

Molecular Targets to Promote Central Nervous System Regeneration

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Abstract: Trauma in the adult mammalian central nervous system (CNS) results in devastating clinical consequences due to the failure of injured axons to spontaneously regenerate. This regenerative failure can be attributed to both a lack of positive cues and to the presence of inhibitory cues that actively prevent regeneration. Substantial progress has been made in elucidating the molecular identity of negative cues present at the CNS injury site following injury. In the past several years, multiple myelin-associated inhibitors including Nogo, Myelin-associated glycoprotein and Oligodendrocyte-myelin glycoprotein have been characterized. Furthermore a neuronal receptor complex and several intracellular substrates leading to outgrowth inhibition have been identified. Rapid progress has also been made in identifying the role of neurotrophins and other positive cues in promoting axonal regrowth. The most recent advances in our understanding of positive stimuli for axon regeneration come from transplantation studies at the CNS lesion site. A number of artificial substrates, tissues, and cells including fetal cells, neural stem cells, Schwann cells and olfactory-ensheathing cells have been tested in animal models of CNS injury. Based on our expanded knowledge of inhibitory influences and on the positive characteristics of various transplants, a number of interventions have been tested to promote recovery in models of CNS trauma. These advances represent the first steps in developing a viable therapy to promote axon regeneration following CNS trauma.

Key Words: Regeneration, myelin-associated inhibitors, astroglial scar, Rho GTPase, cyclic AMP, transplantation, Nogo.

INTRODUCTION

Following traumatic injury to the central nervous system (CNS) a complex series of events ensue that contribute to failed regeneration. Initial damage to the axon is exacerbated by secondary damage due to inflammation and infiltration of immune cells. Apoptosis, Wallerian degeneration, excitotoxicity, ischemia and the immune response participate in expanding the lesion core. Current clinical interventions such as the administration of high intravenous doses of the steroid methylprednisolone are directly aimed at limiting secondary damage (Bracken, 2001). In addition to limiting secondary damage, long distance regeneration of injured nerve fibers must be stimulated to reestablish communication within the nervous system. Efforts to promote long distance regeneration have focused on stimulating the expression of critical growth associated proteins in the cell body and on circumventing the inhibitory environment of the CNS lesion site. Full restoration of function will further require the establishment of appropriate connectivity and myelination of regenerated fibers to mediate reliable electrical conduction.

The response to CNS trauma is often studied in spinal cord injury (SCI) models where significant progress has been made in our understanding of the cellular response to injury. Ultimately, functional loss following spinal cord injury can be attributed to disrupted transmission in long ascending and descending fiber tracts responsible for transmitting motor and sensory information throughout the body. The failure of

severed nerve fibers to spontaneously re-extend to their targets distinguishes the CNS from the peripheral nervous system (PNS) where individual fibers do regenerate to restore function. The ability of the PNS to regenerate has provided a system to compare and contrast the injury response in the CNS and provides hope that the CNS may be stimulated to regenerate and restore function.

In this review we will focus on current developments in our understanding of the biology of long distance axon regeneration at the molecular level. We will review recent advances in the identification of myelin-associated inhibitors, their receptors and their intracellular targets as well as inhibitory components of the glial scar. For each inhibitory component we will review and discuss recent strategies to neutralize these molecules to promote axonal regeneration. We will also review recent advances in the cellular transplantation field. A variety of cellular transplant strategies have been tested for their ability to provide positive stimuli and a growth permissive matrix for axon regeneration. We will discuss the advantages and pitfalls of various transplant strategies. A special emphasis will be put on the potential application of these advances to the development of a therapeutic treatment for SCI.

THE CNS ENVIRONMENT

Classic experiments performed over 20 years ago demonstrated that CNS neurons are capable of long distance regeneration when provided with a growth permissive environment (David and Aguayo, 1981). Since the historical finding that CNS neurons are regeneration competent, the CNS environment has been extensively studied and it is clear

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that multiple influences at the CNS lesion site inhibit regenerative growth. Both myelin debris (Caroni and Schwab, 1988a, b) and an astroglial scar (McKeon *et al.*, 1991; Davies *et al.*, 1997; Davies *et al.*, 1999) actively inhibit regeneration by challenging the severed axon with inhibitory proteins that signal to the axon and prevent growth. Extensive research is therefore being done to identify inhibitory components at the CNS lesion site and to define their mechanism of action. From this research many interventions designed to replace or neutralize the inhibitory environment in the injured CNS have been tested in injury models with the aim of promoting long distance regeneration and functional recovery.

