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Oligodendrocyte progenitors are generated throughout the embryonic mouse brain, but differentiate in restricted foci

Rebecca J. Hardy* and Victor L. Friedrich, Jr

Brookdale Center for Molecular Biology, Mount Sinai Medical School, Box 1126, New York, NY 10029, USA

*Author for correspondence (e-mail: hardy@anton.molbio.mssm.edu)

SUMMARY

Recent evidence from studies mapping the expression of putative oligodendrocyte progenitor specific mRNAs has suggested that oligodendrocyte progenitors arise during embryogenesis, in specific foci of the neuroectoderm. In order to test this hypothesis, we have assayed different regions of the embryonic central nervous system for their ability to generate oligodendrocytes following transplantation into neonatal cerebrum. To allow identification of donor-derived oligodendrocytes in wild-type host brain, we used the MβP transgenic mouse, which expresses *lacZ* in oligodendrocytes, as donor tissue. We found that tissue fragments derived from several levels of the anterior-posterior axis of the neural tube at E14.5 and E12.5, chosen to include (hindbrain, cervical and lumbar spinal cord), or exclude (dorsal telencephalon) putative foci of oligodendrocyte progenitors, all produced oligodendrocytes following transplantation. In addition, these same regions taken from E10.5, prior to the appearance of putative oligodendrocyte progenitor markers, also all yielded oligodendrocytes on transplantation. This indicates that precursor cells that can generate oligodendrocytes are widespread throughout the neuroectoderm as early as E10.5.

We have also used the oligodendrocyte lineage-specific glycolipid antibodies O4, R-mAb and O1 to identify those regions of the developing brain that first support the differentiation of oligodendrocytes from their progenitor cells. We found that the first oligodendrocytes arise in prenatal brain at E14.5, in a restricted zone adjacent to the midline of the medulla. These cells are mitotically inactive, differentiated oligodendrocytes and, using light and electron microscopy, we show that they become functional, myelin-bearing oligodendrocytes. We have mapped the subsequent appearance of differentiated oligodendrocytes in the prenatal brain and show that they appear in a restricted, tract-specific manner.

Our results suggest that oligodendrocytes are generated from neuroectodermal cells positioned throughout the rostrocaudal axis of the neural tube, rather than at restricted locations of the neuroectoderm. By contrast, the differentiation of such cells into oligodendrocytes does occur in a restricted manner, consistent with local regulation of oligodendrocyte progenitor differentiation.

Key words: oligodendrocyte precursors, glycolipid antibodies, myelin, mouse

INTRODUCTION

One of the most fundamental questions facing developmental biologists is how the apparently homogeneous population of neuroectodermal cells that constitute the neural tube differentiate and give rise to the multiple cell types and complex structures of the mature brain. Along the rostrocaudal axis of the neural tube, the neuroectoderm divides into distinct regions: the spinal cord, the rhombencephalon, mesencephalon, diencephalon and telencephalon. Neurons are born from all rostrocaudal levels of these structures; neuroectodermal cells undergo mitosis at the luminal surface of the neural tube and subsequently differentiate into neuroblasts, which migrate away into the mantle and marginal layers of the developing central nervous system (CNS).

Less well studied is the differentiation of the neuroectoderm into neuroglia during embryonic brain development. Oligodendrocytes, the myelin-forming cells of the central nervous system, have been thought to arise postnatally from the subventricular zones (SVZ) of the brain. Retroviral labeling studies have shown that in the neonatal rat cerebrum, oligodendrocytes are generated from the SVZ of the lateral ventricles (Levison and Goldman, 1993; Zerlin et al., 1995). Similarly, immunocytochemical studies have indicated that oligodendrocytes of the cerebellum arise postnatally, from the SVZ of the fourth ventricle (Reynolds and Wilkin, 1988, 1991). Oligodendrocyte progenitors then migrate away from these zones and populate the developing brain. Following cessation of migration, mitotic oligodendrocyte progenitors can be immunolabeled by the mAb O4 (Warrington and Pfeiffer, 1992; Warrington et al., 1993). Postmitoic, differentiated oligodendrocytes continue to bind O4, and also acquire the ability to bind the mAbs R-mAb and O1 (Warrington and Pfeiffer, 1992).

There is some evidence that oligodendrocyte progenitors are present in prenatal brain, however. Messenger RNAs encoding

platelet-derived growth factor alpha-receptor (PDGFαR), which is expressed by oligodendrocyte progenitors in cell cultures, and DM20, a myelin protein found in differentiated oligodendrocytes, are concentrated in several foci in the embryonic CNS. PDGFαR mRNA localizes to ventral spinal cord, medulla and an area within the ventral diencephalon of the E13 rat embryo (Pringle and Richardson, 1993). A similar region of the ventral diencephalon, between the second and third interprosomeric boundary defined by Dlx-1 and Wnt-3 expression (Bulfone et al., 1993), has been shown to express DM20 mRNA at E12.5 in the mouse (Timsit et al., 1995). PDGFαR and DM20 mRNA expression then becomes more widespread throughout the ventral medulla and diencephalon, spreading dorsally around birth. Such expression has been thought to reflect the focal generation of oligodendrocyte progenitors during embryogenesis, and their subsequent migration into developing CNS tissues, although this has not yet been directly demonstrated.

This model of oligodendrogliogenesis suggests that only neuroectodermal cells in specialized regions of the neural tube, as defined by PDGFαR or DM20 mRNA expression, differentiate along the oligodendrocyte lineage. An alternative possibility is that neuroectodermal cells located along the entire length of the rostrocaudal axis of the neural tube generate oligodendrocytes. Differentiation into identifiable oligodendrocyte progenitors may then occur in a spatially and temporally regulated manner, leading to the restricted patterns of putative oligodendrocyte progenitor markers previously observed during prenatal development.

To test this hypothesis, we have transplanted regions of the developing embryonic CNS into newborn hosts to assay for their ability to give rise to oligodendrocytes. In addition, we have used oligodendrocyte lineage-specific anti-glycolipid antibodies to define the regions of the prenatal brain that first support the differentiation of oligodendrocyte progenitors into oligodendrocytes. Here we show that the first oligodendrocytes to appear in the murine brain do so at E14.5, in a restricted location in the developing hindbrain. We have mapped the subsequent appearance of differentiated oligodendrocytes in the prenatal brain and show that such cells appear in a restricted, tract-specific manner. In contrast, transplantation experiments reveal that the capacity of neuroectodermal cells to generate oligodendrocytes is not similarly restricted, but is widespread throughout the rostrocaudal axis of the neural tube.

MATERIALS AND METHODS

Antibodies

The O4 mAb (Sommer and Schachner, 1981) reacts with sulphatide on the surface of differentiated oligodendrocytes and also with an unidentified antigen, POA, on the surface of oligodendrocyte progenitors (Bansal et al., 1989, 1992). R-mAb is specific for oligodendrocytes (Ranchst et al., 1978) and reacts with galactocerebroside as well as several other antigens, including sulphatide (Bansal et al., 1989). The O1 mAb reacts with galactocerebroside on the surface of differentiated, postmitotic oligodendrocytes (Bansal et al., 1989; Warrington and Pfeiffer, 1992). MBP antiserum was a gift of Dr David Colman (Mt Sinai, NY). Anti-2′,bromo-5-deoxyuridine (BrdU) antibodies were purchased from DAKO (Carpinteria, CA) and anti-β-galactosidase (β-gal) antibodies from Chemicon (Temecula, CA). Fluorescent- or biotin-conjugated secondary antibodies and fluorescent- or horseradish peroxidase (HRP)-conjugated streptavidin were purchased from Southern Biotech (Birmingham, AL), Amersham (Amersham, UK) and Jackson ImmunoResearch (West Point, Il).

Transplantation

Pregnant mice (B6D2F1×MβP5) were anesthetized and donor embryos were removed individually and dissected. The region of the brain to be transplanted was identified and a small tissue piece (0.2-0.5 μl) was isolated and drawn into a Hamilton syringe. Recipient mice (C57/BL6×DBA) aged P0-P3 were anesthetized by hypothermia. Following sectioning of overlying skin and cranial bone, the tissue plug was delivered into the left cerebral hemisphere at the level of the subcortical white matter, adjacent to the lateral ventricle (1 mm caudal to bregma, 1 mm deep).

