

R) mRNA is expressed in the ventral half of the spinal cord in two longitudinal columns, one each side of the central canal. Initially, these columns are only two cells wide but the cells subsequently appear to proliferate and disseminate throughout the spinal cord. Our previous studies of PDGF- α R expression in the developing CNS suggested that PDGF- α R may be a useful marker of the oligodendrocyte lineage in situ. The data presented here complement those studies and lead us to propose that the earliest oligodendrocyte precursors in the spinal cord originate in a very restricted region of

the ventricular zone during a brief window of time around embryonic day 14 (E14). In the embryonic brain, migrating PDGF- α R⁺ cells appear to originate in a localized germinal zone in the ventral diencephalon (beneath the foramen of Monro). Our data demonstrate that gene expression and cell fate can be regulated with exquisite spatial resolution along the dorsoventral axis

INTRODUCTION

One of the major challenges in developmental neurobiology is to determine how the enormous variety of neuronal and glial cell types in the mature central nervous system (CNS) is generated from the undifferentiated neuroepithelial cells of the embryonic neural tube. We are approaching this problem by focussing on the development of oligodendrocytes, the myelinating glia of the CNS. During development, oligodendrocytes arise from glial progenitor cells known as O-2A progenitors (see Richardson et al., 1990 for review). There is evidence that O-2A progenitors are migratory cells in vivo, invading developing white matter tracts from nearby germinal zones (Altman, 1966; Paterson et al., 1973; Small et al., 1987; Reynolds and Wilkin, 1988; LeVine and Goldman, 1988a,b), but their sites of origin are, in general, unknown. It is possible that the ultimate origin of the oligodendrocyte lineage, in common with most neuronal cell lineages, is in the germinal zones around the ventricles of the brain and spinal cord (ventricular and subventricular zones; Altman, 1966; Paterson et al., 1973; LeVine and Goldman, 1988a,b). However, it also has been suggested that oligodendrocytes in the spinal cord develop from de-differentiated radial glia (Choi et al., 1983; Choi and Kim, 1985; Hirano and Goldman, 1988; Aloisi et al., 1992). It has not been possible to resolve this or other related issues because of the lack of specific molecular markers that can be used to identify oligodendrocyte lineage cells in sections of CNS tissue. We recently presented evidence that, during late neurogenesis in the rat,

expression of the alpha receptor for platelet-derived growth factor (PDGF- α R) might be restricted to cells of the oligo-

as day zero of the pregnancy. Embryos of approximately the right ages were checked according to the morphological criteria of Long and Burlingame (1938). Two or three animals were examined at each age. In some instances (E12, E14, E16 and E18) animals from separate litters were used; there were no obvious differences. Tissues were fixed for 24 hours in ice cold 4% (w/v) paraformaldehyde in phosphate-buffered saline (PBS) and cryoprotected by immersion in 0.5 M sucrose in PBS for 24 hours at 4°C. Tissues were immersed in OCT embedding compound (BDH) and frozen in aluminium foil boats placed onto dry ice. Tissue was kept at -70°C until required. Frozen sections were cut (15 µm nominal thickness) on a cryostat and were collected on 3-aminopropyltriethoxysilane (APES)-coated glass microscope slides. Sections were air dried for approx. 2 hours at 20°C and postfixed in 4% paraformaldehyde in PBS for 15 minutes at room temperature. After two 5-minute washes in PBS, the sections were dehydrated in increasing concentrations of ethanol (30, 60, 80, 95 and 100% for 1 minute each) and stored at -70°C.

Animals were sectioned in three different planes (coronal, horizontal and sagittal). At E12 and E13, serial coronal and horizontal sections were taken through the animal at approximately 100 µm intervals. For E14, E15 and E16 animals, coronal sections were taken at approximately 100 µm intervals and horizontal sections at approximately 200 µm intervals. At E18 serial coronal sections were taken every 300 µm and horizontal sections every 400 µm.