MYELIN-ASSOCIATED AXON GROWTH INHIBITORS

Original experiments demonstrating that CNS neurons fail to grow on white matter provided the first suggestion that myelin actively prevents axon outgrowth. Subsequent biochemical fractionation of CNS myelin led to the identification of an axon outgrowth inhibitory activity termed NI35/250 (Caroni and Schwab, 1988a, b). A function-blocking monoclonal antibody named IN-1, which recognizes NI35/250, generated great excitement in the regeneration field because it promoted regrowth of injured corticospinal tract (CST) fibers and behavioral recovery when infused into the site of a spinal cord lesion (Caroni and Schwab, 1988b; Schnell and Schwab, 1990). However, this antibody recognizes several proteins in brain homogenates. The protein responsible for the inhibitory activity in this myelin fraction was subsequently identified as Nogo (Chen *et al.*, 2000; GrandPré *et al.*, 2000; Prinjha *et al.*, 2000) and more specific reagents are being developed to validate the original findings obtained using the IN-1 antibody. Further analysis of myelin has demonstrated that two other myelin proteins, Myelin-Associated Glycoprotein (MAG) and Oligodendrocyte Myelin Glycoprotein (OMgp), also inhibit axon outgrowth.

Myelin Associated Glycoprotein

MAG is a member of the immunoglobulin superfamily and was the first myelin-associated protein to be identified as an inhibitor of axonal regeneration (McKerracher *et al.*, 1994; Mukhopadhyay *et al.*, 1994). *In vitro* evidence suggests that MAG is a bi-functional molecule that promotes outgrowth from neurons during development and inhibits the outgrowth of adult neurons. The response to MAG switches at approximately postnatal day 4 in rodents and is controlled by changes in intracellular levels of cAMP (Cai *et al.*, 2001). In spite of the evidence that MAG is a potent outgrowth inhibitor *in vitro*, its relative contribution to the inhibitory environment of the CNS following SCI is controversial. Regeneration of corticospinal fibers in MAG knockout mice is no different from control mice following dorsal hemisection (Bartsch *et al.*, 1995; Li *et al.*, 1996). However, when MAG knockout mice are crossbred with C57Bl/Wld mice, which have PNS regeneration deficits due to delayed Wallerian degeneration, regeneration is improved (Schafer *et al.*, 1996) suggesting that MAG does contribute to the

inhibitory environment *in vivo*. In addition, *in vivo* treatment with MAG antibody preferentially accelerates regeneration of peripheral motor nerves, although there is no effect on sensory nerve regeneration within the PNS (Mears *et al.*, 2003). Finally, inactivation of MAG by chromophore-assisted laser inactivation (CALI; Wong *et al.*, 2003) promotes retinal ganglion cell (RGC) regeneration in chick retina-optic nerve explants. Optic nerve crush on E15 chick retina-optic nerve explants followed by acute inactivation of MAG along the nerve permits significant regrowth of retinal axons past the site of lesion. Together these data suggest that MAG has an inhibitory effect on selective populations of neurons *in vivo*.

The selective effects of MAG may be explained by the various MAG-binding partners. MAG is a sialic acid binding protein and binds the gangliosides GD1a and GT1b (Vinson *et al.*, 2001). Clustering of GT1b inhibits neuronal outgrowth however it is not clear that this represents a physiologic mechanism for MAG inhibition. Rather, GT1b clustering may serve to cluster signaling molecules to discrete membrane compartments to facilitate MAG signaling (McKerracher, 2002). MAG also binds directly to Nogo-66 Receptor (NgR; see below), which acts as a functional receptor mediating MAG inhibition (Domeniconi *et al.*, 2002; Liu *et al.*, 2002).

Nogo

Nogo was identified as the protein responsible for the inhibitory activity of NI35/250, and as the antigen of the IN-1 antibody over 10 years following the original characterization of this myelin fraction (Chen *et al.*, 2000; GrandPré *et al.*, 2000; Prinjha *et al.*, 2000). Three major forms of Nogo (Nogo A, B, C) are generated by alternative splicing (Nogo-A/B) and promoter usage (Nogo-C). Nogo is a member of the reticulon protein family and is highly expressed in the ER and Golgi (Chen *et al.*, 2000; GrandPré *et al.*, 2000; Prinjha *et al.*, 2000). However, Nogo-A is also expressed on the cell surface of oligodendrocytes in the CNS where it mediates outgrowth inhibition (GrandPré *et al.*, 2000; Oertle *et al.*, 2003). *In vitro*, three distinct domains of Nogo-A have inhibitory properties in growth cone collapse and neurite outgrowth assays (Chen *et al.*, 2000; GrandPré *et al.*, 2000; Prinjha *et al.*, 2000; Oertle *et al.*, 2003). Two domains are located in the N-terminal portion of Nogo-A and the third inhibitory domain is a 66 amino acid segment, termed Nogo-66, which is flanked by 2 transmembrane regions in the C-terminus of Nogo-A. 188 amino acids in the C-terminal domain of Nogo-A are shared by Nogo-A/B/C and thus Nogo-66 is present in each of the Nogo isoforms.

The expression of individual Nogo domains at the cell surface is an important issue in understanding its physiologic function in plasticity and regeneration. For myelin-associated inhibitors to play a role in limiting structural plasticity (Buffo *et al.*, 2000; Schnaar, 2003), critical domains must be expressed at the oligodendrocyte cell surface in intact brain to interact with neuronal receptors. However, following spinal cord injury, damaged oligodendrocytes release their contents, thus injured neurons may be exposed to domains of Nogo-A not normally

expressed at the cell surface. Intriguingly, both Nogo-66 (GrandPré *et al.*, 2000) and the amino terminal domain of Nogo (Oertle *et al.*, 2003) can be detected on the oligodendrocyte cell surface suggesting that Nogo exists in two different topologies simultaneously.

