Defective oligodendrocyte development and severe hypomyelination in PDGF-A knockout mice

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Accepted 11 November 1998; published on WWW 7 January 1999

SUMMARY

). It is not known whether all oligodendrocytes are derived from these PDGFRα-progenitors or whether a subset(s) of oligodendrocytes develops from a different, PDGFRα-negative lineage(s). We investigated the relationship between PDGF and oligodendrogenesis by examining mice that lack either PDGF-A or PDGF-B. *PDGF-A* null mice had many fewer *PDGFR*α-progenitors than either wild-type or *PDGF-B* null mice, demonstrating that proliferation of these cells relies heavily (though not exclusively) on PDGF-AA homodimers. *PDGF-A*-deficient mice also had reduced numbers of oligodendrocytes and a dysmyelinating phenotype (tremor). Not all parts of the central nervous system (CNS) were equally affected in the knockout. For example, there were profound reductions in the numbers of *PDGFR*α-progenitors and oligodendrocytes in the spinal cord and cerebellum, but less severe reductions of both cell types in the medulla. This correlation suggests a close link between *PDGFR*α-progenitors and oligodendrogenesis in most or all parts of the CNS. We also provide evidence that myelin proteolipid protein (*PLP/DM-20*)-positive cells in the late embryonic brainstem are non-dividing cells, presumably immature oligodendrocytes, and not proliferating precursors.

Key words: Central nervous system, Oligodendrocyte, Progenitor cell, PDGF, PDGF receptor, Knockout mouse, PLP/DM-20, MBP, Myelin

confirming and extending previous studies. In the spinal cord, *PDGF-A* mRNA was first detected in the floor plate at E11 (Orr-Urtreger and Lonai, 1992), and persisted there until after E12 (data not shown). This is before the appearance of any *PDGFR*α-expressing cells in the ventral cord; *PDGFR*α^+ oligodendrocyte precursors first appear around E12.5 in the mouse, at the luminal surface near the floor plate (Pringle et al., 1996; Hardy, 1997; Calver et19. 19Bwn)A aftnd 3d;
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restricted to a region close to the midline, were also present in the *PDGF-A*