In situ hybridization

Our in situ hybridization procedure, based on that of Lawrence and Singer (1985), has been described previously (Pringle et al., 1989), except that we omitted the preincubation in non-radioactive -thio UTP, and we included an extra wash in 4× SSC for 1 hour at room temperature immediately following the hybridization step. This latter modification seemed to be important for eliminating occasional high backgrounds. Our ³⁵S-labelled RNA probe was generated as described previously by in vitro transcription from an approx. 1.5 kb *SacI-PvuII* fragment encompassing most of the extracellular domain of the rat PDGF- R, cloned into pGEM1. For autoradiography, the slides were coated with Ilford K5 nuclear emulsion, exposed for 1-2 weeks in the dark at 4°C, and developed in Kodak D-19. Some sections were lightly counterstained with Hematoxylin (Gills No.3, Sigma).

Computer imaging

Corresponding bright- and dark-field micrographs were converted to digital images using a video camera and frame grabber connected to a Macintosh computer. The monochrome images were assigned false colours and superimposed using Adobe Photoshop software, and the composite images photographed directly from the computer monitor.

RESULTS

To map the distribution of PDGF- R mRNA in the developing rat CNS, we performed in situ hybridization experiments with a ³⁵S-RNA probe corresponding to part of the extracellular domain of the rat PDGF- R. This probe specifically recognizes PDGF- R mRNA and does not cross-hybridize with PDGF- R mRNA (Pringle et al., 1992), which is also known to be present in the developing rat CNS (Sasahara et al., 1991; Smits et al., 1991). Having previously investigated PDGF- R expression in the CNS of rats aged E16 and above, in this study we concentrated on animals at earlier stages of development. Cryostat sections (15 µm nominal thickness) of embryonic rats

(E12, E12.5, E13, E14, E15, E16 and E18) were hybridized in situ with the PDGF- R probe, as described in Materials and methods. Subsequently, sections were autoradiographed, lightly stained with hematoxylin and examined in the light microscope under bright- and dark-field illumination. Control experiments with ³⁵S-RNA probes homologous to PDGF- R mRNA ('sense' probe) gave no autoradiographic signal above background (Pringle et al., 1992).

PDGF-

did not express PDGF- R (data not shown). The PDGF- R⁺ cells were present in the basal plate all along the length of the spinal cord (Fig. 2A-C), but at more rostral levels the number of PDGF- R⁺ cells in the transverse plane was greater, and individual PDGF- R⁺ cells were present at a distance from the ventricular zone as if they had detached themselves from the central cluster and migrated into the

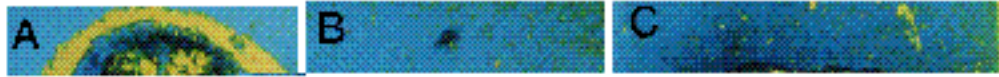


Fig. 2. PDGF- R expression in the E14 rat spinal cord. Transverse sections through an E14 rat spinal cord were subjected to in situ hybridization and autoradiography to visualize PDGF-

might be germinal centers for PDGF- R⁺ cells in the brain that are topologically equivalent to those in the spinal cord. To explore this possibility we cut sections of embryonic rat brain and subjected them to the in situ hybridization procedure with our PDGF- R probe. Fig. 7 shows coronal sections through different parts of the diencephalon of an E13 rat brain photographed under dark-field illumination. There are two separate regions of PDGF- R expression in the ventricular and subventricular zones, one in the dorsal half and one in the ventral half of the diencephalon. In the more anterior section only the dorsal region of expression is visible (arrow in A); this presumably corresponds to the anterior tip of the wedge-shaped zone of PDGF- R expression in the posterior part of the diencephalon, visible in sagittal sections of E13 (not shown) or E14 (Fig. 6A) rats. The existence of a dorsal and a ventral region of PDGF- R expression in the developing diencephalon suggested that, by analogy with the spinal cord, the ventral zone of PDGF- R⁺ might be the source of the many individual PDGF- R⁺ cells that later appear throughout the forebrain and midbrain (Pringle et al., 1992; Fig. 6B). We therefore examined the ventral zone of PDGF- R expression more carefully in series of coronal sections of E14 and E15 rat heads. Three sections through the forebrain of an E15 rat are shown in Fig. 8. At this age there are very few, if any, PDGF- R⁺ cells in the most anterior part of the forebrain (Fig. 8A). More posteriorly (Fig. 8B), individual PDGF- R⁺ cells are visible throughout the developing thalamus and hypothalamus; these cells are mainly located outside the subventricular zones of the lateral ventricles, and are absent from the most dorsal extremities (developing cerebral cortex). Further posteriorly (Fig. 8C), a region of intense PDGF- R expression is encountered in the ven-