The function of Nogo *in vivo* has been studied by examining the phenotype and injury response of Nogo knockout mice however the results of these studies are controversial. Three independent groups have generated Nogo knockout mice. A Nogo-A/B mutant mouse line was studied by Strittmatter and colleagues (Kim *et al.*, 2003). In this line a significant increase in corticospinal sprouting above and below spinal cord dorsal hemisections was observed. This anatomical recovery was accompanied by enhanced functional recovery as assessed by the Basso, Beattie, and Bresnahan (BBB) scale. This study suggests that Nogo-A plays an important role in the failure of regeneration of adult CNS neurons. Intriguingly this regeneration was restricted to young mice suggesting that other plasticity mechanisms may contribute to facilitate regeneration. In a second mouse line generated by Schwab and colleagues (Simonen *et al.*, 2003), Nogo-A expression was disrupted by conventional gene targeting. As compared with the Strittmatter mice, these mice exhibit a compensatory upregulation of Nogo-B expression by oligodendrocytes, and a significant, but less dramatic regenerative response of CST fibers following dorsal hemisections. The more modest phenotype could be attributed to the compensatory increase in Nogo-B, which contains the Nogo-66 inhibitory domain. Two other Nogo knockout lines were generated by Tessier-Lavigne and colleagues (Zheng *et al.*, 2003). One line lacks Nogo-A and -B (Nogo-A/B mutant) and the second line lacks all three isoforms (Nogo-A/B/C mutant). In these two lines, no improvement in anatomical or functional recovery following spinal cord lesions was found. While there is no clear explanation for the different responses to injury in these Nogo knockout lines, it is conceivable that differences in genetic background or unidentified modifiers modulate the effect of Nogo-A.

Oligodendrocyte Myelin Glycoprotein

OMgp is the most recently identified myelin-associated inhibitor. OMgp is a GPI-anchored protein containing a leucine-rich repeat region and ser/thr repeats in the C-terminus. Consistent with a function in inhibiting CNS regeneration, OMgp is expressed by oligodendrocytes and inhibits neurite outgrowth *in vitro* (Kottis *et al.*, 2002; Wang *et al.*, 2002b). The *in vivo* function of OMgp has not yet been fully addressed. Analysis of OMgp mRNA expression demonstrates that it is expressed at late stages of myelination suggesting that it may arrest oligodendrocyte proliferation (Vourc'h *et al.*, 2003). Intriguingly, OMgp (Habib *et al.*, 1998) and Nogo (Oertle *et al.*, 2003) are also expressed by neurons where their functions remain unknown.

Other Myelin-Associated Inhibitors

Understanding the relative contribution of Nogo, MAG and OMgp to myelin inhibition is still an active area of study. Generation of double and triple knockout combina-

tions of Nogo, MAG and OMgp will determine if these proteins together account for the full inhibitory activity of myelin. While it is conceivable that other myelin-associated proteins may have inhibitory activity, the major myelin inhibitory activities identified by biochemical fractionation have been accounted for. Nonetheless other evidence suggests that chondroitin sulfate proteoglycans, which have been previously identified as inhibitory components of the glial scar, are also present in myelin and may contribute to myelin inhibition (Niederost *et al.*, 1999).

SIGNALING FROM MYELIN ASSOCIATED INHIBITORS

Receptors for Myelin-Associated Inhibitors

A critical step in elucidating the mechanisms of action of myelin-associated inhibitors is the identification of corresponding axonal receptors. The Nogo-66 receptor (NgR) is a common receptor mediating inhibition by Nogo-66, MAG and OMgp. NgR is a GPI-linked, leucine-rich repeat (LRR) protein and it is expressed in multiple types of neurons within the brain (Fournier *et al.*, 2001). Originally identified as a receptor for Nogo-66, NgR was subsequently identified as a functional binding partner for MAG and OMgp. This is particularly surprising because Nogo, MAG and OMgp are structurally unrelated proteins. To understand the basis of this multi-ligand binding, the crystal structure of NgR has been solved (Barton *et al.*, 2003; He *et al.*, 2003). NgR is a banana-shaped molecule consisting of short strands that together form a long parallel sheet that spans the concave surface of the molecule. The convex side of the receptor is formed by the loops connecting the strands and several small helices. Two homologues of NgR (NgRH1 and NgRH2) that do not bind to Nogo ligand (Barton *et al.*, 2003; Pignot *et al.*, 2003) and several NgR fish homologues (Klinger *et al.*, 2003) have been identified. Based on analyses of these homologues, several candidate residues have been identified that may directly bind to inhibitory ligands. Site directed mutagenesis of NgR will be required to confirm ligand binding sites and this will be an important step in developing reagents to block ligand-receptor interactions. Detailed characterization of NgR mRNA demonstrates that NgR is not expressed by all neuronal subtypes raising the possibility that other unidentified neuronal receptors may exist for myelin-associated inhibitors (Hunt *et al.*, 2002; Josephson *et al.*, 2002).

