Building bridges with astrocytes for spinal cord repair
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Abstract
Simultaneous suppression of glial scarring and a general enhancement of axonal outgrowth has now been accomplished in an adult rat model of spinal cord transection. Transplantation of a novel astrocyte cell type derived from glial-restricted precursors in vitro raise the eventual possibility of cellular therapy for spinal cord injury.

The functional regeneration of neuronal processes in the injured nervous system poses a formidable challenge. During development, axons - the long processes of neurons - grow long distances through complex terrains in stereotypical patterns to connect with the appropriate targets and facilitate effective communication. Much energy has been expended by researchers in investigating how axonal growth and connectivity are guided and regulated. While a complete understanding is still a long way off, it is clear that these are complex processes, involving multiple molecular cues that occur in stereotyped sequences. Many of the cues mediating axonal growth and guidance are lost in the adult central nervous system (CNS) and these processes are further disrupted by injury, resulting in disoriented axons. The injury itself releases inhibitors of axon growth from white matter (bundled tracts of axons) [1,2] and local endogenous glial cells, the supporting non-neuronal cells of the nervous system, respond to the insult with increased production of a variety of growth inhibitors [3,4]. In addition to these environmental changes, there are intrinsic differences in the growth responses of immature and adult axons - adult axons grow less strongly. In consequence, it seems that the key to functional regeneration in the injured adult spinal cord is the simultaneous modification of multiple inhibitory cues - a demanding task that requires a particularly special type of glial cell. In a recent paper in Journal of Biology, Davies et al. [5] describe the identification of such cells and their transplantation to promote a remarkable regeneration of adult axons after spinal cord transection in the rat.

The cells utilized by Davies et al. [5] are termed GRP-derived astrocytes. This unusual name derives from the origins of the cells and reflects recent advances in our understanding of the cellular development of the CNS. Classical morphological studies identified the major epochs of neural development, in which neurons arise before glial cells [6]. Evidence that all major cell types might be derived from multipotent stem cells emerged from in vitro assays in which 'neurosphere'-producing cells were isolated, passaged and shown to generate neurons and the glial cell types astrocytes and oligodendrocytes [7]. These observations prompted an intensive search to define intermediate cell types between a multipotent stem cell and the fully differentiated cellular products. Using a series of in vitro approaches, Davies et al. [5] identified precursor cells derived from multipotent stem...
The striking axonal regeneration seen by the authors following GRP-derived astrocyte transplantation raises the critical issue of what is special about these cells. It seems likely that one of the main keys to enhanced axonal regeneration is modulation of the endogenous host cells’ response to injury rather than the provision of specific molecular promoters of axonal elongation by the transplanted cells. For example, the regeneration-promoting abilities of GRP-derived astrocytes are not restricted to particular populations of neurons. Davies et al. [5] severed the rubrospinal tract - a population of axons that runs from the brain and is involved in relaying information that controls muscle function. Animals with injuries to the rubrospinal tract had lost the ability to coordinate their fore- and hindlimbs precisely. After transplantation of GRP-derived astrocytes to the site of the lesion, the team observed increased regrowth of the rubrospinal tract axons into the injury site compared with untreated animals, and enhanced recovery of locomotor function.

In other experiments the authors found that GRP-derived astrocytes enhanced the growth of axons from transplants of sensory dorsal-root ganglion neurons through a lesion. These axons, which normally derive from neurons located outside the spinal cord, are likely to use distinct molecular cues for outgrowth compared with the rubrospinal tract axons, and it is unlikely that both sets of cues are expressed at the same time by the GRP-derived astrocytes. Rather, the GRP-derived astrocytes appear to modulate shared responses of adult CNS cells to injury, and provide an environment that recapitulates essential properties of the developing CNS.

Two aspects seem particularly important. The first is the suppression of glial scarring that normally accompanies injury to the CNS. Glial scars result in excessive growth in size of astrocytes (hypertrophy) and upregulation of the production of various proteoglycans that inhibit axonal growth. The onset of the scarring response is significantly delayed by transplanting GRP-derived astrocytes, which perhaps allows a window of opportunity for regenerating axons to traverse the lesion site. Second, transplantation of GRP-derived astrocytes imposes a striking linear orientation on host glial cells such that they provide a more uniform environment through which the regenerating axons grow more easily.

Davies et al. [5] provide two main insights that will be important in approaches to promoting spinal cord repair by cell transplantation. First, the selection of the appropriate cell type is critical for regeneration. Cells that are too immature or uncommitted are relatively ineffective, presumably because their fate can be dictated by signals at the injury site. Cells
that are too mature, as in the host, are relatively ineffective, presumably because they are programmed to form glial scars. Thus, the level of commitment or cell differentiation is key. Second, creation of a ‘regeneration-permissive’ environment is not neuron-specific. The commonalities of axon outgrowth seem sufficient for different types of neurons to be able to benefit from the same treatment. These observations suggest that axon regeneration is perhaps fundamentally different from initial axonal pathfinding during development, which appears to be essentially neuron-specific [10].

This study also raises a number of interesting questions on the in vivo correlates of GRP-derived astrocytes. For example, do they represent a distinct cell population in the intact CNS or are they simply a product of in vitro ‘cell engineering’? The origin and lineages of glial cells in the CNS has been extensively studied, particularly in the spinal cord. Although several models have been proposed linking neurons and oligodendrocytes in a common lineage [11,12], more recent studies suggest that this is unlikely [13,14]. Earlier studies had linked astrocytes and oligodendrocytes in a common lineage [15], and previous work [16] from two of the authors of Davies et al. [5] demonstrated a more primitive glial precursor that generates different types of astrocytes and oligodendrocytes. The molecular cues used by Davies et al. [5] to generate GRP-derived astrocytes are operative in the intact CNS but are likely to be used in concert with multiple other signals to specify other cell types, including neurons [17-20]. The origins of astrocytes in vivo remain unclear. Indeed, in other regions of the CNS astrocytes have been proposed to represent stem cells on the basis of the expression of GFAP, and clonal studies in vitro suggest a significant diversity among spinal cord astrocytes [21]. The precise assessment of lineage associations between cells of the CNS and identification of intermediate cell types require novel approaches and the generation of new molecular markers.

Ultimately, it will be essential to unravel the cellular and molecular bases of the phenomena described by Davies et al. [5]. What is it about GRP-derived astrocytes that facilitates their orientation and what are the molecular mechanisms by which they promote axonal growth? These questions will not be answered easily. The model of cell transplantation into the injured spinal cord is extremely complex. Multiple cell interactions are occurring simultaneously and early interactions are likely to establish cascades of subsequent events. Such complexity limits the use of modern DNA array-based discovery approaches, and insights are more likely to come from cell-based strategies. The hope is that identification of critical upstream steps in the regulation of the host glial response to CNS injury that are modulated by GRP-derived astrocytes might lead to the identification of key regulators that could be targets for pharmacological therapeutics.

Regardless of whether there is a precise in vivo counterpart of the GRP-derived astrocytes and of the molecular mechanisms by which these cells promote axon elongation, the studies by Davies et al. [5] reveal both the importance of cellular maturity in promoting axonal regeneration and provide a source of cells for effective therapeutic approaches aimed at adult spinal cord regeneration.

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