Transplant recipients were killed 21 days following transplantation by perfusion through the left ventricle with 4% paraformaldehyde in 0.1 M Pipes buffer. Brains were postfixed overnight in the same fixative at 4°C. The left hemisphere was cut into 75 μm parasagittal

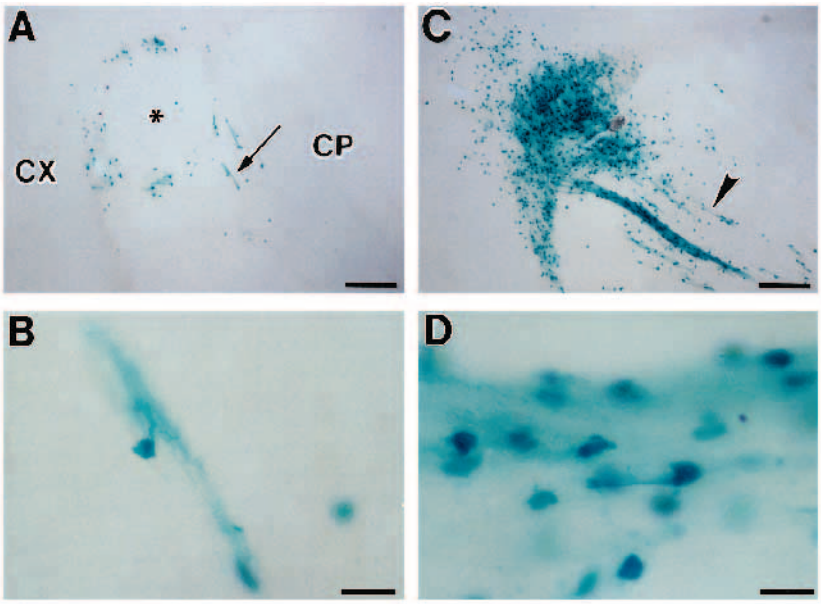

Fig. 1. Transplanted oligodendrocytes derived from tissue fragments from E10.5 dorsal telencephalon (A,B) or E12.5 hindbrain (C,D). (A) In this example, X-Gal$^+$ donor-derived oligodendrocytes are found at the periphery of the transplant (asterisk), which lies in the white matter between the cerebral cortex (CX) and caudoputamen (CP). X-Gal$^+$ oligodendrocytes can also be seen away from the transplant site where they lie adjacent to host caudoputamen axon bundles (arrow). (B) The oligodendrocyte marked by an arrow in (A) is attached via a process to its dependant myelin sheaths, represented by hazy blue staining. (C) This transplant lies in a similar location but contains numerous X-gal$^+$ oligodendrocytes, both within the transplant and at some distance from it. Donor-derived oligodendrocytes have located caudoputamen axon bundles where they participate in their myelination (arrowhead). (D) High magification of myelinating X-gal$^+$ oligodendrocytes derived from the same transplant. Scale bars: 250 μm (A,C); 25 μm (B,D).

sections on a vibratome. These were then processed for X-gal color detection as previously described (Friedrich and Lazzarini, 1993). Sections from some specimens were double-immunolabeled using antibodies against β-gal and myelin basic protein (MBP) (see below).

Tissue preparation and immunocytochemistry

Embryos aged E12.5, E13.5, E14.5, E16.5 and E18.5 were used for immunocytochemistry; the day of vaginal plug was taken as day 0.5. Immunolabeling was carried out on both unfixed and fixed tissue, as previously described (Warrington and Pfeiffer, 1992; Hardy and Friedrich, 1996). Briefly, for unfixed tissue: brains and spinal cords were dissected out, embedded in 4% low melting point agarose and 300 μm thick sections cut on a vibratome. Sections were then incubated in Earle's balanced salt solution with 20 mM Hepes and 36 mM glucose (EBSS-Hepes) with 5% bovine serum albumin (BSA) for 30 minutes, followed by primary antibody diluted in 1% BSA in EBSS-Hepes for 3 hours and then fluorochrome-conjugated secondary antibody for 3 hours. Sections were then fixed overnight in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. All steps were carried out at 4°C.

For fixed tissue, dissected prenatal brains were immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 for 24-48 hours at 4°C. Neonatal mice (P0), were perfused through the left ventricle with the same fixative and brains removed and postfixed overnight at 4°C. They were then embedded in low melting agarose as above and 75-100 μm thick sections cut on the vibratome. Sections were immunolabeled exactly as previously described for the immunolabeling of oligodendrocytes in fixed postnatal tissue (Hardy and Friedrich, 1996). Briefly, sections were blocked in 10% normal goat serum (NGS) in 1% gelatin, 5% BSA and 0.05% sodium azide in phosphate-buffered saline (PBS) for 30 minutes, incubated overnight with primary antibodies diluted in the same buffer and then in fluorochrome- or biotin-conjugated secondary species or subclass specific antibodies for 3 hours at room temperature and with fluorochrome- or HRP-conjugated steptavidin for 1 hour at room temperature. All steps were followed by three washes in PBS (20 minutes each). Sections were then mounted on slides and coverslips placed on top using 2.5% diazobicycloxane in glycerol/PBS (9:1).

Immunolabeled sections were examined and photographed by conventional transmission microscopy (Figs 1, 3B) or epifluorescence microscopy (Fig. 3A), or using a Leica TCS4D confocal laser scanning micorscope.

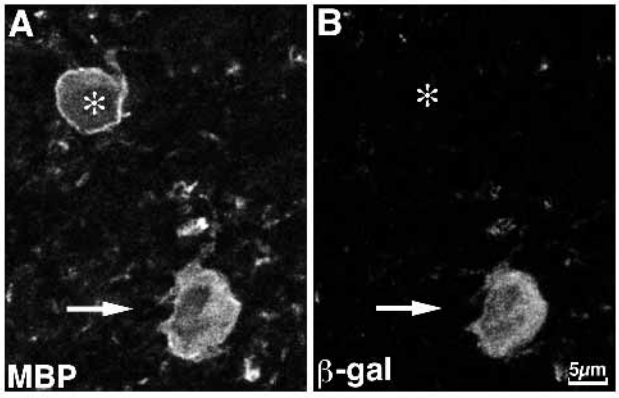

Fig. 2. Immunocytochemical characterization of donor-derived cells using double immunofluorescence with antibodies to MBP (A) and β-gal (B). A host $MBP^+/\beta\text{-gal}^-$ oligodendrocyte (asterisks) lies near a donor-derived $MBP^+/\beta\text{-gal}^+$ oligodendrocyte (arrows) in the cerebral cortex, 21 days following transplantation. Donor tissue: E12.5 dorsal telencephalon.

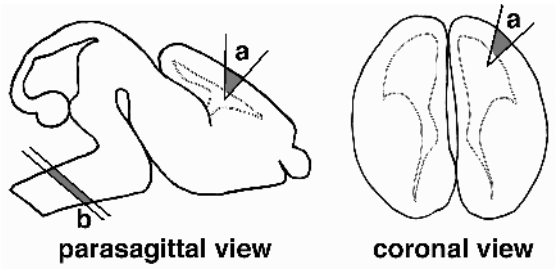

Fig. 3. Schematic diagram of location of donor tissue fragments in the embryonic brain. Tissue fragments from E10.5, E12.5 and E14.5 brain were dissected from either dorsal telencephalon (a) or hindbrain (b), as indicated by shaded areas.

Electron microscopy

Tissue was prepared by routine methods (Friedrich and Mugnani, 1981). Briefly, prenatal brains were immersion-fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 for 48 hours and 100 μm thick sections cut on a vibratome. Sections were then fixed sequentially in buffered solutions of gluteraldehyde, osmium tetroxide and uranyl acetate, dehydrated and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and photographed on a Zeiss EM-10.

RESULTS

We reasoned that if oligodendrocyte progenitors arise from the neuroepithelium in restricted regions of the neural tube, then the ability to generate oligodendrocytes would initially be confined to these regions. To test this hypothesis, we transplanted fragments of embryonic brain and spinal cord, from various regions and developmental stages, into the brains of neonatal recipients, and assayed them 3 weeks later for donor-derived oligodendrocytes.

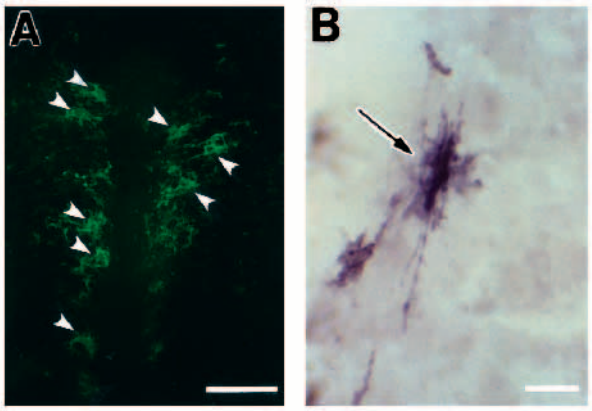

Fig. 4. Anti-glycolipid antibodies recognize cells in prenatal brain. (A) Transverse section of E14.5 hindbrain, immunolabeled using O1 mAb and viewed by conventional epifluoresence microscopy. $O1^+$ cells (arrowheads) are restricted to a narrow zone flanking the midline raphe. At this low magnification, the copious processes of labeled cells obscure their cell bodies. (B) R-mAb immunolabeling with peroxidase detection labels the same population of cells (arrow). A, 300 μm unfixed section; B, 100 μm paraformaldehyde-fixed section. Scale bars: 100 μm (A); 50 μm (B).