The GPI-linked nature of NgR suggests that it complexes with a co-receptor to mediate intracellular transduction of myelin-associated inhibitory signals. Consistent with this model, the p75 neurotrophin receptor (p75^{NTR}) binds to NgR forming a receptor complex that mediates signaling by Nogo-66, MAG and OMgp (Wang *et al.*, 2002a; Wong *et al.*, 2002). Consistent with a role for p75^{NTR} in myelin inhibition, neurons from p75^{NTR} null mice exhibit increased growth capacity on myelin substrates as compared with neurons from control mice (Walsh *et al.*, 1999; Wang *et al.*, 2002a). p75^{NTR} affects the activity of the small guanosine triphosphatase (GTPase) Rho, which is known to effect the neuronal cytoskeleton, and likely mediates axon inhibition via this interaction (see below).

Myelin-Associated Inhibitors and Intracellular Signaling

The intracellular machinery involved in mediating the inhibitory function of myelin-associated proteins is also an active area of research. cAMP and Rho GTPase are two key intracellular targets that have been implicated in myelin-dependent inhibition. Several studies have described the importance of the cAMP-Protein kinase A (PKA) pathway in the neuronal response to myelin-associated inhibitors of axon growth (Snider *et al.*, 2002). Decreased levels of cAMP during development coincide with the developmental switch to MAG inhibition. Further, infusion of cAMP analogues *in vivo* promotes spinal and sensory axon regeneration (Neumann *et al.*, 2002; Qiu *et al.*, 2002b). cAMP levels can be elevated by treatment with neurotrophins, thus priming with neurotrophins can promote neurite outgrowth on myelin substrates *in vitro* (Cai *et al.*, 1999). There is also evidence that cAMP-mediated increases in neurite outgrowth on myelin are mediated by increase in the expression of Arginase I and polyamines (Cai *et al.*, 2002). This is consistent with several studies demonstrating a positive role for polyamines in the growth and development of the nervous system as well as in axonal regeneration (Slotkin and Bartolome, 1986; Gilad *et al.*, 1996).

Rho GTPases play an important role in the transduction of extracellular signals into changes in the cytoskeleton (Hall, 1998). Well characterized members of the Rho family of GTPases are Rho, Rac and Cdc42 (Bishop and Hall, 2000). In neurons, Rho activation is generally associated with growth cone collapse and inhibition of outgrowth while Rac and Cdc42 activation often correlate with growth cone attraction and axon extension (Giniger, 2002). There is clear biochemical evidence that Rho is activated in response to MAG, Nogo and CSPG stimulation (Fournier *et al.*, 2000; Niederost *et al.*, 2002; Borisoff *et al.*, 2003; Dubreuil *et al.*, 2003). Blockade of Rho activation with C3 transferase partially circumvents myelin- and CSPG-dependent inhibition. Furthermore, blockade of a downstream effector of Rho, Rho associated kinase (aka ROK), with a pharmacological antagonist promotes axon outgrowth on inhibitory substrates (Dergham *et al.*, 2002; Niederost *et al.*, 2002; Fournier *et al.*, 2003).

Rho activation is likely mediated via the NgR/p75^{NTR} receptor complex. p75^{NTR} binds directly to the Rho guanine nucleotide dissociation factor (RhoGDI) that maintains Rho in an inactive state (Yamashita and Tohyama, 2003). The p75^{NTR} RhoGDI interaction is strengthened by stimulation with known myelin-associated inhibitors. Thus, ligand-mediated p75^{NTR} sequestration of RhoGDI may facilitate Rho activation and neurite outgrowth inhibition. Rho also represents a potential target for crosstalk with the cAMP pathway. Previous work indicates that PKA activation increases RhoA extraction from membranes by RhoGDI (Forget *et al.*, 2002). Increased intracellular cAMP could therefore directly inhibit myelin-dependent activation by phosphorylating RhoA.

THE GLIAL SCAR

In addition to the presence of myelin-associated inhibitors, there is a progressive response from glial cells

surrounding a CNS injury, which contributes to the inhibitory environment (Morgenstern *et al.*, 2002). This process is called glial scarring and is characterized by the proliferation and migration of astrocytes into the lesion site where they extend tightly packed processes and deposit extracellular matrix creating a mechanical barrier for regeneration. Inhibition of glial cell proliferation during the period of glial scar formation can improve CNS regeneration. Treatment of adult rats with cytosine-D-arabino-furanoside to reduce glial proliferation following a unilateral transection of the medial forebrain bundle reduces the number of glial cells populating the lesion site and increases the number of axons distal to the lesion (Rhodes *et al.*, 2003). Reactive astrocytes within the glial scar also express CSPGs (Asher *et al.*, 2001), which chemically inhibit axonal outgrowth. A number of CSPGs are upregulated following CNS injury including neurocan, versican, brevican, decorin and biglycan (McKeon *et al.*, 1991; Asher *et al.*, 2000; Asher *et al.*, 2001). Neutralizing individual components of the scar such as Col III and IV with either an iron chelator or a function blocking antibody increases regeneration after injury in the fimbria fornix (Stichel *et al.*, 1999). Modest effects on the growth of severed nigrostriatal axons have been observed with hyaluronidase treatment to cleave neurocan, versican and brevican (Moon *et al.*, 2003). However, as with myelin-associated inhibitors, several components of the scar may need to be neutralized in order to render the environment more conducive for axon regrowth. Chondroitinase treatment of reactive astrocytes harvested from animals following various injury paradigms (McKeon *et al.*, 1991) and of frozen spinal cord sections (Zuo *et al.*, 1998) improve the capacity of these substrates to support neurite outgrowth in culture.

