et al., 1989, 1992).

Oligodendrocyte differentiation in chick spinal cord cultures derived from early embryos appears similar to that *in vivo* suggesting that, as in the rodent CNS (Williams et al., 1985), the differentiation of these cells is not dependent on morphogenesis of the intact spinal cord. However, in cultures derived from older embryos, the timing of oligodendrocyte differentiation is delayed compared to either intact spinal cord or cultures derived from younger animals. The reason for the inhibition of oligodendrocyte development in older cultures is unclear. One possibility is that it reflects a transient disruption of either cell-cell interactions or diffusible signals that subsequently regulate oligodendrocyte differentiation in the chick spinal cord.

The ventral origin of oligodendrocyte precursors appears to be a characteristic of vertebrate development. In rat spinal cord, indirect analysis of proliferating cell populations suggests that cells located in the region of the ventral ventricular zone dorsal to the ventral midline give rise to spinal cord oligodendrocytes (Warf et al., 1991; Noll and Miller, 1993). Similarly, *in situ* hybridization with probes to either the PDGF receptor (Pringle and Richardson 1993), which is thought to be specific for oligodendrocyte precursors in the late embryonic rat CNS (Pringle et al., 1992), or to the myelin protein CNP have



Fig. 8. O4 antibody appears to label an antigen other than sulfatide on the surface of immature chick spinal cord oligodendrocyte precursors. Total lipids extracted from E8+1DIV ventral spinal cord cultures (lane 2) and E9 lumbar chick spinal cord (lane 3) along with purified sulfatide standard (50 ng, lane 1) were separated by TLC and immunostained with O4. In both cultured cells and spinal cord samples, the major band labeled by O4 antibody migrates between the doublet of purified sulfatide. Immunofluorescent labeling of parallel chick spinal cord preparations demonstrated many O4+ but virtually no R-mab+ cells. The indistinct higher band in lane 3 is non-specific which is purple in color (specific bands are brown) and appear in areas of excess lipid.

indicated that the earliest localization of labeled cells is in the region of the ventral ventricular zone (Yu et al., 1994). While clearly defining the location of origin of spinal cord oligodendrocytes, these previous studies did not elucidate the cellular characteristics and morphology of the labeled cells. In the developing chick spinal cord, the ability to utilize O4 immunolabeling at the ultrastructural level clearly demonstrates that individual cells in a distinct region of the ventral spinal cord acquire specific characteristics associated with the oligodendrocyte lineage while still an integral part of the ventricular lining of the central canal. These observations provide direct support for the hypothesis that the ventricular zone of the developing vertebrate CNS is not a homogenous assembly of cells but rather is composed of microdomains that give rise to distinct cell types (Hamburger, 1948; Wenger, 1950; Leber et al., 1990; Yu et al., 1994).

The factors that regulate the initial development of oligodendrocyte precursors in the ventral ventricular region of the spinal cord remain unclear. It seems likely that the spatially restricted appearance is in part regulated by the dorsal-ventral axis of the spinal cord. A number of studies have implicated the ventrally located notochord in controlling dorsal-ventral polarity of the vertebrate spinal cord (van Stratten et al., 1985; Yamada et al., 1991, 1993). For example, transplantation of an additional notochord to ectopic sites adjacent to the lateral or dorsal spinal cord induces an additional floorplate and pool of motor neurons (van Stratten et al., 1988; Yamada et al., 1993). Preliminary studies suggest that the notochord may also play a role in regulating oligodendrocyte development in the spinal cord although the molecular mediators are unknown (Orentas and Miller, 1994). One attractive hypothesis to account for the initial ventral appearance of spinal cord oligodendrocyte precursors is that the ventrodorsal axis of the spinal cord is established by environmental factors from the notochord (Tanabe et al., 1994). As a result of this ventralization, specific as yet unidentified, local signals induce cells at the ventral ventricular zone to become committed to the oligodendrocyte lineage.