Donor tissue in these experiments was taken from mice homozygous for the MβP transgene. This transgene drives expression of the *E. coli lacZ* gene under the control of a 1.9 kb promoter/enhancer fragment from the MBP gene, and is an effective marker for oligodendrocytes (Gow et al., 1992). We have previously demonstrated that CNS tissue fragments from perinatal MβP mice give rise to *lacZ*-expressing oligodendrocytes when transplanted into brains of recipients of the same age (Friedrich and Lazzarini, 1993). Donor-derived oligodendrocytes migrate away from the implantation site, integrate with host brain and myelinate host axons.

Embryonic CNS produces oligodendrocytes when implanted into postnatal brain

In this study, we have transplanted tissue plugs from the CNS of embryos from E10.5 through E14.5 into the cerebral hemispheres of early postnatal recipients.

Transplanted plugs were placed at the dorsolateral angle of the SVZ, adjacent to the SVZ; as this region is known to generate oligodendrocyte progenitors perinatally (Levison and Goldman, 1993), we anticipated that it would provide factors to promote the survival, migration and differentiation of transplant-derived oligodendrocyte progenitors. Recipients were killed 3 weeks after implantation and brain sections were stained with X-gal to visualize donor-derived oligodendrocytes.

Implants were often visible as disruptions of local host architecture. While many X-gal$^+$, transplant-derived oligodendrocytes were located within and at the edges of implants, in all cases labeled cells were also located away from implants, in places which, by their location and structure, clearly belonged to the recipients. Fig. 1 shows two examples, illustrating sparse (Fig. 1A,B) and copious (Fig. 1C,D) production of transplant-derived oligodendrocytes. In both cases, labeled oligodendrocytes are found away from the implant, within or adjacent to axon bundles of the host caudoputamen (Fig. 1). Thus, in these animals, precursors derived from the transplants have migrated into host tissue and differentiated into oligodendrocytes. Some X-gal$^+$ oligodendrocytes emit processes that enter caudoputamen axon bundles (Fig. 1A,B). Diffuse staining of axon bundles in the vicinity of X-gal$^+$ cell bodies resembles staining in native MβP transgenic mice (Gow et al., 1992), and reflects the formation of myelin on host axons by the transplant-derived cells (Friedrich and Lazzarini, 1993; Friedrich et al., 1993). The number of X-gal$^+$ oligodendrocytes varied between specimens but did not correlate with either the developmental age or the origin of donor tissue.

To confirm the identification of X-gal$^+$ cells as oligodendrocytes, we double-immunolabeled sections with antibodies to MBP and to β-gal. As expected, we found that all oligodendrocytes positive for β-gal also contain MBP. Transplant-derived, MBP$^+$/β-gal$^+$ oligodendrocytes were intermingled with host-derived, MBP$^+$/β-gal$^-$ oligodendrocytes (Fig. 2).

These data demonstrate that tissue from embryonic CNS generates oligodendrocytes when transplanted into postnatal brain. Furthermore, cells within the donor tissue emigrate to host brain parenchyma and differentiate into mature oligodendrocytes. Donor-derived oligodendrocytes appear functionally indistinguishable from host oligodendrocytes since they participate in the myelination of host axons.

The capacity to generate oligodendrocytes is widespread along the rostrocaudal axis of the embryonic CNS

We initially assayed several regions of E14.5 embryos for their ability to produce oligodendrocytes following transplantation. It is at this age that differentiated oligodendrocytes first appear, in a restricted zone in the hindbrain (see below). We found that coronal slices of hindbrain (Fig. 3) produced X-gal$^+$ oligodendrocytes following transplantation. However, fragments of dorsal telencephalon (a in Fig. 3), which contain no oligodendrocytes at E14.5 (see below), also gave rise to oligodendrocytes in our assay. Since foci of putative oligodendrocyte progenitors appear first appear in brain at around E12.5 (Pringle and Richardson, 1993; Timsit et al., 1995), we tested fragments of E12.5 CNS that we expected to include (coronal slices of hindbrain, cervical, lumbar spinal cord) or exclude (dorsal telencephalon; see a in Fig. 3) putative oligodendrocyte progenitors. We found that all of these regions gave rise to oligodendrocytes when transplanted (e.g. Fig. 1C,D). In addition, all of these regions taken at E10.5, prior to any expression of putative oligodendrocyte progenitor markers (Pringle and Richardson, 1993), were able to generate oligodendrocytes following transplantation (e.g. Fig. 1A,B).

Differentiated oligodendrocytes are present in the prenatal brain

As demonstrated above, the focal distribution of putative oligodendrocyte progenitor markers that has been reported by others, does not correlate with the distributed potential of CNS tissue to generate oligodendrocytes. We wondered if there was a relationship between these foci and the appearance of differentiated oligodendrocytes. Oligodendrocyte progenitor differentiation has been thought to begin around the time of birth in the rodent (Abney et al., 1981; Levine and Goldman, 1988; Reynolds and Wilkin, 1988; Hardy and Reynolds, 1991); however, some genes normally found in differentiated oligodendrocytes are expressed prenatally in the CNS (Yu et al., 1994; Timsit et al., 1995). We therefore used the oligodendrocyte lineage-specific anti-glycolipid antibodies, O4, R-mAb and O1, to locate cells of the oligodendrocyte lineage in prenatal mouse brain.

In murine brain from E14.5 onwards, a population of cells are strongly immunolabeled by all three of these monoclonal antibodies. Fig. 4 shows immunoreactive cells in the E14.5 hindbrain, detected by standard epifluorescence microscopy and by peroxidase immunolabeling. The earliest stage of development at which we observed immunoreactive cells was E14.5; E12.5 and E13.5 brain contained no O4$^+$, R-mAb$^+$ or O1$^+$ cells. O4, RmAb and O1 each bind sets of surface antigens specific for the oligodendrocyte lineage (Bansal et al., 1989); however, under certain conditions they may also bind to nonspecific intracellular antigens (Warrington and Pfeiffer, 1992). To check for nonspecific intracellular immunolabeling, we examined sections by confocal microscopy, which provided resolution and clarity sufficient to distinguish surface from internal labeling. Thin optical sections through labeled cell bodies showed that, for each of the three monoclonal antibodies, immunoreactivity is restricted to the cell surface (Fig. 5B).

All O4$^+$, R-mAb$^+$ and O1$^+$ cells in prenatal brain morphologically resemble oligodendrocytes of early postnatal brain,