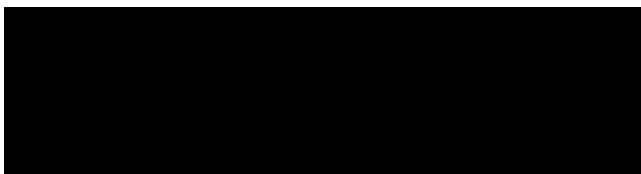




Fig. 4. PDGF- R expression in the E16 rat spinal cord. Transverse sections through an E16 spinal cord were subjected to in situ hybridization and autoradiography; bright- and dark-field images are displayed as outlined in the legend to Fig. 2. Sections from the thoracic (A) and cervical levels (B) are shown. The approximate levels of these sections are indicated by black arrowheads in Fig. 6B. PDGF- R cells are no longer present at the ventricular surface; instead, many individual cells are distributed throughout the gray and white matter, mainly in the ventral half of the cord.

tricular and subventricular zones of the third ventricle, particularly beneath the ducts that connect the third and the lateral ventricles (foramen of Monro). Outside this germinal center, in the ventral half of the forebrain, there are many individual PDGF- R^+ cells, which we presume originated in the ventricular zone just described. These individual PDGF- R^+ cells later propagate in the anterior and dorsal directions to populate the entire cerebral cortex by the day of birth (Pringle et al., 1992).

DISCUSSION

A focus of PDGF- αR expression in the ventricular zone of the embryonic spinal cord: the origin of the oligodendrocyte lineage?

The most salient finding of the present work is the discovery of a transient longitudinal column of PDGF- R

expression in the basal ventricular zone of the embryonic spinal cord. In cross-section, this column appears as an island of PDGF- R mRNA in the midline of the spinal cord or, where the central canal is dilated, as two islands flanking the midline. Because of the systematic temporal lag in development along the length of the spinal cord, we have been able to observe these foci of PDGF- R^+ cells over about one-and-a-half days of development in one animal. In the lumbar region of the cord at E14, the region of PDGF- R expression is tiny, comprising only two cells in the cross-sectional (coronal) plane. It is not possible to distinguish these PDGF- R^+ cells from their PDGF- R^- neighbours by morphology using the light microscope. At more rostral levels (later developmental time) the island of PDGF- R expression enlarges, presumably as a result of cell proliferation, and individual PDGF- R^+ cells appear at a distance from the midline, apparently having detached themselves from the central focus of cells and migrated into



Fig. 5. PDGF- R expression in the E18 rat spinal cord. Transverse sections through the thoracic region of an E18 spinal cord (A) and brainstem (B) were treated as outlined in the legend to Fig. 1. PDGF- R^+ cells are distributed throughout the cross-section of the cord, including the most dorsal region. The ventral-dorsal gradient of PDGF- R^+ cells has almost, but not quite, disappeared. Scale bar (in A), 250 μ m.

the gray matter. Two days later, at E16, the cluster of PDGF- R⁺ cells has disappeared from the ventricular zone; instead, there are many PDGF- R⁺ cells throughout the gray and white matter in the ventral half of the spinal cord, although there are still relatively few in the dorsal half. By E18, PDGF- R⁺ cells are distributed more-or-less uniformly throughout the cross-section of the cord. This temporal sequence of events strongly suggests that PDGF- R⁺ cells originate at a defined point in the ventricular zone and subsequently migrate away from there into the surrounding tissue. The fact that PDGF- R⁺ cells are present in the ventricular layer for only a day or two suggests that the founding PDGF- R⁺ cells and/or their progeny move away from the ventricular zone soon after they are formed and that most proliferation of these cells takes place outside the ventricular layer.