and P19 knockout animals the density of myelin was much

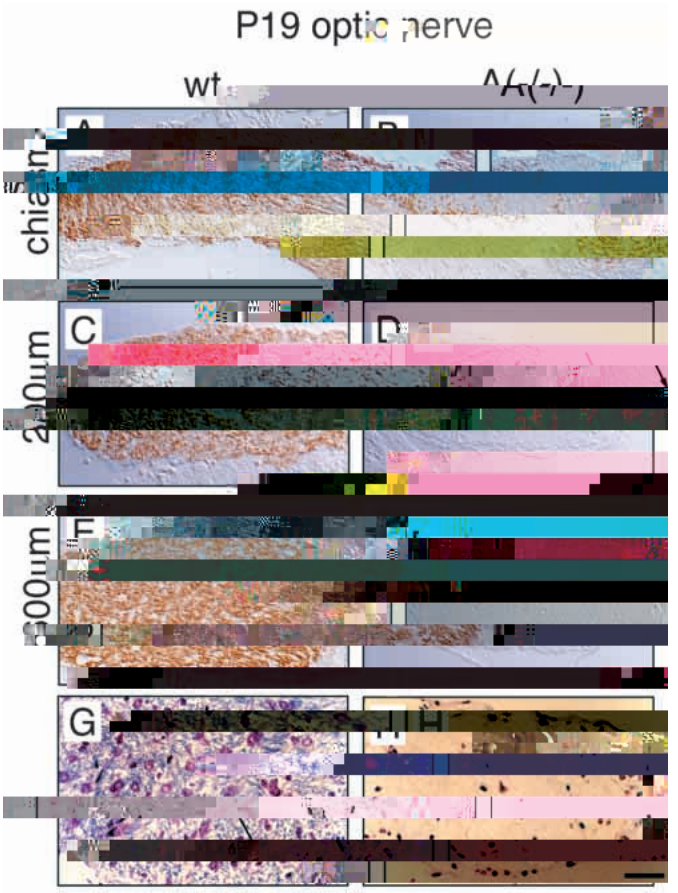

Fig. 8. Loss of MBP-positive nerve fibres and oligodendrocytes in *PDGF-A* knockout optic nerve. Wild-type (A,C,E,G) and *PDGF-A* null (B,D,F,H) sections of P19 optic nerve were compared with MBP immunolabelling (A-F), or Luxol Fast Blue/Cresyl Violet (G,H) to visualize cells (purple/violet) and myelin (blue). MBP-positive fibres are present but less numerous in the optic chiasm of null mutants compared to wild type. With increasing distance from the chiasm towards the eye, MBP-positive fibres gradually disappear in the

Calver et al., 1998). Our studies of PDGF knockout mice described here provide striking, direct evidence that PDGF is important for oligodendrocyte development in vivo. However, some O-2A progenitor cells do accumulate in the absence of embryonic PDGF-A, particularly in the brainstem. It is possible that other growth factors normally co-operate with PDGF-A and exert a limited effect in its absence. It is also possible that maternal PDGF-AA might cross the placenta and partially complement the embryonic PDGF-A deficiency, although why this would preferentially affect the brainstem is not obvious.

In the *PDGF-A* knockouts, a reduction in the number of *PDGFR*α$^+$ progenitor cells present at birth was followed by a parallel reduction in the number of oligodendrocytes and the amount of myelin that formed postnatally. Those regions that experienced the greatest loss of *PDGFR*α$^+$ progenitors in the embryo – spinal cord, optic nerve and cerebellum – were also those regions where myelin loss was most severe. Where the loss of *PDGFR*α$^+$ precursors was less pronounced, in the brainstem and parts of the cerebral cortex, for example, there was a more modest loss of myelin internodes. This correlation suggests a link between *PDGFR*α$^+$ progenitors and myelinating oligodendrocytes and is compatible with the idea that oligodendrocytes in all regions of the CNS develop from a single class of *PDGFR*α$^+$ progenitors. Note that one would not necessarily expect a perfect correlation between progenitors and oligodendrocytes since their numbers are controlled separately, progenitors by mitogens (mainly PDGF; this study) and oligodendrocytes by survival factors (Barres et al., 1992; Calver et al., 1998; this study).

There is a population of *PDGFR*α-negative, *PLP/DM-20*-positive cells in the late embryonic brainstem and cervical spinal cord (Timsit et al., 1995; Peyron et al., 1997; this study). The location of these cells – close to the ventricular surface – led to the suggestion that the *PLP/DM-20*-positive cells and the *PDGFR*α$^+$ cells are distinct types of oligodendrocyte progenitors that belong to independent cell lineages (Timsit et al., 1995; Peyron et al., 1997; Spassky et al., 1998). To test whether the *PLP/DM-20*-positive cells are dividing, as expected for a progenitor cell population, we labelled E15