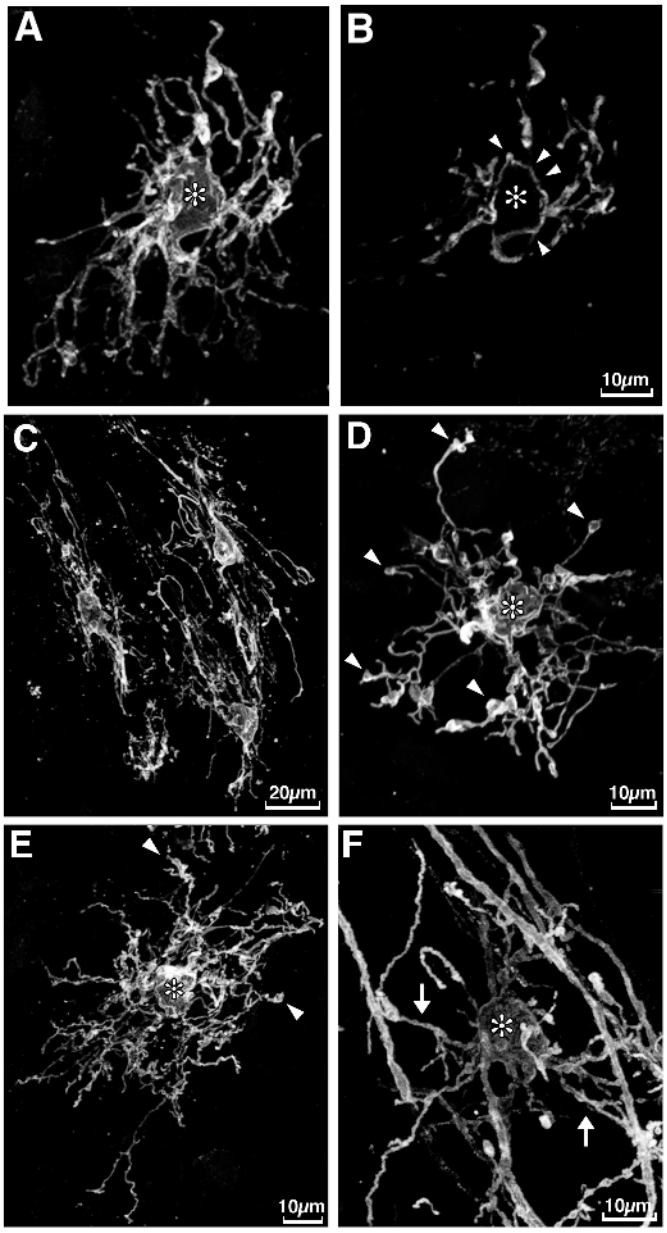

Fig. 5. Confocal microscopy of immunolabeled cells in prenatal hindbrain using O1 (A-D) or R-mAb (E,F). The positions of cell bodies are marked with asterisks. (A) Plane projection of serial optical sections through a highly arborized cell characteristic of an oligodendrocyte. A single optical section from this series (B) shows that the immunoreactivity is restricted to the cell surface (B; arrowheads) and that the interior of the cell body is devoid of labeling. Sometimes the multiple irregular processes of labeled cells are roughly oriented in the same direction (C), probably along axon tracts. Other cells have highly arborized, more radially oriented processes (D), often with membraneous expansions along or at the termini of processes (D,E; arrowheads). Cells with this highly arborized morphology persist throughout prenatal development (E). Begining at E16.5, some hindbrain oligodendrocytes have begun the process of myelination and have connecting processes to myelin sheaths (F; arrows). All images except B are plane projections of serial optical sections. (A-D) 300 μm unfixed sections from E14.5; (E,F) 75 μm paraformaldehyde-fixed sections from E16.5 (E) or P0 (F).

which have been previously described following immunolabeling with the same three antibodies (Warrington and Pfeiffer, 1992; Hardy and Friedrich, 1996). Fig. 5 shows several examples of labeled cells from E14.5 (A-D) or E16.5 (E) medulla. Labeled somas are typically around 10 μm in diameter and emit 5-10 thin primary processes. These processes branch many times to form a complex arbor, radiating some 50 μm from the cell body. These radiating processes of prenatal oligodendrocytes frequently possess focal membraneous expansions, either along their length or at the extremities (Fig. 5A,D,E). These features are characteristic of developing oligodendrocytes in postnatal brain (Hardy and Friedrich, 1996), indicating that the cells immunolabeled prenatally belong to the oligodendrocyte lineage. Furthermore, as described below, some prenatal oligodendrocytes initiate axon ensheathment, visible both by confocal light and by thin section electron microscopy, at E16.5.

$O4^+$ cells in prenatal brain are not mitotic

Many studies have shown that oligodendrocyte progenitors bind O4 prior to their differentiation into R-mAb^+/$O1^+$ oligodendrocytes, and that such $O4^+$/R-mAb^- cells are mitotic, whereas differentiated $O4^+$/R-mAb^+/$O1^+$ oligodendrocytes are not (Gard and Pfeiffer, 1989; Warrington and Pfeiffer, 1992). Interestingly, we find that the first $O4^+$ cells to appear in prenatal brain at E14.5 also bind R-mAb (>200 $O4^+$ cells counted in each of two experiments); no $O4^+$/R-mAb^- cells are seen at this or earlier times, although $O4^+$/R-mAb^- cells are observed later at E18.5 and P0. Hence, in contrast to perinatal tissue, the appearance of differentiated $O4^+$/R-mAb^+ oligodendrocytes at E14.5 is not preceeded by that of $O4^+$/R-mAb^- progenitor cells. To determine whether the $O4^+$/R-mAb^+/$O1^+$ oligodendrocytes present at E14.5 were postmitotic, we injected pregnant (14.5 days gestation) mice with the thymidine analog BrdU, killed embryos 2 hours later, and immunolabeled sections. A short 2 hour survival period was used to reduce the likelihood that mitotic $O4^-$/R-mAb^-/$O1^-$ cells might incorporate BrdU and subsequently gain the ability to bind these antibodies, prior to fixation. $BrdU^+$ cells were abundant in the ventricular zone surrounding the fourth ventricle, and were also sparsely distributed throughout the medulla. Double-immunolabeling revealed that no $O4^+$, R-mAb^+ or $O1^+$ oligodendrocytes in E14.5 embryonic brain had incorporated BrdU (>200 of each $O4^+$, R-mAb^+ and $O1^+$ cells counted; e.g. Fig. 6). We cannot rule out that these cells are dividing very slowly and so do not accumulate detectable levels of BrdU during the 2 hour pulse. However, as they go on to mature and produce myelin sheaths (see below), it seems likely that these earliest born oligodendrocytes are postmitotic cells.

Differentiated oligodendrocytes appear in specific locations in the prenatal brain

We have mapped the location of differentiated oligodendrocytes, as defined by immunolabeling with O1 and R-mAb antibodies, in serial sections of brain from E14.5 to birth (Fig. 7). At E14.5, oligodendrocytes are present only in the developing hindbrain, where they are strikingly restricted to a narrow zone on either side of the midline (Fig. 7, E14.5 a; Fig. 8A,B). This paramedian zone extends caudally into the cervical spinal cord, where it is located ventral to the central canal (Fig. 9), and

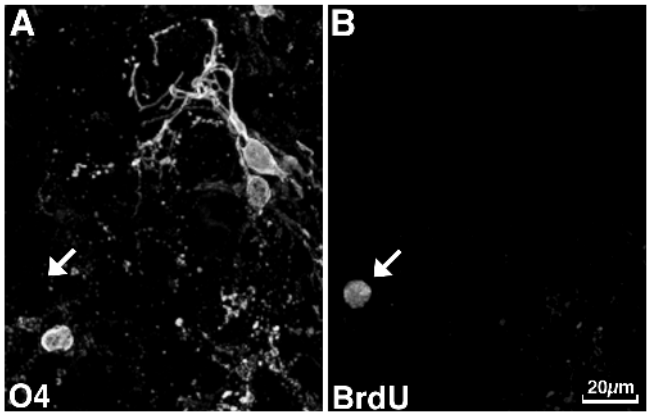

Fig. 6. $O4^+$ cells present at E14.5 do not proliferate. Double-immunolabeling of prenatal brain following a 2hr pulse of BrdU, with O4 mAb (A) and antibodies to BrdU (B). $O4^+$ cells have not incorporated the label during this time. A nearby mitotic cell is $O4^-$ (arrows).

rostrally to the pons (Fig. 7, E14.5 b,e). No oligodendrocytes are present in midbrain, diencephalon or telencephalon at this time (Fig. 7, E14.5 c,d,e).

At subsequent developmental times, oligodendrocytes are found in one of two discontinuous groups, a caudal group (first seen at E14.5) in the pons, medulla and spinal cord and a rostral group (first seen at E16.5) in the rostral diencephalon and the telencephalon. These two groups remain distinct throughout prenatal development, separated by the caudal diencephalon, which is free of oligodendrocytes until birth (Fig. 7e).

At E16.5, oligodendrocytes in the more caudal group are still found adjacent to the midline. Oligodendrocytes are more numerous in the medulla and some are now found near, but usually not within, the ventricular zone of the fourth ventricle (Fig. 7, E16.5 a; Fig. 8C,D). The caudal group of oligodendrocytes extends more rostrally in the pons, but cells remain restricted to the paramedian zone (Fig. 7, E16.5 b). Most oligodendrocytes at E16.5 are similar in morphology to those seen at E14.5 (Fig. 5); however a few cells in the medulla extend processes to axons and have begun axon ensheathment (see below). The rostral group of oligodendrocytes first appears at E16.5; it consists of a small number of cells in the hypothalamus, located near the third ventricle at the level of the optic chiasm, and occasional cells in the internal capsule (Fig. 7, E16.5 c).