ANTAGONIZING AXON OUTGROWTH INHIBITION

Virtually every identified step in axon outgrowth inhibition, from ligands to receptors to intracellular substrates, has been targeted to promote regeneration (Fig. 1, Table 1). These interventions have validated the role of many of proteins in axon inhibition and have led to varying degrees of anatomical and functional recovery in rodent models of spinal cord injury. Function blocking antibody strategies have been primarily used to neutralize myelin-associated inhibitory ligands. The IN-1 monoclonal antibody was raised against a potent inhibitory fraction of CNS myelin (NI35/250) (Caroni and Schwab, 1988b). Treatment of lesioned axons in multiple models of CNS injury with hybridoma cells secreting the IN-1 antibody significantly improves anatomical and functional recovery (Caroni and Schwab, 1988b; Schnell and Schwab, 1993; Schnell *et al.*, 1994). Treatment with IN-1 antibody also enhances sprouting of unlesioned fibers (Thallmair *et al.*, 1998; Z'Graggen *et al.*, 1998; Buffo *et al.*, 2000; Raineteau *et al.*, 2001). This suggests that some repair could be achieved by sprouting of undamaged fibers mediating functional compensation for lesioned pathways. Although, the application of this antibody may appear promising therapeutically, the IN-1 antibody is not a specific blocker for Nogo-A and it has low antigen affinity. Recently a new version of IN-1 antibody was designed using a soluble

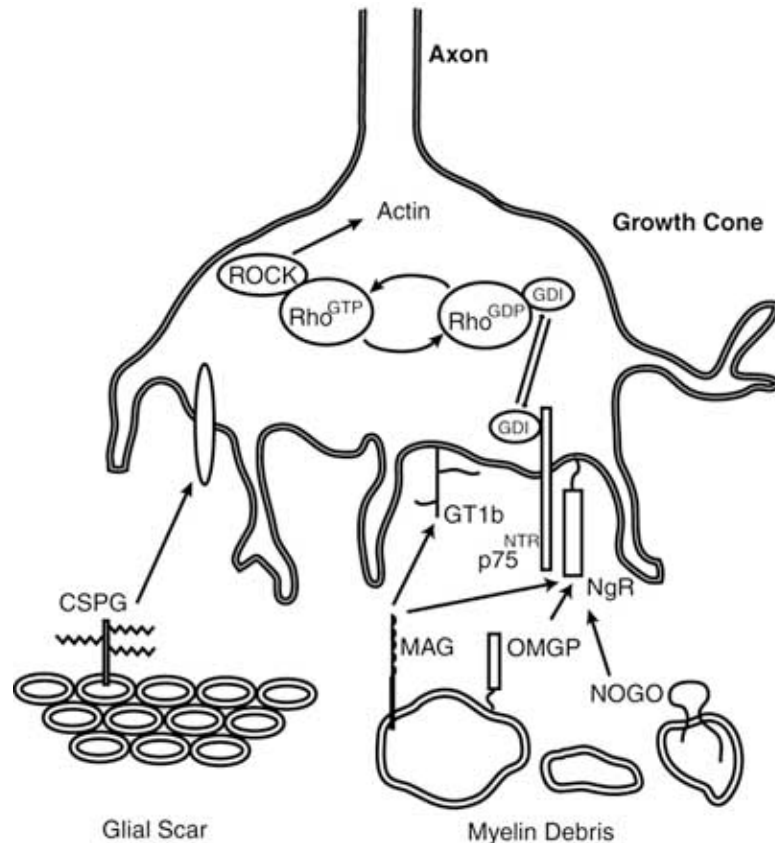


Fig. (1). Potential molecular targets to promote CNS regeneration. Schematic representation of CNS inhibitory molecules, their receptors and intracellular targets. The growth cone of a severed axon encounters inhibitory cues present in the glial scar and CNS myelin debris. Binding of MAG, Nogo and OMgp to NgR, as well as exposure to CSPGs, leads to Rho activation and actin cytoskeleton rearrangements that in turn cause growth cone collapse and neurite retraction. (CSPG, chondroitin sulfate proteoglycan; MAG, myelin-associated glycoprotein; GDI, guanine nucleotide dissociation factor; ROCK, Rho-associated kinase; NgR, Nogo-66 Receptor).

fragment of Nogo-A (IN-1 F(ab)), which shows a significantly stronger neutralizing effect on Nogo-A *in vitro* (Fiedler *et al.*, 2002). Also, more specific antibodies against recombinant Nogo are now being developed and tested *in vivo* (Oertle *et al.*, 2003). A function blocking antibody to MAG (Mears *et al.*, 2003) has also been tested for its capacity to promote regeneration. This antibody specifically promotes regeneration of motor peripheral nerves in the PNS but has no effect on sensory neurons.

