FUNCTIONS OF ADULT OLIGODENDROCYTE PRECURSOR CELLS IN BRAIN INJURY AND REPAIR*

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ABSTRACT

Oligodendrocytes populate the nervous system in great numbers, and are vital to the protection of neurons due to their role in myelin production. Previously unrecognized as a unique cell type, oligodendrocyte precursor cells (OPCs) have now been identified via their expression of a specific marker antigen, NG2. In the following discussion, the characteristics of OPCs are elucidated within the context of research on how new myelin is produced within damaged and demyelinated areas of rodent brains as well as axon regeneration in areas of spinal cord damage. Identifying the specific role of OPCs in remyelination and in regrowth of axons through glial scar tissue are key processes in achieving a greater understanding of the pathophysiology of multiple sclerosis and other brain disorders.


GLIAL CELLS—THE SUPPORT SYSTEM OF THE NEURON

The nervous system consists of 2 categories of cells: neurons and glial cells. Neurons are responsible for the communication and hence thought-processing actions of the central nervous system (CNS). It is the function of glial cells to support and protect neurons, specifically by physically surrounding, anchoring, and insulating them, supplying nutrition and oxygen, and destroying and eliminating damaged or dead neurons. Within the CNS, the 4 types of glial cells include astrocytes, microglia, oligodendrocytes, and oligodendrocyte precursor cells (OPCs). This article will focus on the oligodendrocytes and OPCs, because it is the role of these specific glial cells to provide support to axons and to produce the myelin sheath, which in turn provides insulation and enhances conduction of action potentials.

Previously unrecognized as a unique cell type, OPCs comprise 8% to 9% of the cell population of the rodent CNS, and express a marker antigen (the NG2 chondroitin sulfate proteoglycan) that differentiates these cells from myelinating oligodendrocytes, astrocytes, or microglia.1 Studies in rodents have revealed that oligodendrocytes arise from an identified precursor initially generated in the last third of gestation. These precursor cells migrate extensively throughout the CNS, divide as they migrate, and once they reach their final destination, they undergo a slow, morphological differentiation, eventually becoming myelinating cells.2 However, not all of these cells complete this pathway. It seems that many of these cells, at the pro-oligodendrocyte stage, do not complete this differentiation, but rather, become so-called adult OPCs (Figure 1).

Adult OPCs have several distinct characteristics, including, but not limited to, their ability to develop into oligodendrocytes when isolated from adult tissues (see Sidebar). Their processes contact synapses and nodes of Ranvier. They may be postsynaptic targets for glutaminergic and gamma aminobutyric acid-ergic neurons involved in glutamatergic transmission at synapses, although their precise role in the CNS is still unknown. OPCs are about as abundant as microglia, comprising approximately 3% to 8% of all the nuclei in the brain. Thus, the key question remains as to why...
These cells are so abundant. Although they can act as precursors or progenitors for the oligodendrocyte lineage, the numbers of cells present far exceed what might be necessary for this function, and their proximity to synapses tend to indicate that they must have other important functions.

**Functions of OPCs**

It is known that OPCs are extremely sensitive to the pathophysiological state of the brain, and that they react to many different types of experimentally induced insults by a fairly reproducible set of changes in cell shape. They re-enter the cell cycle and proliferate locally at sites of injury, establishing reactive changes that are extremely transient. This knowledge, coupled with the fact that OPCs can turn into oligodendrocytes, prompted the question as to whether adult OPCs participate in the repair of demyelinating lesions.

A study was conducted to analyze the reactions of OPCs to a transient demyelination of the rat brain stem induced by the injection of ethidium bromide. This resulted in a rapid 21% decline in the number of OPCs within affected fiber tracts such as the spinal tract of the trigeminal nerve. The surviving OPCs had altered morphology and began to increase in number. Their change in numbers indicated a rapid response to the pathophysiological state of the brain. The occurrence of demyelination generated a sufficient number of OPCs to participate in the repair of the demyelinated lesions. In other words, concurrent with the death of oligodendrocytes and the loss of myelin from the effects of the toxin, there was a large increase in the OPC population, only within these demyelinated areas, and as myelin was replaced, there was again a decrease in the number of OPCs. Although the ethidium bromide killed some OPCs, those that survived rapidly divided, continued to increase in number, and then as remyelination occurred they declined again, although they were still maintained at a slightly elevated number.