At E18.5, oligodendrocytes in the medulla are more widespread and include for the first time a number of cells in the

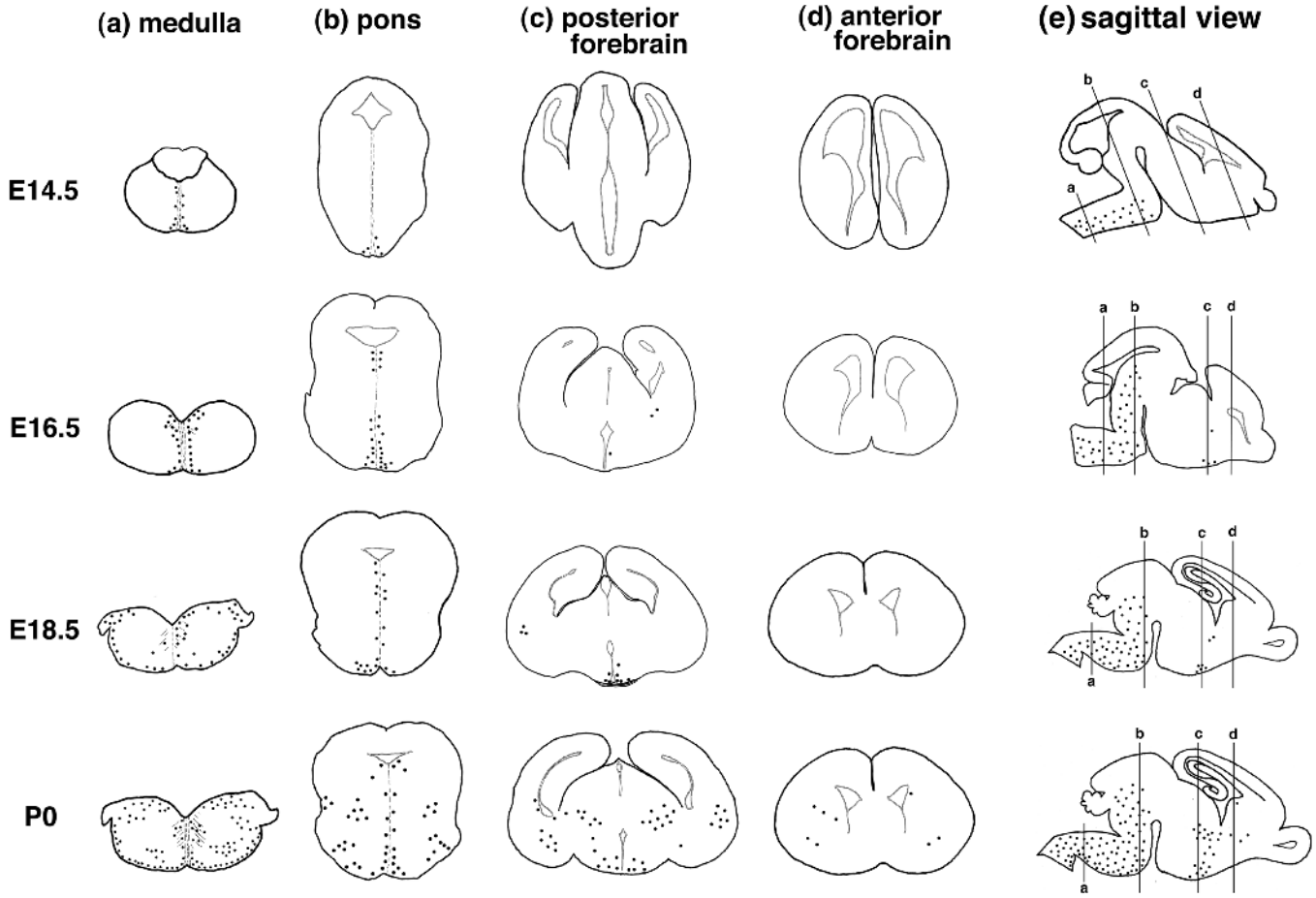

Fig. 7. Location of oligodendrocytes during prenatal brain development. Closed circles represent the location of oligodendrocytes labeled by O4, O1 and R-mAb antibodies in transverse sections of E14.5, E16.5, E18.5 and P0 mouse brain, at the level of the medulla (a), pons (b), posterior forebrain (c) and anterior forebrain (d). e represents oligodendrocytes from transverse sections projected onto a mid-sagittal view. $O4^+/O1^-/R\text{-}mAb^-$ cells are not shown.

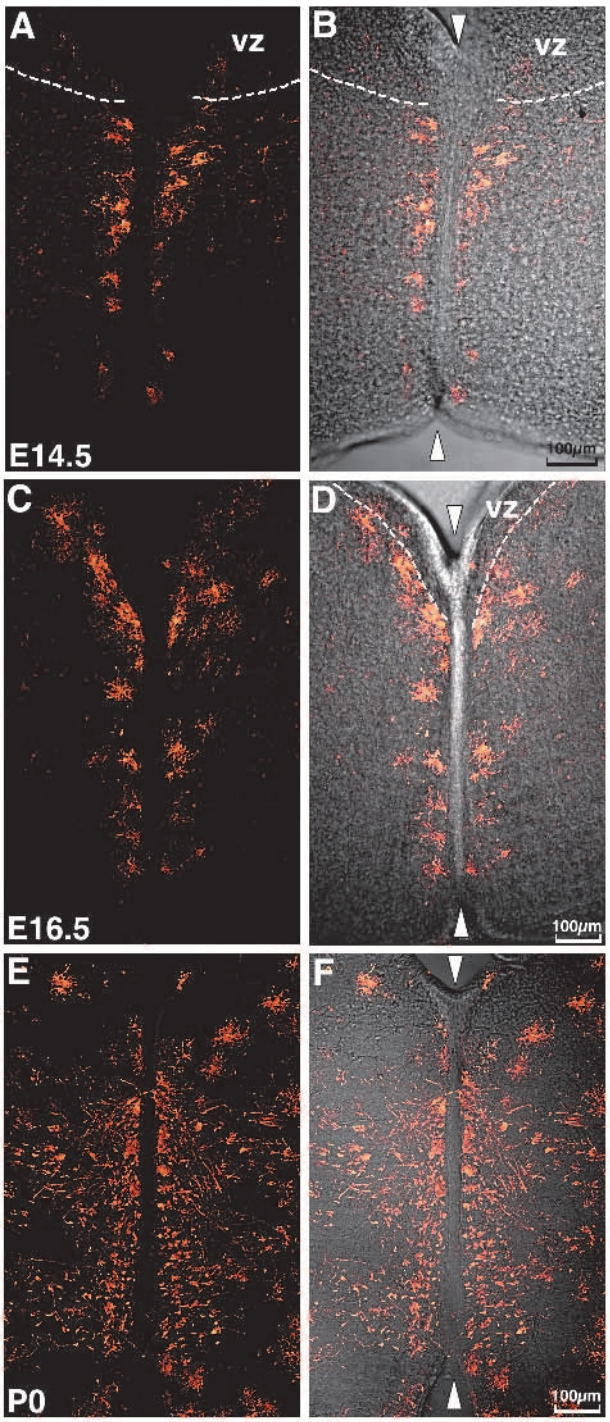

Fig. 8. Transverse sections of medulla during prenatal development, immunolabeled with O1 (A-D) or R-mAb (E,F).
(A,C,E) Immunofluorescence images are represented in a pseudocolor glowscale. (B,D,F) The corresponding transmitted light images overlaid with the glowscale immunofluorescence images; arrowheads mark the midline raphe. (A,B) At E14.5, oligodendrocytes are restricted to a narrow zone flanking the midline raphe, and the ventricular zone (vz) is devoid of labeled cells. (C,D) At E16.5, oligodendrocytes are still restricted to the midline ventrally, although they are now more numerous and appear dorsally and laterally, as well as along the inner margin of the reduced ventricular zone. By birth (E,F), oligodendrocytes are widespread throughout the medulla and those flanking the midline are now involved in myelination.