Two NgR antagonists have been developed in an effort to neutralize myelin-associated axon growth inhibitors. Based on the finding that Nogo-66 consists of two distinct domains, one mediating binding to the NgR and one mediating inhibitory activity, a NgR antagonist named NEP 1-40 was developed (GrandPré *et al.*, 2002). NEP1-40 is a 40 residue amino-terminal peptide fragment of Nogo-66, which blocks Nogo-66 binding to NgR. This peptide partially blocks the inhibitory activity of CNS myelin and Nogo-66 *in vitro*. The antagonist also promotes regeneration *in vivo* when applied to rats intrathecally at the time of injury (GrandPré *et al.*, 2002) or when applied systemically 1 week after spinal

cord lesion (Li and Strittmatter, 2003). Although more work remains to be done, the efficacy of this reagent in promoting recovery when applied post-injury raises hope that this type of reagent may be effective for treating chronic spinal cord patients. NEP1-40 may selectively block Nogo-66 and not effect MAG or OMgp action (Domeniconi *et al.*, 2002; GrandPré *et al.*, 2002), thus a more efficacious reagent might be produced by blocking NgR binding to all of the ligands. For this purpose, another antagonist has been developed termed NgREcto, which likely blocks NgR binding to all of the myelin-associated inhibitors. NgREcto is a soluble truncated form of NgR lacking the unique C-terminal domain that appears necessary for the inhibitory activity of the receptor. NgREcto antagonizes the neurite outgrowth inhibitory effects of Nogo and myelin *in vitro* (Fournier *et al.*, 2002), however its *in vivo* effects have not yet been reported.

p75^{NTR} is a coreceptor for NgR therefore blockade of p75^{NTR} function or NgR/p75^{NTR} interactions may efficiently block inhibitory signals from multiple myelin inhibitors. A p75^{NTR} antibody, which disrupts the interaction between

Table 1. Various Reagents have been Developed to Target and Antagonize Inhibitory Molecules Involved in the Inhibition of CNS Regeneration. Antagonists have been Tested in *In Vitro* Assays of Neurite Outgrowth and Growth Cone Collapse and in Animal Models of SCI Injury

Antagonist	Target
C3 transferase ^{1,2,3}	Rho A
Chondroitinase ABC ^{4,5}	Chondroitin Sulfate Proteoglycans
cytosine-D-arabinofuranoside ⁶	Proliferating Glial Cells
db-cAMP ^{7,8,9,10}	Protein Kinase A
IN-1 Ab ^{11,12,13}	NI35/250
IN-1 F(ab) ¹⁴	NI35/250
Myelin Vaccination ¹⁵	Myelin Inhibitors
NEP1-40 ^{16,17}	NgR
NgREcto ¹⁸	NgR
Nogo-66/MAG vaccination ¹⁹	Nogo-66/MAG
p75 ^{NTR} antibody ²⁰	p75 ^{NTR}
p75 ^{NTR} siRNA ²¹	p75 ^{NTR}
PEP5 ²²	p75 ^{NTR}
Y-27632 ^{23,24,25}	Rho Kinase

(¹ Lehmann *et al.*, 1999, ² Dergham *et al.*, 2002, ³ Fournier *et al.*, 2003, ⁴ Moon *et al.*, 2001, ⁵ Bradbury *et al.*, 2002, ⁶ Rhodes *et al.*, 2003, ⁷ Cai *et al.*, 2002, ⁸ Neumann *et al.*, 2002, ⁹ Qiu *et al.*, 2002b, ¹⁰ Cui *et al.*, 2003, ¹¹ Caroni and Schwab, 1988b, ¹² Schnell and Schwab, 1993, ¹³ Schnell *et al.*, 1994, ¹⁴ Fiedler *et al.*, 2002 ¹⁵ Huang *et al.*, 1999, ¹⁶ GrandPre *et al.*, 2002, ¹⁷ Li and Strittmatter, 2003, ¹⁸ Fournier *et al.*, 2002 ¹⁹ Sicotte *et al.*, 2003, ²⁰ Wong *et al.*, 2002, ²¹ Higuchi *et al.*, 2003 ²² Yamashita and Tohyama, 2003 ²³ Dergham *et al.*, 2002, ²⁴ Fournier *et al.*, 2003, ²⁵ Borisoff *et al.*, 2003).