However, the question was whether these OPCs were really turning into myelinating oligodendrocytes. Answering this question has been rather difficult because the transition from a progenitor, or a precursor cell, to a true myelinating cell is very rapid and thus difficult to observe. Yet, it is certain that there is a correlation between the proliferation of OPCs and the loss of myelin, and as the myelin is restored the number of OPCs again declines, leaving us with strong circumstantial evidence that these are the cells that are at least one of the populations of cells that repair and remyelinate demyelinated lesions.

**Characteristics of Adult Oligodendrocyte Precursor Cells**

- PDGF-α receptor positive, O4 positive in vivo
- GFAP-negative, OX42-negative
- Develop into oligodendrocytes when isolated from adult tissues
- Comprise 3% to 8% of all cells in the CNS
- Proliferate slowly in vivo
- Processes contact synapses and nodes of Ranvier
- Postsynaptic targets for glutaminergic and GABAergic neurons

PDGF = platelet-derived growth factor; GFAP = glial fibrillary acidic protein; CNS = central nervous system; GABA = gamma aminobutyric acid.
Reynolds et al also studied the relationship between the presence of OPCs and the occurrence of remyelination in animal models and also in human tissue. As has been previously discussed, antibodies to the NG2 chondroitin sulfate proteoglycan was used to follow the response of endogenous OPCs to demyelination. In this series of experiments, the authors discovered some new evidence on the response of OPCs to the chronic inflammatory demyelinating environment seen in recombinant myelin oligodendrocyte glycoprotein-induced experimental allergic encephalomyelitis in rats. NG2-expressing OPCs responded to the inflammatory demyelination in this model by becoming reactive and increasing in number. The response of OPCs appeared to involve following successive relapses but did not always lead to remyelination, with areas of chronic demyelination observed in the spinal cord. In adult human CNS tissue, OPCs, as identified via the NG2 antibody label, were also seen in chronic multiple sclerosis lesions in both white and gray matter. Thus, research seems to suggest there are at least 2 signals involved in activation of OPCs: one from demyelination as indicated by our work, and one from inflammation, as evidenced from the experiments of Reynolds et al.

Based upon the experimental evidence thus far, several conclusions may be made regarding the behavior of OPCs. First, OPCs react to several different types of experimentally induced demyelination, and these reactions are localized to the demyelinated areas. Second, large increases in the numbers of OPCs accompany demyelination and more importantly, it would appear that reactive OPCs might be capable of differentiating into myelin-forming cells (see Sidebar).

**WHY DO REMYELINATION AND AXON REGENERATION FAIL?**

The question then arises as to why, since large numbers of these cells are found within both active and chronic multiple sclerosis plaques, does remyelination fail? Senescence among OPCs may be rate limiting for continuing remyelination. A second possibility, however, comes from the fact that these cells have calcium-permeable alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. AMPA is an excitatory amino acid, and, as we have seen, there is an excitotoxic component in long-term multiple sclerosis lesions. It is possible that the glutamate released during the neuronal degenerative aspects of multiple sclerosis is acting to kill those cells—the OPCs—that are most capable of developing into myelin-forming cells and thus that have the most potential to actually repair the lesions.

To change focus, it should be recognized that OPCs react to many different types of injury, not simply demyelination, and in particular they participate in the creation of the glial scar that forms at sites of injury, both in the brain and spinal cord. When scarring occurs in an adult rat brain, reactive NG2-positive OPCs will form a dense plaque within an array of hypertrophic astrocytes. The glial scar is a highly complex tissue. It contains many different cell types, depending upon the nature of the injury, including an extracellular matrix of both growth-promoting molecules as well as growth-inhibiting molecules. This matrix is a rich magnet for all sorts of small molecules, trophic factors, cytokines, chemokines, and, most important, it contains a large number of molecules known to inhibit axon growth during development. These include chondroitin sulfate proteoglycans (such as NG2), myelin-associated growth inhibitors, and repulsive axon guidance molecules (Figure 2). Thus, it is generally accepted that the glial scar is both a physical and biochemical barrier to successful axon regeneration and repair, as will be discussed in further detail below.