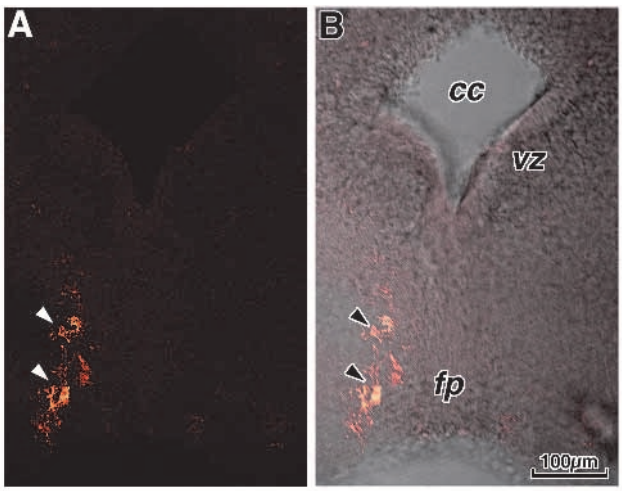

Fig. 9. Transverse section of prenatal cervical spinal cord at E14.5 immunolabeled with O1 mAb. Oligodendrocytes are also restricted to ventromedial structures in the cervical spinal cord. In this section, several $O1^+$ oligodendrocytes (arrowheads) are seen lateral to the floorplate (fp). The ventricular zone (vz) surrounding the central canal (cc) is devoid of labeled cells. Oligodendrocytes lying on the other side of the floorplate are out of the plane of focus.
(A) immunofluorescence image; (B) corresponding transmitted light image with O1 immunofluorescence overlay.

marginal white matter and a much smaller number scattered through the reticular formation (Fig. 7, E18.5 a). The paramedian concentration of oligodendrocytes remains and contains an increased number of forming myelin sheaths. Oligodendrocytes are now more numerous in the hypothalamus and internal capsule but are still restricted in the forebrain to the level of the optic chiasm (Fig. 7, E18.5 c,e).

At birth, oligodendrocytes in the medulla and pons are distributed sparsely throughout the reticular formation and are markedly concentrated in the marginal white matter and in the paramedian zone (Fig. 7, P0 a). Forming myelin sheaths are still more numerous, limited primarily to the paramedian zone, where the first oligodendrocytes appeared at E14.5. In the neonatal forebrain, oligodendrocytes are more numerous in the internal capsule and hypothalamus and make their first appearance in subcortical white matter and in the caudoputamen (Fig. 7, P0 c,d). The rostral group extends more anteriorally than previously, with oligodendrocytes residing both in the rostralmost parts of the internal capsule and adjacent to the SVZ of the lateral ventricle (Fig. 7, P0 e).

The first oligodendrocytes to differentiate prenatally become myelinating cells

Newly differentiated oligodendrocytes in postnatal brain begin to express myelin proteins 1-2 days after their final division (Reynolds and Wilkin, 1991). They then undergo characteristic morphological changes, culminating in their elaboration of cytoplasmic tubes around axons (Fig. 5F; Hardy and Friedrich, 1996). To determine if the first oligodendrocytes that appear prenatally undergo this cascade of events, we followed oligodendrocytes adjacent to the medullary midline from E14.5 to birth. We found that these cells undergo progressive changes consistent with their development into mature, myelinating cells, exactly as

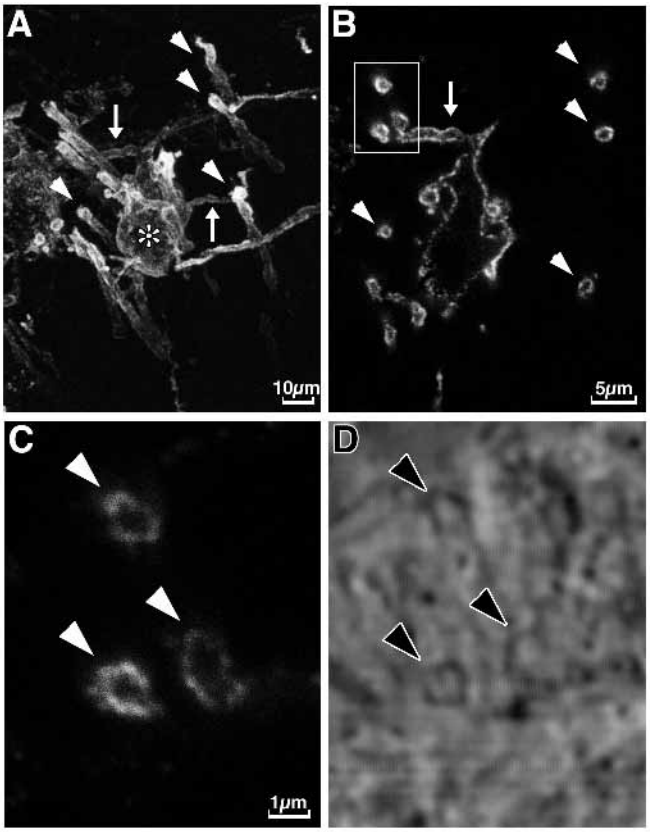

Fig. 10. Early myelin formation in the E16.5 medulla. (A) Plane projection of serial optical sections taken through an R-mAb$^+$ oligodendrocyte at E16.5, located near the medullary midline. Processes can be seen connecting the cell body (asterisk) to R-mAb$^+$ rod-like structures (arrowheads). (B) A single optical section from this series at the level of the cell body. The rod-like structures appear as rings (arrowheads) and are the precursors of myelin sheaths. In this section, one ring (included in boxed area) can clearly be seen connected to the cell body by a process (arrow). (C,D) A high resolution single optical section of several rings (boxed in B) viewed simultaneously with fluorescence (C) and transmitted light (D). The forming myelin sheaths, which are R-mAb$^+$, are refractile and are visible by transmitted light (arrowheads in C and D).

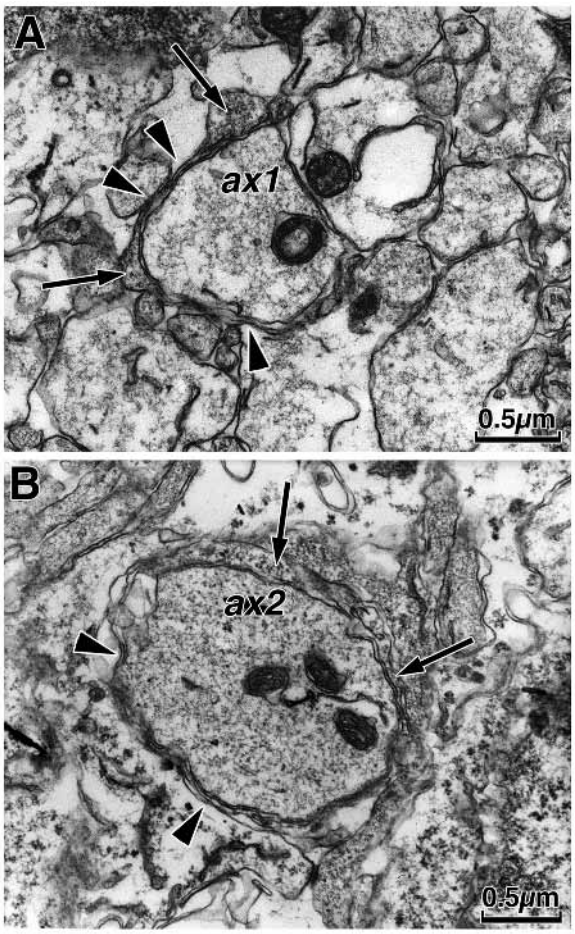

Fig. 11. Early stages of axon ensheathment in E16.5 medulla. (A) A profile of an axon (ax1) partially ensheathed by a lamellar oligodendrocyte process (arrows), from which cytoplasm has been extruded in some regions (arrowheads). (B) Another axon (ax2), completely encircled by multiple cytoplasmic lamellae (arrows), showing partial compaction (arrowheads). Both of these examples are located near to the midline medullary raphe.

observed in postnatal oligodendrocytes. At E14.5, oligodendrocytes do not express the myelin proteins CNP and MBP, but these are present in cell bodies and processes 2 days later at E16.5 (data not shown). In addition, surface-immunolabeling using glycolipid antibodies revealed the first morphological features of myelination by oligodendrocytes at this time (Fig. 10). Some oligodendrocyte cell bodies extend processes to rod-shaped structures (Fig. 10A, arrowheads). These rods appear as rings when viewed in cross section at high resolution (Fig. 10B,C); furthermore, the rings are visible by ordinary transmitted light illumination, confirming that they represent substantial structures in the tissue (Fig. 10D). These features are identical to those seen at the initiation of axon ensheathment in postnatal brain (Hardy and Friedrich, 1996), and indicate that some oligodendrocytes in E16.5 brain have already begun myelination. We examined tissue from the medullary midline of E16.5 brain by electron microscopy and found a small number of axons surrounded by spirally wrapped cytoplasmic lamellae (Fig. 11), an early stage in the formation of myelin sheaths (Peters et al., 1991).