The successful myelination of the entire spinal cord appears to be critically dependent on the migration of oligodendrocyte precursors. Precursors have to migrate both laterally from the ventral ventricular zone to the developing lateral columns and dorsally to the developing dorsal columns. The temporal cor-

relation of the invasion of dorsal regions of the spinal cord with O4+ cells and the acquisition of that tissue to give rise to oligodendrocytes strongly suggest that O4 labels premigratory and migratory chick oligodendrocyte precursors. By contrast, in rodent, the majority of oligodendrocyte precursor migration is accomplished by immature precursors prior to their expression of O4 and GC immunoreactivity (Noble et al., 1988; Warrington et al., 1993). Analysis of the distribution of O4+ cells at different stages of development of the chick spinal cord effectively charts their pathways of migration and suggests that such migrational pathways are not random. The majority of ventral-to-dorsal migration occurs in the intermediate gray matter of the spinal cord. The cell and molecular nature of the ventral-to-dorsal migrational pathway is unclear. One possibility is that oligodendrocyte precursors, like some neuron precursors, may utilize the axons of the commissural neurons during this phase of migration (Phelps et al., 1990; Phelps and Vaughn, 1993). However, radial migration to the developing ventral and ventrolateral columns requires a different cellular substrate. Radial glial cells, present in the spinal cord during embryonic development (Hirano and Goldman, 1988) and other regions of the CNS, have been proposed to guide radial migrations of neuron precursors (Rakic, 1971; Hatton, 1990). By analogy, the radial migration of spinal cord oligodendrocyte precursors from a ventricular origin may utilize radial glial guides. Consistent with this hypothesis, earlier studies have clearly demonstrated that immature spinal cord oligodendrocyte precursors in the developing white matter have a striking radial orientation (Hirano and Goldman, 1988; Choi et al., 1983; Choi and Kim, 1985).

In conclusion, these studies provide direct evidence for the ventral ventricular origin and subsequent dorsal migration of oligodendrocyte precursors during development of the vertebrate spinal cord. The ability to specifically identify these cells both *in vivo* and *in vitro* by O4 labeling in the chick CNS will allow for the future characterization of the signaling mechanisms responsible for the induction of vertebrate spinal cord oligodendrogenesis.