It is likely that the PDGF- R⁺ cells originating in the basal ventricular zone represent glial cells, not neurons. Cells in the basal plate are known to give rise to motor neurons, which come to reside in the ventral horns of the spinal cord, but motor neuron production ceases by E13, about

one day before we first detect PDGF- R expression in the basal ventricular zone (Nornes and Das, 1974; Altman and Bayer, 1984). Indeed, most mitotic activity in the basal plate has ceased, and the ventricular zone has largely regressed, before PDGF- R⁺ cells first appear in the basal plate at E14. Thus, the appearance of PDGF- R⁺ cells in that region of the spinal cord is a relatively late neurogenic event, suggesting that these cells might be glial rather than neuronal in nature. This conclusion is supported by the observation that most proliferation of PDGF- R

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PDGF- R⁺ cells must be glial cells, since they are present in the white matter of the spinal cord which is devoid of neuronal cell bodies. Blood vessels are readily apparent in these spinal cord sections, often being marked by rows of cells with elongated nuclei. These cells, which presumably represent microvascular endothelial cells and/or circulating blood cells, are always negative for PDGF- R.

We believe that the PDGF- R⁺ cells arising in the basal ventricular zone include the earliest precursors of the oligodendrocyte lineage. First, it is known that O-2A progenitor cells, the immediate precursors of oligodendrocytes, express PDGF- R in vitro and in vivo (Hart et al., 1989; McKinnon et al., 1990); we have recently presented circumstantial evidence from in situ hybridization that, during later neurogenesis (after E16), PDGF- R expression might be restricted to the O-2A lineage (Pringle et al., 1992). Second, the time and site of origin of PDGF- R⁺ cells in the ventral half of the spinal cord is entirely consistent with

what is known about the appearance of oligodendrocyte precursors in the cord. Warf et al. (1991) divided the spinal cord of embryonic rats into longitudinal segments which they further dissected into ventral and dorsal halves. They dissociated the cells and looked for the presence of oligodendrocytes, either immediately following dissociation or after maintaining the cells in culture for some time. At E14 they found no oligodendrocytes anywhere in the spinal cord, but oligodendrocytes developed in cultures of cells derived from all rostrocaudal levels, implying that oligodendrocyte precursors, but not mature oligodendrocytes, were present all along the spinal cord at E14. However, only the ventral half of the E14 spinal cord had the capacity to give rise to oligodendrocytes in culture. During later development (after E16), the dorsal half of the spinal cord gradually acquired the capacity to generate oligodendrocytes in vitro, although the numbers of oligodendrocytes forming in cultures of dorsal cells were significantly less than in cultures of ventral cells until E18. These data implied that oligodendrocyte precursors first appear at E14 in the ventral half of the spinal cord and later spread to the dorsal half. Thus, there are striking similarities between the results of Warf et al. (1991) and our own data on PDGF- R expression, strongly suggesting that at least some of the PDGF- R⁺ cells that we observe in the basal plate corre-

tricular zone). This region of the ventricular zone gives rise to interneurons of the substantia gelatinosa, the last major population of neurons to form in the spinal cord (Nornes and Das, 1974; Altman and Bayer, 1984). The first interneurons become post-mitotic at the cervical level of the cord around E14, and the peak of interneuron production occurs around E15 (Nornes and Das, 1974; Altman and Bayer, 1984). Therefore, PDGF- R expression in the alar ventricular zone appears to precede interneuron differentiation. PDGF A-chain is expressed by many cells in the developing spinal cord from E14 to adulthood (Yeh et al., 1991; H. Mudhar and W.D.R., unpublished data). It is possible, therefore, that PDGF might act as a mitogen or survival factor for interneuron precursors in the later stages of their development. It will be interesting to discover if other populations of spinal cord neurons that are born earlier than interneurons (e.g. motor neurons) also express PDGF- R before their final mitotic division.

Evidence for a localized source of migrating PDGF-

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