Indeed, there are generally 2 explanations put forth as to why CNS neurons do not regenerate. One potential explanation is that the adult CNS neurons have a very

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**OPCs: Conclusions**

- OPCs react to several different types of experimentally induced demyelination.
- The reactions of OPCs are localized to the demyelinated areas.
- Large increases in the numbers of OPCs accompany demyelination.
- Reactive OPCs may be capable of differentiating into myelin-forming cells.
- Senescence among OPCs may be rate limiting for continuing remyelination.

OPCs = oligodendrocyte precursor cells.
low intrinsic capacity to regrow, but a second reason may be that the environment of the glial scar is extremely inhospitable to regeneration. This raises the question as to whether the presence of large numbers of OPCs in the glial scar might be the explanation for blocked axon regeneration, given the fact that OPCs express an abundant array of growth-inhibitory substances.

To test this hypothesis, a series of tissue culture experiments were conducted that revealed the membranes of OPCs are nonpermissive for axon growth. The next step was to determine whether the NG2 antigen present in OPCs was responsible for this phenomenon. Treatment of the OPC membranes with anti-NG2 antibodies that block its functions partially restored growth permissiveness. Further analyzing the structure of NG2, it was discovered that it is a fairly straightforward molecule with a multitude of functions, which are listed in the Sidebar. However, the specific property of NG2 of interest in this research was its ability to inhibit axon growth. A series of experiments were designed in which rats underwent surgery to damage their spinal cords, and then antibodies against NG2 were allowed to penetrate the damaged area. Utilizing function-blocking and non-function-blocking antibodies of NG2, the question posed was whether NG2 at the glial scar played a role in creating the negative environment for axon regeneration. The evidence from this series of experiments suggests that NG2 is indeed the responsible agent, because treating these lesions with function-blocking antibodies allows axons to grow in areas that they do not normally grow through.

A further investigation was conducted to look at the effects of so-called “priming” on CNS regrowth, and specifically what might happen to damaged axons in the presence of NG2. If, prior to making the spinal cord lesion, the peripheral branch of these neurons is cut, this causes a series of changes in the cell body among which is the generation of an increase in cyclic AMPA, and these cells are now invigorated, or primed, and are able to grow past the lesion site. What then would happen if the process of lesion priming were combined with blocking the scar with NG2-neutralizing antibodies?

In control animals it was determined that lesion priming caused a robust regrowth of these damaged axons, but it was also found that they tended to grow in ectopic locations. However, if priming were combined with anti-NG2 treatment, it was observed that many of the axons now grew through the white matter, rostral to the lesion, in...
a more topographically appropriate manner. Thus, it appears that lesion priming does allow the axons to grow, but it does not desensitize them to whatever inhibitors might be present in the glial scar. Furthermore, when at least one class of inhibitors is blocked, a more topographically appropriate pattern of axon regrowth occurs, and, of course, this is the fundamental issue that needs to be addressed in the future to assure meaningful, functional recovery for patients with multiple sclerosis.

**SUMMARY**

To date, research into the causes and potential treatments for multiple sclerosis has been directed toward one particular group of glial cells: the OPCs. It has been discovered that OPCs play a role in remyelination, and that these and other NG2-expressing cells also populate the glial scar. Adult OPCs present an interesting paradox. On the one hand, they play a positive role in remyelination. However, in the cases of physical damage to the CNS, reactive OPCs may have a detrimental effect. An antigen present on the surface of OPCs, NG2 participates in the inhibition of axon regeneration after injury caused by the glial scar. Therefore, preventing interactions between damaged neurons and NG2 promotes regrowth through the glial scar, and this, along with mechanisms of remyelination, are promising avenues of research in the attempt to aid those suffering from the debilitating effects of multiple sclerosis.

**REFERENCES**