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GLIAL CELLS IN THE RAT OPTIC NERVE AND SOME THOUGHTS ON REMYELINATION IN THE MAMMALIAN CNS

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SUMMARY

Studies on the rat optic nerve in the past 5 years have produced two surprises. First, they demonstrated that there are two biochemically, developmentally and functionally distinct types of astrocytes in the optic nerve, and probably in white matter tracts throughout the CNS: one seems to be responsible for inducing endothelial cells to form the blood–brain barrier while the other seems to service nodes of Ranvier. Second, they showed that oligodendrocytes and type-2 astrocytes develop from a common bipotential (O-2A) progenitor cell that seems to migrate into the developing optic nerve, and may well migrate all over the CNS to wherever myelination is required; this implies that the neuroepithelial cells of the optic stalk are restricted to forming type-1 astrocytes.

Some of the findings in the optic nerve may be relevant to the problem of CNS regeneration after injury. These include the following. (1) Reactive gliosis in white matter tracts seems to be mainly a function of type-1 astrocytes. (2) Proliferating O-2A progenitor cells are present in the adult CNS, raising the possibility that they may be able to produce new oligodendrocytes and type-2 astrocytes following injury and thereby aid regeneration. (3) Type-1 astrocytes seem to be able to respond to environmental signals and form localized barriers that block the migration of O-2A progenitor cells; it is conceivable that the same barriers block the migration of regenerating axonal growth cones.

INTRODUCTION

We have been studying glial cell development and function in the rat optic nerve for almost 10 years. Some of our findings may be helpful in understanding the role of glial cells in regeneration of the mammalian central nervous system (CNS) following injury. We shall first briefly review some of these findings and then consider their possible relevance to remyelination in the mammalian CNS.

Key words: astrocytes, oligodendrocytes, optic nerve, remyelination.

THE THREE TYPES OF MACROGLIAL CELLS IN RAT OPTIC NERVE ARISE BY TWO LINEAGES

The optic nerve was chosen for study because it lacks intrinsic neurones, making it one of the simplest parts of the CNS. It contains three types of macroglial cells – oligodendrocytes and two types of astrocytes (Raff *et al.* 1983*a*): type-1 astrocytes form the glial limiting membrane at the periphery of the nerve while type-2 astrocytes are found in the interior of the nerve (Miller & Raff, 1984).

In vitro studies suggest that the three types of macroglial cells arise by two distinct lineages: oligodendrocytes and type-2 astrocytes develop from a common, bipotential (O-2A) progenitor cell (Raff, Miller & Noble, 1983*b*) whereas type-1 astrocytes develop from a different precursor cell (Raff, Abney & Miller, 1984). Type-1 astrocytes first appear at embryonic day 16 (E16), oligodendrocytes on the day of birth (E21) and type-2 astrocytes between postnatal days 8 and 10 (P8–10) (Miller *et al.* 1985).

O-2A PROGENITOR CELLS APPARENTLY MIGRATE INTO THE DEVELOPING OPTIC NERVE

Recent evidence indicates that O-2A progenitor cells are motile. When a fragment of normal newborn mouse CNS is transplanted into the brain of a *shiverer* mutant mouse, which is genetically unable to make myelin basic protein (MBP) (Barbarese, Neilson & Carlson, 1983; Roach *et al.* 1983; Roach, Takahashi, Pravtcheva & Hood, 1985), MBP^+ myelin is later found widely distributed in the host CNS, indicating that oligodendrocytes or their precursors can migrate large distances from the graft (Lachapelle *et al.* 1984). Time-lapse microcinephotography studies of neonatal optic nerve cultures suggest that it is the progenitor cell rather than the oligodendrocyte that is migratory: in such cultures, progenitor cells, which have a characteristic bipolar morphology (Temple & Raff, 1986), migrate actively until they differentiate into oligodendrocytes, at which point locomotion stops (Small, Riddle & Noble, 1987).

Indirect evidence suggests that O-2A progenitor cells migrate during normal CNS development and that those in the developing rat optic nerve do not arise from the neuroepithelial cells forming the optic stalk but instead migrate into the nerve from the brain. In E17 rats, O-2A progenitor cells are found at the chiasma-end but not at the eye-end of the optic nerve. By birth, they are found at the eye-end, but only in small numbers compared to the chiasma-end. By the second postnatal week, they are evenly distributed along the nerve (Small, 1986; Small, Riddle & Noble, 1987). Type-1 astrocytes, however, first appear at the eye-end of the nerve (Small, 1986; Small, Riddle & Noble, 1987), making it unlikely that the gradient of O-2A progenitor cells found in the developing nerve reflects a gradient of neuroepithelial cell differentiation along the nerve.