p75^{NTR} and NgR, circumvents myelin inhibition (Wong *et al.*, 2002). Further, p75^{NTR} interactions with Rho-GDI have been blocked with a peptide ligand that is associated with the fifth alpha helix of p75^{NTR} (Pep5). Pep5 interferes with the interaction between p75^{NTR} and Rho-GDI, prevents the activation of Rho and promotes neurite outgrowth (Yamashita and Tohyama, 2003). Others have proposed the use of small interfering RNA (siRNA) to suppress endogenous p75^{NTR} protein levels by triggering posttranscriptional gene silencing (Higuchi *et al.*, 2003). RNA interference (RNAi) is achieved by using siRNAs to silence gene expression in a sequence-specific manner. p75^{NTR} knockdown by siRNA may provide a more accurate method to study p75^{NTR} function than traditional studies of knockout mice where there are likely differences in gene expression that modify the cellular context. RNAi allows for the reliable knockdown of p75 levels in a temporally controlled fashion. A limitation of RNAi has been the transient nature of its activity in mammalian cells, and this issue needs to be resolved before it represents a viable therapeutic approach. Recent advances in vector-based small interfering RNA systems may help realize the potential of such a technique (Brummelkamp *et al.*, 2002).

Infusion of Chondroitinase ABC into CNS lesion sites to remove chondroitin sulfate GAG chains has been attempted in an effort to neutralize the inhibitory glial scar. Chondroitinase ABC treatment improves regenerative growth in the lesioned nigrostriatal pathway (Moon *et al.*, 2001) and in the lesioned spinal cord (Bradbury *et al.*, 2002). However, the distance of CST fiber regeneration following chondroitinase ABC treatment is modest compared to that following IN-1 antibody treatment (Schnell and Schwab, 1990; Schnell *et al.*, 1994). This is likely due to the myelin-associated inhibitors that fibers encounter at the edge of the neutralized scar. This highlights the importance of a multifaceted approach to treating CNS injuries. It should also be noted that while Chondroitinase ABC neutralizes many CSPGs, some CSPGs such as NG2 retain their inhibitory properties following treatment since their inhibitory activity is in the protein core domain (Jones *et al.*, 2002).

Development of antagonists to intracellular targets represents one of the most promising ideas to promote regeneration since convergent targets of multiple inhibitory and growth-promoting pathways are being identified. For example there is evidence that the small GTPase RhoA is activated in response to both myelin-associated inhibitors and components of the glial scar (Niederost *et al.*, 2002; Fournier *et al.*, 2003; Monnier *et al.*, 2003). Rho activation can be blocked with C3 transferase, an enzyme from *Clostridium botulinum* that blocks Rho function by ADP-ribosylation of its effector domain (Dillon and Feig, 1995). Treatment of neurons with C3 transferase promotes neurite outgrowth *in vitro* on substrates of myelin-associated inhibitors (Lehmann *et al.*, 1999; Winton *et al.*, 2002; Fournier *et al.*, 2003) and CSPGs (Monnier *et al.*, 2003). Infusion of C3 at the site of dorsal hemisection *in vivo* also promotes regeneration following spinal cord injury, however these effects are dependent on the mode of delivery (Dergham *et al.*, 2002; Fournier *et al.*, 2003). C3 itself is not membrane permeable, and the development of cell-permeable forms of C3 should add new insights into the role of Rho in outgrowth inhibition *in vivo* (Winton *et al.*, 2002; Dubreuil *et al.*, 2003). Blockade of Rho activation may further improve recovery following spinal cord injury via a neuroprotective effect. It is striking that mice treated with C3 following a dorsal hemisection function significantly better than control animals as early as 24 hours following injury. Due to the time course, this effect cannot be attributed to long distance growth of injured fibers. The early behavioral benefit may be due to a neuroprotective effect of Rho inactivation (Dubreuil *et al.*, 2003). Rho activation following spinal cord injury enhances p75^{NTR}-mediated apoptosis and this is prevented with C3 delivery. Together these studies implicate Rho as a key signaling intermediate in axon outgrowth inhibition. The Rho pathway has also been targeted with Y-27632, a purine derivative that inhibits Rho-associated kinase (ROK), a serine-threonine protein kinase that is activated by Rho (Ishizaki *et al.*, 1997; Uehata *et al.*, 1997). Inactivation of ROK with Y-27632 promotes neurite outgrowth on permissive substrates such as laminin (Kato *et al.*, 1998; Bito *et al.*, 2000). Further, Y-27632 promotes neuronal outgrowth on myelin and CSPG substrates *in vitro* and in models of spinal cord injury (Dergham *et al.*, 2002;

Borisoff *et al.*, 2003; Fournier *et al.*, 2003; Monnier *et al.*, 2003).

Treatments to elevate cAMP levels also promote regeneration on myelin substrates *in vitro* (Cai *et al.*, 2001) and enhance regeneration *in vivo*. Injection of dibutyryl-cAMP, a membrane permeable analogue of cAMP, into the dorsal root ganglion (DRG) significantly increases the regeneration of injured central sensory branches (Neumann *et al.*, 2002; Qiu *et al.*, 2002a). Moreover, these sensory neurons are able to overcome the inhibitory influence of CNS myelin when subsequently cultured *in vitro*. Further, co-application of CPT-cAMP, another cAMP analogue, with ciliary neurotrophic factor increases regeneration of axotomized RGCs into peripheral nerve autografts (Cui *et al.*, 2003). The tradeoff with targeting intracellular signaling substrates such as Rho and cAMP is that they are common to multiple cell types and side effects on other cells will occur at the lesion site. Thus, the identification of neuronal-specific intracellular substrates of axon outgrowth inhibitors remains an important pursuit.