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REFERENCES

- Armstrong, R. C., Dorn, H. H., Kufta, C. V., Friedman, E. and Dubois-Dalcq, M. (1992). Pre-oligodendrocytes from adult human CNS. *J. Neurosci.* **12**, 1538-1547.
- Bansal, R., Stefansson, K. and Pfeiffer, S. E. (1992). Proligodendroblast antigen (POA), a developmental antigen expressed by A007/O4-positive oligodendrocyte progenitors prior to the appearance of sulfatide and galactocerebroside. *J. Neurochem.* **58**, 2221-2229.
- Bansal, R., Warrington, A. E., Gard, A. L., Ranscht, B. and Pfeiffer, S. E. (1989). Multiple and novel specificities of monoclonal antibodies O1, O4, and R-mAb used in the analysis of oligodendrocyte development. *J. Neurosci. Res.* **24**, 548-557.
- Bignami, A., Eng, L. F., Dahl, D. and Uyeda, C. T. (1972). Localization of the glial fibrillary acidic protein in astrocytes by immunofluorescence. *Brain Res.* **43**, 429-435.
- Bogler, O., Wren, D., Barnett, S. C., Land, H. and Noble, M. (1990). Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of the oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. *Proc. Nat. Acad. Sci. USA* **87**, 6368-6372.
- Bottenstein, J. E. and Sato, G. H. (1979). Growth of a rat neuroblastoma cell line in serum-free supplemented medium. *Proc. Nat. Acad. Sci. USA* **76**, 514-517.
- Bunge, R. P. (1968). Glial cells and the central myelin sheath. *Physiol. Rev.* **48**, 197-251.
- Choi, B. H. and Kim, R. C. (1985). Expression of glial fibrillary acidic protein by immature oligodendroglia and its implications. *J. Neuroimmunol.* **8**, 215-235.
- Choi, B. H., Kim, R. C., Lapham, L. W. (1983). Do radial cells give rise to both astroglia and oligodendroglial cells? *Dev. Brain Res.* **8**, 119-130.
- Eisenbarth, G. S., Walsh, F. S. and Nirenberg, M. (1979). Monoclonal antibody to a plasma membrane antigen of neurons. *Proc. Nat. Acad. Sci. USA* **76**, 4913-4917.
- Fok-Seang, J. and Miller, R. H. (1994). Distribution and differentiation of A2B5+ glial precursors in the developing rat spinal cord. *J. Neurosci. Res.* **37**, 219-235.
- Fujita, S. (1965). An autoradiographic study on the origin and fate of the subpial glioblast in the embryonic chick spinal cord. *J. Comp. Neurol.* **124**, 51-60.
- Gard, A. L. and Pfeiffer, S. E. (1989). Oligodendrocyte progenitors isolated directly from developing telencephalon at a specific phenotypic stage: myelinogenic potential in a defined environment. *Development* **106**, 119-132.
- Gard, A. L., and Pfeiffer, S. E. (1990). Two proliferative stages of the oligodendrocyte lineage (A2B5+O4- and O4+GalC-) under different mitogenic control. *Neuron* **5**, 615-625.
- Greenspan, J. B., Stern, J. L., Pustilnik, S. M. and Pleasure, D. (1990). Cerebral white matter contains PDGF-responsive precursors to O-2A cells. *J. Neurosci.* **10**, 1866-1873.
- Hamburger, V. (1948). The mitotic patterns in the spinal cord of the chick embryo and their relation to histogenetic processes. *J. Comp. Neurol.* **88**, 221-284.
- Hamburger, V. and Hamilton, H. (1951). A series of normal stages in the development of the chick embryo. *J. Morph.* **88**, 49-92.
- Hardy, R. and Reynolds, R. (1991). Proliferation and differentiation potential of rat forebrain oligodendroglial progenitors both *in vitro* and *in vivo*. *Development* **111**, 1061-1080.
- Hatton, M. E. (1990). Riding the glial monorail: a common mechanism for glial guided neuronal migration in different regions of the developing mammalian brain. *Trends in Neurosci.* **13**, 179-184.
- Hirano, M. and Goldman, J. E. (1988). Gliogenesis in rat spinal cord: evidence for origin of astrocytes and oligodendrocytes from radial precursors. *J. Neurosci. Res.* **21**, 155-167.
- Leber, S. M., Breedlove, M. and Sanes, J. (1990). Lineage, arrangement and death of conally related motoneurons in chick spinal cord. *J. Neurosci.* **10**, 2451-2462.
- McKinnon, R. D., Matsui, T., Dubois-Dalcq, M. and Aaronson, S. A. (1990). FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* **5**, 603-614.
- Noble, M., Murray, K., Stroobant, P., Waterfield, M. D. and Riddle, P. (1988). Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* **333**, 560-562.
- Noll, E. and Miller, R. H. (1993). Oligodendrocyte precursors originate at the ventral ventricular zone dorsal to the ventral midline in the embryonic rat spinal cord. *Development* **118**, 563-573.
- Orentas, D. M. and Miller, R. H. (1994). Environmental regulation of oligodendrocyte induction in the embryonic chick spinal cord. *J. Devel. Neurosci. Supp.* **222** S-B.
- Phelps, P. E., Barber, R. P., Brennan, V. M. M., Salvaterra, P. M. and Vaughn, J. E. (1990). Embryonic development of four different subsets of cholinergic neurons in rat cervical spinal cord. *J. Comp. Neurol.* **291**, 9-26.
- Phleps, P. E. and Vaughn, J. E. (1993). Electron microscopic evidence that commissural fibers may guide neuronal migration in developing rat cervical spinal cord. *Soc. Neurosci. Abs.* **19**, 875.
- Placzek, M., Tessier-Lavigne, M., Jessell, T. M. and Dodd, J. (1990). Orientation of commissural axons *in vitro* in response to a floor plate derived chemoattractant. *Development* **110**, 19-30.
- Placzek, M., Yamada, T., Tessier-Lavigne, M., Jessell, T. M. and Dodd, J. (1991). Control of dorso-ventral pattern in vertebrate neural development, induction and polarizing properties of the floor plate. *Development* **113** Supplement 2, 105-122.