FUNCTIONS OF THE MACROGLIAL CELLS IN OPTIC NERVE

While it is clear that oligodendrocytes are responsible for myelinating retinal ganglion cell axons in the optic nerve, the functions of the astrocytes are less certain. Our findings suggest that type-1 astrocytes have a number of functions in addition to forming the glial limiting membrane. They put feet on blood vessels and can induce endothelial cells to form 'tight' vessels (Janzer & Raff, 1987), strongly suggesting that these glial cells are responsible for inducing capillary and venule endothelial cells in the CNS to form the blood–brain barrier. Experiments in culture suggest that type-1 astrocytes secrete growth factors that stimulate O-2A progenitor cells to proliferate (Noble & Murray, 1984; Raff, Abney & Fok-Seang, 1985). There is also indirect evidence that type-1 astrocytes are responsible for glial scarring in CNS white matter: the astrocytes forming the glial scar in adult rat optic nerve 20 weeks after nerve transection, for example, have the antigenic phenotype of type-1 astrocytes (Miller *et al.* 1986). Although we cannot exclude that type-2 astrocytes change their antigenic phenotype in response to nerve transection and come to resemble type-1 astrocytes, quantitative immunohistochemical analyses of cut nerves suggest that type-2 astrocytes (and oligodendrocytes) eventually die in transected nerves and that type-1 astrocytes are mainly responsible for the gliosis (Miller *et al.* 1986). The same results are obtained following stab lesions in the corpus callosum (Miller *et al.* 1986). Similarly, very few O-2A lineage cells are found in optic nerves examined 2 and 8 weeks after neonatal transection; most of the cells in such cut nerves appear to be type-1 astrocytes (David, Miller, Patel & Raff, 1984). We originally interpreted these observations on adult and neonatal optic nerve transection as suggesting that O-2A lineage cells depend on axons for their long-term survival. However, another possibility is that the degeneration of cut axons removes the pathway by which O-2A progenitor cells normally migrate into the optic nerve. This could explain both the drastic reduction in O-2A lineage cells that follows neonatal nerve transection (David *et al.* 1984) and the slow decrease in these cells that follows adult nerve transection (Miller *et al.* 1986), assuming that small numbers of progenitor cells continue to migrate into the adult optic nerve to replace senescent oligodendrocytes or type-2 astrocytes. The presence of proliferating O-2A progenitor cells in adult rat optic nerve (ffrench-Constant & Raff, 1986*a*) is consistent with the view that there is normally a slow turnover of oligodendrocytes and type-2 astrocytes in the adult nerve.

What are the functions of type-2 astrocytes? We have provided indirect evidence that they extend fine processes that run longitudinally in the nerve and collectively envelop the exposed axonal plasma membrane at nodes of Ranvier (ffrench-Constant & Raff, 1986*b*). The functions of such perinodal astrocyte processes are unknown: in principle, they could help both to nourish the adjacent axon and to stabilize the local extracellular ion concentration in the face of repeated nerve impulses. Whatever the functions of type-2 astrocytes, it seems sensible to view the O-2A cell lineage, in the optic nerve at least, as specialized for myelinating axons and constructing nodes of

Ranvier: oligodendrocytes develop first and form myelin sheaths, while type-2 astrocytes develop later and extend processes to the nodes of Ranvier.

In vitro experiments suggest that oligodendrocyte differentiation is the constitutive pathway of O-2A progenitor cell development, which is triggered automatically when a progenitor cell stops dividing (Raff *et al.* 1985; Temple & Raff, 1986), whereas type-2 astrocyte differentiation depends on inducing factors that appear relatively late in the developing optic nerve (Raff *et al.* 1985; S. Hughes & M. Raff, in preparation); perhaps the myelinating oligodendrocytes control the production of the inducing factors that drive the type-2 astrocyte pathway once myelination has begun.