DISCUSSION

We have demonstrated that in murine brain, the first oligodendrocytes to differentiate from their progenitor cells do so at E14.5. In addition, we show that these earliest born oligodendrocytes are restricted to a narrow zone flanking the glial structure forming the midline of the medulla, the medullary raphe. The first oligodendrocytes to appear in the developing diencephalon do so 2 days later, also in specific loci: the floor of the hypothalamus near the third ventricle and the internal capsule. Oligodendrocytes subsequently appear throughout the brain in a pattern consistent with differentiation of progenitors in specific axonal tracts. We have also demonstrated that the first myelin sheaths to form in the prenatal brain do so in a manner consistent both spatially and temporally with their production by the earliest born oligodendrocytes. Once prenatal

oligodendrocytes differentiate from their progenitor cells they begin to express myelin proteins and commence myelin sheath production, exactly as has been described for oligodendrocytes during early postnatal development (Roussel and Nussbaum, 1981; Hartman et al., 1982; Reynolds and Wilkin, 1988; Hardy and Reynolds, 1991).

Oligodendrocyte progenitor differentiation is controlled by locally derived signals

Our finding that the appearance of the first oligodendrocytes in the prenatal brain is not preceeded by the appearance of $O4^+$ mitotic oligodendrocyte progenitors is somewhat surprising. The $O4^+$/R-mAb$^-$ oligodendrocyte progenitor has been shown to be an important and biologically distinct stage of the oligodendrocyte lineage, both in cell culture (Gard and Pfeiffer, 1989; Hardy and Reynolds, 1993b) and postnatally in vivo (Warrington and Pfeiffer, 1992; Warrington et al., 1993). Our evidence suggests that this stage of the lineage is either absent or very short-lived during the genesis of the earliest oligodendrocytes. This is in contrast to perinatal forebrain, where $O4^+$/R-mAb$^-$ oligodendrocyte progenitors are widely distributed throughout grey and white matter regions, prior to the appearance of differentiated oligodendrocytes (Warrington and Pfeiffer, 1992; R. J. H., unpublished observations). Hence the rate of the progression of precursor cells through the defined stages of the oligodendrocyte lineage is dependant upon their local environment. In cell culture, different stages of the oligodendrocyte lineage have distinct responses to external stimuli (Hardy and Reynolds, 1993b; Pfeiffer et al., 1993), and this may be one mechanism whereby the local intercellular environment regulates the timing of oligodendrocyte generation.

The presence of the earliest born oligodendrocytes in medial hindbrain is logical in the context of several previous studies. DM20 mRNA localizes to identical hindbrain regions at the same stages of embryogenesis (Timsit et al., 1995). In postnatal CNS, DM20 expression is restricted to mature oligodendrocytes and myelin (reviewed in Macklin, 1992) and it now seems likely that DM20 mRNA found prenatally is expressed, not by oligodendrocyte progenitors as was previously suggested, but by the differentiated, postmitotic oligodendrocytes that we have described here. In addition, the appearance of oligodendrocytes at these sites is preceded by the expression of the putative oligodendrocyte progenitor marker, PDGFαR mRNA, which is initially restricted to cells adjacent to the midline of the medulla several days earlier (Pringle and Richardson, 1993). Thus PDGFαR mRNA-expressing oligodendrocyte progenitors in this region differentiate into oligodendrocytes within a few days.

While this schedule appears to operate in developing hindbrain and ventral hypothalamus, not all PDGFαR mRNA-expressing cells undergo a similar progression. For example, expression of PDGFαR mRNA in the diencephalon at around E12.5 is not quickly followed by the appearance of differentiated oligodendrocytes in this region; oligodendrocytes do not appear in corresponding structures until after birth (Pringle and Richardson, 1993; this study). Hence the significance of this expression, and the DM20 mRNA expression also present in this region at this time, is not clear. It is possible that such expression is not in fact related to glial cell development. PDGFαR mRNA and protein have been detected in neuronal populations, both prenatally and in the adult CNS (Cheng and Mattson, 1995; Vignais et al., 1995) and DM20 mRNA has been localized to myocardial cells in the heart (Campagnoni et al., 1992).

The local regulation of oligodendrocyte progenitor differentiation seems a logical mechanism and one that has previously been discussed (Levison and Goldman, 1993; Franklin and Blakemore, 1995); oligodendrocytes are generated from their progenitor cells where they are needed, in regions where axons require myelination. Many studies have revealed that neurons can influence oligodendrogliogenesis in a variety of ways (reviewed in Hardy and Reynolds, 1993a). Therefore maturing axons may signal neighbouring oligodendrocyte progenitors to differentiatiate and/or mature, leading to their own myelination. Other factors may modulate such regulation; for example, the Pax3 transcription factor has been shown to suppress differentiation of myelinating Schwann cells (Kioussi et al., 1995). As Pax3 mRNA has been localized to dorsal regions of the embryonic hindbrain (reviewed by St-Onge et al., 1995), a similar role for Pax3 in suppression of oligodendrocyte differentiation is consistent with the restriction of oligodendrocytes to ventral hindbrain regions we have described.

Potential for oligodendrogliogenensis is widespread along the rostocaudal axis of the neural tube

The restriction of putative oligodendrocyte progenitor markers to specific foci in the embryonic brain has led to the suggestion that neuroectodermal cells only differentiate along the oligodendrocyte lineage in restricted regions of the developing brain (Pringle and Richardson, 1993; Timsit et al., 1995). This model predicts that oligodendrocyte progenitors generated within these foci form pools of cells from which all oligodendrocytes are derived. We now show that oligodendrocytes can arise from regions of embryonic brain which lie outside these foci. It therefore seems likely that precursor cells destined to become oligodendrocytes are derived from neuroectodermal cells along the entire rostrocaudal axis of the neural tube, not only at restricted sites. These cells, yet to express defined oligodendrocyte-lineage specific markers, presumably migrate out from the ventricular zones of the developing neural tube, either dorsally, ventrally or laterally, to populate the presumptive brain.

The production of oligodendrocytes at sites distant from foci of PDGFαR mRNA expression has precedents in the developing cerebellum and spinal cord. It has been demonstrated that oligodendrocyte precursors already populate distal portions of the cerebellum (Gonye et al., 1994) at a time which precedes the expression of PDGFαR mRNA in these regions (Pringle et al., 1992). In the prenatal spinal cord, several lines of evidence have indicated that oligodendrocyte progenitors are generated exclusively in the ventral half of the cord and subsequently migrate into the dorsal half. For example, the capacity to generate oligodendrocytes in culture is restricted to ventral spinal cord at early developmental times, a property only acquired by dorsal spinal cord at later stages (Warf et al., 1991), or following contact with notochord (Trousse et al., 1995). In addition, a variety of putative oligodendrocyte progenitor markers, including PDGFαR mRNA, are initially restricted to ventral spinal cord during embryogenesis, suggesting a putative origin of spinal cord oligodendrocytes in the ventral neuroepithelium of the central canal (Yu et al., 1994). However, recent evidence suggests that, at times when

PDGFαR mRNA-expressing cells are restricted to ventral cord, both dorsal and ventral halves of the spinal cord may contain cells that can differentiate into oligodendrocytes following transplantation (Cameron-Curry and LeDourarin, 1995).

In the light of these results and those of the present study, it now seems likely that precursor cells that are destined to differentiate into oligodendrocytes are substantially more widespread than PDGFαR mRNA-expressing cells. Thus the patterns of PDGFαR mRNA expression previously observed probably represent the differentiation, in a spatially and temporally restricted manner, of earlier precursor cells that are already positioned throughout the neural tube. What is not clear at present is whether these precursor cells are already committed to the oligodendrocyte lineage, or whether they are multipotential. Several studies have described multipotential precusor cells derived from the embryonic cerebrum which can, at least in culture, give rise to astrocytes, oligodendrocytes and neurons (Reynolds and Weiss, 1992; Davis and Temple, 1994; Williams and Price, 1995). Whether such cells are restricted to germinal zones, or whether they retain their multipotentiality following their migration into the developing brain, remains to be determined. While the mechanisms that control the differentiation of precursor cells into PDGFαR mRNA-expressing oligodendrocyte progenitors are presently unknown, our transplantation results demonstrate that the signals which ellicit this differentiation in the embryonic CNS are also present in postnatal brain.

Our results suggest that oligodendrocytes are generated from neuroectodermal cells present throughout the rostrocaudal axis of the neural tube, as is the case with neurons, rather than at restricted locations of the neuroectoderm. These neuroectodermally-derived cells then migrate away from germinal zones and populate the developing brain, where they produce oligodendrocytes in a restricted manner, consistent with the local regulation of oligodendrocyte progenitor differentiation.