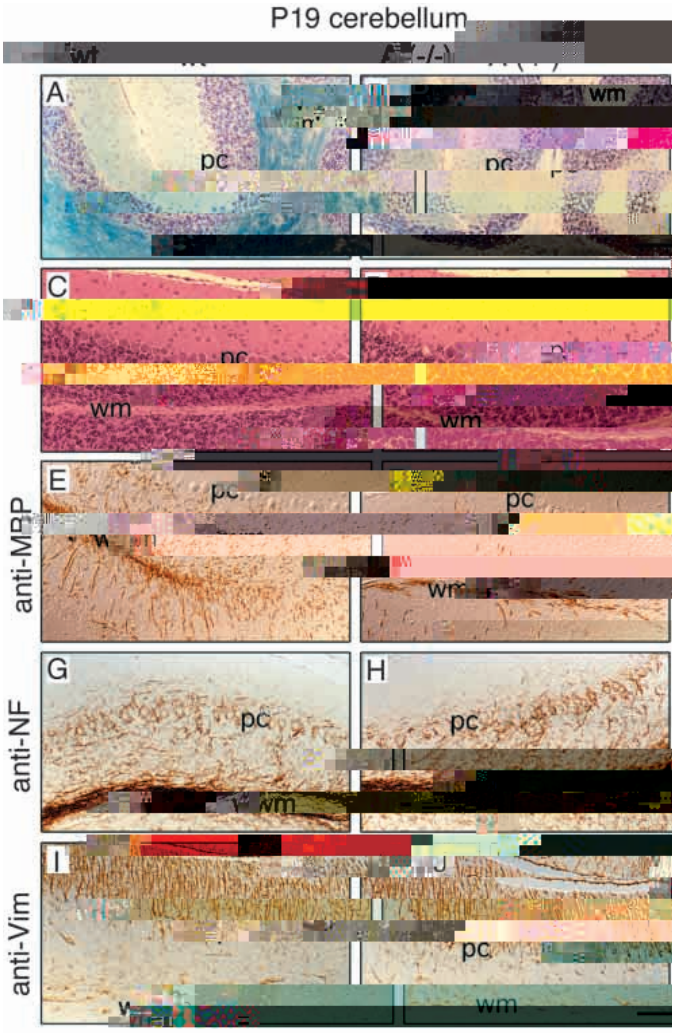

Fig. 10. Morphology and cellular composition of *PDGF-A* null cerebellum. Sagittal sections from cerebellar folia were stained with Luxol Fast Blue/Cresyl Violet (A,B) or with Hematoxylin and Eosin (C,D). Immunohistochemistry was performed with anti-MBP (E,F), anti-neurofilament (G,H) or anti-vimentin (I,J). There is a dramatic loss of myelin and MBP immunoreactivity in the foliar white matter (wm) in *PDGF-A* null mice (compare A,B and E,F). However, overall morphology appears normal, and there are no obvious differences in the distribution of Purkinje cells (pc) or granule cells (I,J). Bars, 100 μm.

embryos with a short pulse of BrdU. We found that few if any (<2%) of the *PLP/DM-20*-positive cells incorporated BrdU, whereas a significant proportion (approx. 20%) of *PDGFRα*$^+$ progenitors incorporated label. Hardy and Friedrich (1996) previously found that *PLP/DM-20*-positive cells in the embryonic mouse spinal cord did not incorporate BrdU either. Therefore, we suggest that the *PLP/DM-20*-positive cells in the E15 spinal cord and brain are not proliferating progenitor cells but post-mitotic oligodendrocytes that are derived from *PDGFRα*$^+$ O-2A progenitors. This interpretation is consistent with the observation that the embryonic *PLP/DM-20*-positive cells co-express other markers of differentiated oligodendrocytes such as MBP and CNP (Peyron et al., 1997; data not shown). The *PLP/DM-20* cells might be a specialized class of non-myelinating oligodendrocytes, of unknown function, or they might be nascent, pre-myelinating oligodendrocytes that are awaiting signals to mature into fully fledged myelinating cells later in development.

Note that there are *PLP/DM-20*-positive cells present in the brain as early as E9 (Timsit et al., 1992; Ikenaka et al., 1993; Peyron et al., 1997; Spassky et al., 1998), much earlier than the embryos we examined in this study and long before the first appearance of *PDGFRα*$^+$ O-2A progenitors around E12-E13. At E9, the *PLP/DM-20*-positive cells are clustered in the ventricular zones (VZ) of the ventral diencephalon and elsewhere (Timsit et al., 1992; Peyron et al., 1997; Spassky et al., 1998). These weakly labelled cells clustered in the VZ are clearly different in nature from the scattered, strongly *PLP/DM-20*-positive cells in the late embryonic spinal cord and brainstem, discussed above. The early *PLP/DM-20*-positive cells in the VZ are undoubtedly neuroepithelial precursors but it remains to be determined what types of cells they generate during later development. It is possible that they are already dedicated to the production of oligodendrocytes from as early as E9. It is perhaps more likely that they are pluripotent precursors whose later progeny might include oligodendrocytes as well as neurons and/or astrocytes. In either case, we imagine that they must downregulate *PLP/DM-20* while upregulating *PDGFRα* as they proliferate and migrate away from the VZ, then strongly upregulate *PLP/DM-20* once again as they leave the cell cycle and differentiate into oligodendrocytes.