SPECIALIZED TYPE-1 ASTROCYTES IN THE LAMINA CRIBOSA REGION OF THE OPTIC NERVE MAY BE RESPONSIBLE FOR PREVENTING O-2A PROGENITOR CELLS FROM ENTERING THE RETINA

If O-2A progenitor cells are motile and migrate down the optic nerve from the chiasma towards the eye during development (Small, Riddle & Noble, 1987), why do they not migrate into the retina, differentiate into oligodendrocytes (and type-2 astrocytes) and myelinate the retinal ganglion cell axons in the nerve fibre layer? Such myelination would be disastrous as it would make the retina opaque and, thereby, severely impair vision. Several lines of evidence suggest that something in the lamina cribosa region of the optic nerve (where the nerve pierces the sclera) acts as a barrier to prevent the migration of progenitor cells into the retina, an hypothesis originally proposed by Berliner (1931). First, no O-2A progenitor cells, oligodendrocytes or type-2 astrocytes are found in the developing rat retina (C. ffrench-Constant, R. Miller, J. Burne & M. Raff, in preparation). Second, the lamina cribosa region of the nerve is unmyelinated and contains very few oligodendrocytes (Hildebrand, Remahl & Waxman, 1985) or type-2 astrocytes (C. ffrench-Constant, R. Miller, J. Burne & M. Raff, in preparation) , suggesting that few O-2A progenitor cells enter it. Third, in rabbits, which lack a lamina cribosa (Berliner, 1931), the retina contains O-2A lineage cells (C. ffrench-Constant, R. Miller, J. Burne & M. Raff, in preparation) and the central part of the retina is myelinated by oligodendrocytes (Davis, 1929; Berliner, 1931; Narang & Wisniewsky, 1977).

What is the nature of the barrier in the lamina cribosa region of the nerve? A strong possibility is that it is a property of the specialized astrocytes that are the main glial cells in this part of the nerve. These cells extend processes that form a dense meshwork across the nerve, perpendicular to the axons (Skoff, Knapp & Bartlett, 1986); the processes contain an unusually large number of glial filaments compared to astrocytes elsewhere in the nerve (Skoff *et al.* 1986), reminiscent of astrocyte processes in a glial scar (Vaughn & Pease, 1970). These astrocytes have the antigenic phenotype of type-1 astrocytes and are already present at birth (C. ffrench-Constant, R. Miller, J. Burne & M. Raff, in preparation), more than a week before the first type-2 astrocytes appear in the nerve (Miller *et al.* 1985). It is not clear what makes the type-1 astrocytes in this region of the nerve different from those elsewhere

in the nerve. It is possible that either the penetration of vascularized scleral connective tissue into the nerve, which is characteristic of the lamina cribosa region (Fine & Yanoff, 1979), or the leakage of proteins into the nerve from nearby choroidal blood vessels (Tso *et al.* 1975; Flage, 1977; Kistler & LaVail, 1981) induces the astrocytes in this region to adopt a special character, much as injury induces them to form a glial scar. But what is the special character of the type-1 astrocytes in the lamina cribosa region of the nerve that inhibits O-2A progenitor cell migration? One possibility is that the barrier is a mechanical one. Another is that it is a chemical barrier: for example, the type-1 astrocytes in the lamina may not secrete an adequate amount of growth factor to keep the O-2A progenitor cells proliferating; this could cause the latter cells to stop dividing and differentiate and, as a consequence, to stop migrating (Small, Riddle & Noble, 1987).

WHY IS REMYELINATION RELATIVELY INEFFICIENT IN THE CNS?

Why is the process of remyelination following injury relatively inefficient in the mammalian CNS compared to the peripheral nervous system? At one level, the answer seems clear: whereas myelinating Schwann cells can apparently respond to nerve injury by dedifferentiating into a state where they can proliferate, migrate and remyelinate, most myelinating oligodendrocytes apparently cannot dedifferentiate in this way (for example, see Mirsky *et al.* 1980). But why have mammalian oligodendrocytes not evolved the capacity to dedifferentiate if this would enable them to remyelinate axons more efficiently following injury? Perhaps there has been little evolutionary pressure on them to become more efficient at remyelination because inefficient axonal regeneration rather than inefficient remyelination usually limits functional recovery following CNS injury.

Since proliferating O-2A progenitor cells are present in the adult CNS (ffrench-Constant & Raff, 1986*a*), they could in principle migrate into demyelinated regions, differentiate into oligodendrocytes and help to remyelinate the demyelinated axons. If so, then why is remyelination so inefficient in demyelinating diseases such as multiple sclerosis? It is possible that the reactive gliosis associated with the demyelinated lesions prevents O-2A progenitor cells from entering the lesions, just as the gliotic-like type-1 astrocytes in the lamina cribosa region of the optic nerve seem to prevent progenitor cells from entering the retina (C. ffrench-Constant, R. Miller, J. Burne & M. Raff, in preparation). Is it possible that the same type of reactive type-1 astrocytes also block neuronal growth cone migration and thereby limit axonal regeneration after CNS injury?

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