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REFERENCES

Abney, E. R., Bartlett, P. P. and Raff, M. C. (1981). Astrocytes, ependymal cells and oligodendrocytes develop on schedule in dissociated cell cultures of embryonic rat brain. *Dev. Biol.* **83,** 301-310.

Bansal, R., Steffanson, K. and Pfeiffer, S. E. (1992). 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.

Bulfone, A., Puelles, L., Porteus, M. H., Frohman, M. A., Martin, G. R. and Rubenstein, J. L. R. (1993). Spatially restricted expression of Dlx-1, Dlx-2(Tes-a), Gbx-2 and Wnt-3 in the embryonic day 12.5 mouse forebrain defines potential transverse and lonitudinal boudaries. *J Neurosci* **13,** 3155-3172.

Cameron-Curry, P. and LeDourarin, N. M. (1995). Oligodendrocyte precursors originate from both the dorsal and the ventral parts of the spinal cord. *Neuron* **15,** 1299-1310.

Campagnoni, C. W., Garbay, B., Micevych, P., Pribyl, T., Kampf, K., Handley, V. W. and Campagnoni, A. T. (1992). DM20 mRNA splice product of the myelin protoelipid protein gene is expressed in the murine heart. *J. Neurosci. Res.* **33,** 148-155.

Cheng, B. and Mattson, M. P. (1995). PDGFs protect hippocampal neurons against energy deprivation and oxidative injury: evidence for induction of antioxidant pathways. *J. Neurosci.* **15,** 7095-7104.

Davis, A. and Temple, S. (1994). A self-renewing multipotential stem cell in embryonic rat cerebral cortex. *Nature* **372,** 263-266.

Franklin, R. J. M. and Blakemore, W. (1995). Glial-cell transplantation and plasticity in the O-2A lineage-implications for CNS repair. *Trends Neurosci.* **18,** 151-156.

Friedrich, V. L. and Lazzarini, R. A. (1993). Restricted migration of transplanted oligodendrocytes or the progenitors, revealed by transgenic marker MβP. *J. Neural Transpl. Plasticity* **4,** 139-146.

Friedrich, V. L. and Mugnani, E. (1981). Preparation of neural tissues for electron microscopy. In *A Handbook of Neuroanatomical Tract Tracing Techniques*, (ed. L. Heimer and M. Robards), pp. 377-406. New York: Plenum.

Friedrich, V. L. J., Holstein, G. R., Li, X., Gow, A., A, K. K. and A, L. R. (1993). Intracellular distribution of transgenic bacterial β-galactosidase in central nervous system neurons and neuroglia. *J. Neurosci. Res.* **36,** 88-98.

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.

Gonye, G. E., Warrington, A. E., DeVito, J. A. and Pfeiffer, S. E. (1994). Oligodendrocyte precursor quantitation and localization in perinatal brain using a retrospective bioassay. *J. Neurosci.* **14,** 5365-5372.

Gow, A., Friedrich Jr., V. L. and Lazzarini, R. A. (1992). Myelin basic protein gene contains seperate enhancers for oligodendrocyte and Schwann cell expression. *J. Cell Biol.* **119,** 605-616.

Hardy, R. and Friedrich, V. (1996). Progressive remodeling of oligodendrocyte process arbor during myelinogenesis. *Dev. Neurosci.*, in press.

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.

Hardy, R. and Reynolds, R. (1993a). Neuron-oligodendroglial interactions during central nervous system development. *J. Neurosci. Res.* **36,** 121-126.

Hardy, R. and Reynolds, R. (1993b). Rat cerebral cortical neurons in primary culture release a mitogen specific for early (Gd3+/O4-) oligodendrocyte progenitors. *J. Neurosci. Res.***34,** 589-600.

Hartman, B. K., Agrawal, H. C., Agrawal, D. and Kalmbach, S. (1982). Development and maturation of central nervous system myelin: comparison of immunohistochemical localization of proteolipid protein and basic protein in myelin and oligodendrocytes. *Proc. Nat. Acad. Sci., USA* **79,** 4217-4220.

Kioussi, C., Gross, M. K. and Gruss, P. (1995). *Pax3*: A paired domain gene as a regulator in PNS myelination. *Neuron* **15,** 553-562.

Levine, S. M. and Goldman, J. E. (1988). Spatial and temporal patterns of oligodendrocyte differentiation in rat cerebrum and cerebellum. *J. Comp. Neurol.* **277,** 441-455.

Levison, S. M. and Goldman, J. E. (1993). Both oligodendrocytes and astrocytes develop from progenitors in the subventricular zone of postnatal rat forebrain. *Neuron* **10,** 201-212.

Macklin, W. B. (1992). The myelin proteolipid protein gene and its expression. In *Myelin: Biology and Chemistry* (ed. R. E. Martenson), pp. 257-276. Florida: CRC Press.

Peters, A., Palay, S. L. and Webster, H. D. (1991). *The Fine Structure of the Nervous System*, third edition. New York: Oxford University Press.

Pfeiffer, S. E., Warrington, A. E. and Bansal, R. (1993). The oligodendrocyte and its many cellular processes. *Trends Cell Biol.* **3,** 191-197.

Pringle, N. 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.

Pringle, N. P., Mudhar, H. S., Collarini, E. J. and Richardson, W. D. (1992). PDGF receptors in the rat CNS:during late neurogenesis, PDGF-alpha receptor expression appears to be restricted to glial cells of the oligodendrocyte lineage. *Development* **115,** 535-551.

Ranchst, B., Clapshaw, P. A., Price, J., Noble, M. and Siefert, W. (1978).

Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. *Proc. Nat. Acad. Sci. USA* **79,** 2709-2713.

Reynolds, B. A. and Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* **255,** 1707-1710.

Reynolds, R. and Wilkin, G. P. (1988). Development of macroglial cells in rat cerebellum II: An in situ immunohistochemical study of oligodendrocyte lineage from precursor to mature myelinating cell. *Development* **102,** 409-425.

Reynolds, R. and Wilkin, G. P. (1991). Oligodendroglial progenitor cells but not oligodendroglia divide during normal development of the rat cerebellum. *J. Neurocytol.* **20,** 216-224.

Roussel, G. and Nussbaum, J. L. (1981). Comparative localization of Wolfgram W1 and myelin basic proteins in the rat brain during ontogenesis. *Histochem. J.* **13,** 1029-1047.

Sommer, I. and Schachner, M. (1981). Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: and immunocytochemical study in the central nervous system. *Dev. Biol.* **83,** 311-327.

St-Onge, L., Pituello, F. and Gruss, P. (1995). The role of *Pax* genes during murine development. *Sem. Dev. Biol.* **6,** 285-292.

Timsit, S., Martinez, S., Allinquant, B., Peyron, F., Puelles, L. and Zalc, B. (1995). Oligodendrocytes originate in a restricted zone of the embryonic ventral neural tube defined by DM-20 mRNA expression. *J. Neurosci.* **15,** 1012-1024.

Trousse, F., Giess, M., Soula, s., Ghandou, r. S., Duprat, A.-M. and Cochard, P. (1995). Notochord and Floor plate stimulate oligodendrocyte differentiation in cultures of the chick dorsal neural tube. *J. Neurosci. Res.* **41,** 552-560.

Vignais, L., Nait-Oumesmar, B. and Baron-Van Evercooren, A. (1995). PDGF alpha receptor is expressed on mature neurones of the central nervous system. *Neuroreport* **6,** 15-19.

Warf, B. C., Fok-Seang, J. and Miller, R. H. (1991). Evidence for the ventral origin of oligodendrocyte precursors in the rat spinal cord. *J. Neurosci.* **11,** 2477-2488.

Warrington, A. E., Barbarese, E. and Pfeiffer, S. E. (1993). Differential myelinogenic capacity of specific developmental stages of the oligodendrocyte lineage upon transplantation into hypomyelinating hosts. *J. Neurosci. Res.* **34,** 1-13.

Warrington, A. E. and Pfeiffer, S. E. (1992). Proliferation and differentiation of $O4^+$ oligodendrocytes in postnatal rat cerebellum: analysis in unfixed tissue slices using anti-glycolipid antibodies. *J. Neurosci. Res.* **33,** 338-353.

Williams, B. and Price, J. (1995). Evidence for multiple precursor cell types in the embryonic rat cerebral cortex. *Neuron* **14,** 1181-1188.

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 ventricular zone of the neural tube. *Neuron* **12,** 1353-1362.

Zerlin, M., Levison, S. W. and Goldman, J. E. (1995). Early patterns of migration, morphogenesis and intermediate filament expression of subventricular zone cells in the postnatal rat forebrain. *J. Neurosci.* **15,** 7238-7249.

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