Setting aside these arguments, are there any grounds for thinking that there might be distinct lineages and/or subclasses of oligodendrocytes? Different morphological subtypes of oligodendrocytes have been described (del Rio Hortega, 1942; Hildebrand et al., 1993; Butt et al., 1994, 1997). For example, oligodendrocytes that ensheath large-diameter axons have large cell bodies and elaborate only one or a few short myelin internodes, while those that ensheath small-diameter axons have smaller cell bodies and generate many longer internodes. There are also reported biochemical differences between large and small oligodendrocytes in vivo (e.g. Butt et al., 1995). In addition, two putative morphological variants of oligodendrocytes were recently described in cultures of rat spinal cord cells (Fanarraga and Milward, 1997). It is not known whether these variations reflect heterogeneous cell lineages, or developmental plasticity within a single lineage. However, there is evidence that O-2A lineage cells from the optic nerve are indeed plastic and are capable of myelinating a wide range of axonal diameters in vivo (Fanarraga et al., 1998). Plasticity is also indicated by the fact that a single oligodendrocyte can simultaneously myelinate axons belonging to different functional subclasses of neurons (Belichenko and Celio, 1997). At present, therefore, the case for different oligodendrocyte lineages appears weak.

It has been reported (Vignais et al., 1995; Nait-Oumesmar et al., 1997) that many post-mitotic neurons in the developing and adult brain express PDGFRα. We ourselves have not observed reproducible labelling of neurons by in situ hybridization for *PDGFRα*. Other workers have also reported finding PDGFRα immunoreactivity and/or mRNA in O-2A progenitors but not neurons (Nishiyama et al., 1996; Ellison et al., 1996). We do not know the reason for this discrepancy. Setting aside the question of *PDGFRα* expression by post-

mitotic neurons, there is evidence that it is expressed by some neuronal precursors in the embryonic VZ (Pringle and Richardson, 1993; Williams et al., 1997). We therefore looked for morphological abnormalities that might reflect defects in neuronal development, by serially sectioning brains of postnatal *PDGF-A* knockout mice. We did not detect any abnormalities. We also labelled neurons with antibodies against neurofilament but again found no abnormalities. Finally we counted numbers of cerebellar Purkinje neurons and hippocampal neurons, which were reported to express *PDGFR*α strongly (Vignais et al., 1995; Nait-Oumesmar et al., 1997), but found no significant differences in the numbers of these cells. Therefore, while we do not rule out subtle alterations to neuronal development or phenotype, or the loss of specific neuronal subpopulations, it seems that loss of PDGF-A does not have a major effect on neuronogenesis.

In general, it seems that regions that are more distant from the periventricular germinal zones, such as optic nerve, cerebellum and superficial cerebral cortex, are also those regions that showed the most severe loss of myelin in the *PDGF-A* knockout mice. This was well illustrated in the optic nerve, where there was a gradation of myelin in the knockout, declining from the optic chiasm toward the retina. Therefore, loss of PDGF-A might inhibit long-range migration of O-2A progenitors as well as inhibiting their proliferation. An analogous situation has been described for some other types of progenitor cells that are PDGF-dependent in vivo (for review see Lindahl and Betsholtz, 1998). One example relates to lung development. Alveolar smooth muscle cells (SMC) are situated in lung alveolar septa and are the most distally located SMC associated with the respiratory tract. All respiratory tract SMC seem to originate from a population of PDGFRα-positive mesenchymal progenitors (Lindahl et al., 1997b) but it is only the alveolar SMC that are lost in *PDGF-A* knockout lungs (Boström et al., 1996). The developing respiratory epithelium expresses PDGF-A, which probably drives proliferation of the alveolar SMC progenitors and possibly also their migration to the developing alveolar saccules. Angiogenesis provides another example. During this process, PDGF-B is expressed by the sprouting vascular endothelial cells and attracts PDGFRβ-positive vascular SMC progenitors, which migrate from pre-existing blood vessels along the new -1 6euo8.7or en Tj2-positoovveolar saccu 6euo8.riessy to l arJ T* 0.335). The dahBr no s1.11

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