- Pringle, N. P., Mudhar, H. S., Collarini, E. J. and Richardson, W. D.** (1992). PDGF receptors in the CNS, during late neurogenesis, expression of PDGF alpha receptors appears to be restricted to glial cells of the oligodendrocyte lineage. *Development* **115**, 535-551.
- Pringle, N. P. and Richardson, W. D.** (1993). A singularity of PDGF alpha-receptor expression in the dorsoventral axis of the neural tube may define the origin of the oligodendrocyte lineage. *Development* **117**, 525-533.
- Rakic, P.** (1971). Neuron-glial relationships during granule cell migration in the developing cerebellar cortex. A Golgi and electronmicroscopic study in *Maccacus rhesus*. *J. Comp. Neurol.* **141**, 238-312.
- Raff, M. C., Mirsky, R., Fields, K. L., Lisak, R. P., Dorfman, S. H., Silberberg, D. H., Gregson, N. A., Liebowitz, S. and Kennedy, M. C.** (1978). Galactocerebroside is a specific cell surface antigenic marker for oligodendrocytes in culture. *Nature* **274**, 813-816.
- Raff, M. C., Miller, R. H. and Noble, M.** (1993). A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture medium. *Nature* **303**, 390-396.
- Ranscht, B., Clapshaw, P. A., Price, J., Noble, M. and Siefert, W.** (1982). Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. *Proc. Natl. Acad. Sci. USA* **79**, 2709-2713.
- Singh, H. and Pfeiffer, S. E.** (1985). Myelin associated galactolipids in primary cultures from dissociated fetal rat brain: biosynthesis, accumulation and cell surface expression. *J. Neurochem.* **45**, 1371-1381.
- Small, R. K., Riddle, P. and Noble, M.** (1987). Evidence for migration of oligodendrocyte-type-2 astrocyte progenitor cells into the developing rat optic nerve. *Nature* **328**, 155-157.
- Sommer, I. and Schachner, M.** (1981). Monoclonal antibodies (O1-O4) for oligodendrocyte cell surfaces: an immunocytochemical study in the central nervous system. *Dev. Biol.* **83**, 311-327.
- Tanabe, Y., Placzek, M., Edlund, T. and Jessell, T. M.** (1994). Floor plate and motor neuron induction by *vhh-1*, a vertebrate homolog of hedgehog expressed by the notochord. *Cell* **76**, 761-775.
- van Stratten, H. M. W., Hekking, J. W. M., Wietz-Hoessels, E. L., Thors, F. and Drukker, J.** (1988). Effect of the notochord on the differentiation of a floor plate area in the neural tube of the chick embryo. *Anat. Embryol.* **177**, 317-324.
- van Stratten, H. M. W., Thors, F., Wietz-Hoessels, E. L. Hekking, J. W. M. and Drukker, J.** (1985). Effect of a notochordal implant on the early morphogenesis of the neural tube and neuroblasts: histometrical and histological results. *Dev. Biol.* **110**, 247-254.
- Warf, B. C., Fok-Seang, J. and Miller, R. H.** (1991). Evidence for the ventral origin of oligodendrocytes in the rat spinal cord. *J. Neurosci.* **11**, 2477-2488.
- Warrington, A. E., Barbarese, E. Pfeiffer, S. E.** (1993). Differential myelinogenic capacity of specific stages of the oligodendrocyte lineage upon transplantation in to hypomyelinating hosts. *J. Neurosci. Res.* **34**, 1-13.
- Wenger, E. L.** (1950). An experimental analysis of relations between part of the brachial spinal cord of the embryonic chick. *J. Exp. Zool.* **114**, 51-81.
- Williams, B. P., Abney, E. R. and Raff, M. C.** (1985). Macrogial cell development in embryonic brain: Studies using monoclonal antibodies, fluorescence activated cell sorting and cell culture. *Dev. Biol.* **112**, 126-134.
- Wolswijk, G. and Noble, M.** (1989). Identification of an adult-specific glial progenitor cell. *Development* **105**, 387-400.
- Yamada, T., Pfaff, S. L., Edlund, T., and Jessel, T. M.** (1993). Control of cell pattern in the neural tube: Motor neuron induction by diffusible factors from notochord and floor plate. *Cell* **73**, 673-686.
- Yamada, T., Placzek, M., Tanaka, H., Dodd, J. and Jessel, T. M.** (1991). Control of cell pattern in the developing nervous system: polarizing activity of the floor plate and notochord. *Cell* **64**, 635-647.
- Yu, W. P., Collarini, E. J., Pringle, N. P. and Richardson, W. D.** (1994). Embryonic expression of myelin genes: evidence for a focal source of oligodendrocyte precursors in the ventral ventricular zone of the developing spinal cord. *Neuron* **12**, 1353-1362.

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