Neutralizing Myelin-Associated Inhibitors by Vaccination

Therapeutic vaccination is also being investigated as an approach for treating spinal cord injury. This approach stimulates the animal's own immune system to produce antibodies against myelin-associated inhibitors. A vaccination strategy offers two major advantages. First, vaccination with purified myelin would theoretically stimulate the production of function blocking antibodies to multiple inhibitors. Second, vaccination may circumvent delivery issues associated with monoclonal antibody treatment. Hybridoma cells (Schnell and Schwab, 1993) and osmotic mini pumps (Brosamle *et al.*, 2000) are two delivery methods that have been used to introduce monoclonal antibodies to the cerebrospinal fluid (CSF). These methods have been effective in experimental models but in humans these invasive delivery methods may cause problematic secondary effects. Myelin vaccination has been tested by immunizing mice twice weekly with mouse spinal cord homogenates and regeneration of large numbers of CST fibers after dorsal hemisection of the spinal cord was reported (Huang *et al.*, 1999). Antisera from immunized mice promote neurite outgrowth on myelin substrates *in vitro*. Similarly, optic nerve regeneration can be stimulated in rats vaccinated with spinal cord homogenates (Ellezam *et al.*, 2003). These studies have been extended to study the effect of immunizations with a cocktail of purified Nogo-66 and MAG in a mouse spinal cord lesion model (Sicotte *et al.*, 2003). In this case, long distance regeneration and sprouting of injured CST fibers was also enhanced, but to a lesser degree than with myelin vaccination. Serum from mice immunized with the Nogo-66/MAG cocktail recognizes recombinant MAG and Nogo on slot blots demonstrating that antibodies to the immunogens are produced and therefore function-blocking antibodies may contribute to the positive effects on regeneration. There is also evidence that T-cell-mediated protective autoimmunity contributes to improved regeneration in various vaccination protocols. Vaccination with peptides from myelin proteins including Nogo and Myelin Basic Protein (MBP) promotes recovery in

a spinal cord injury model via a T-cell-mediated mechanism (Hauben *et al.*, 2001). Passive transfer of a T cell line directed against a Nogo peptide or an MBP peptide after spinal cord contusion in rats also ameliorates regeneration confirming the contribution of T-cells. The neuroprotective effect of T-cells may be mediated by their ability to remove inhibitory molecules or to secrete neurotrophic factors. It is also encouraging that in this study recovery was significantly promoted following a posttraumatic immunization with a peptide derived from Nogo-A (Hauben *et al.*, 2001).

While the positive effects of vaccination in these studies are encouraging, one of the problems associated with generating antibodies against myelin is that there is a risk of causing autoimmune diseases against myelin, such as experimental autoimmune encephalomyelitis (EAE). EAE is a T cell-mediated disease induced by Th1 cells that produce proinflammatory cytokines. The choice of immunogen will be critical in addressing this issue since various peptides demonstrate different propensities to induce autoimmune disease. EAE has been traditionally prevented by immunizing mice with myelin in Incomplete Freund's adjuvant (IFA; Rivero *et al.*, 1997; Huang *et al.*, 1999) however, IFA is not licensed for use in humans. Immunizations with aluminum hydroxide adjuvant, which can be used in humans, have been successfully tested in mice without inducing EAE (Sicotte *et al.*, 2003). The efficacy and safety of Alum adjuvant has also been reported using myelin-oligodendrocyte glycoprotein-alum vaccination (Wallberg *et al.*, 2003).

CELLULAR TRANSPLANTS – BRIDGING THE GAP

The use of cellular transplants at the CNS lesion site has been used as a mechanism to provide a growth promoting tissue-bridge for nerve fiber growth. Initial transplantation studies utilized peripheral nerve grafts as substrates for growth and provided the first demonstration that CNS neurons are capable of regeneration (David and Aguayo, 1981; Richardson *et al.*, 1984; Friedman and Aguayo, 1985; So and Aguayo, 1985). Many types of cells, tissues and artificial substrates have been subsequently transplanted into the injured spinal cord including fetal cells, neural stem cells (NSCs), Schwann cells and Olfactory Ensheathing Cells (OECs), where they have been assayed for their ability to "bridge the gap" or promote regenerative growth of spinal cord tracts (Kwon and Tetzlaff, 2001; Bunge, 2002; Kocsis *et al.*, 2002; Lakatos and Franklin, 2002). While a full survey of the cellular transplant literature is well beyond the scope of this review, most types of transplants offer several benefits in spinal cord injury paradigms, which will briefly be discussed (Fig. 2). First, glial cell transplants often provide neurotrophic support to promote cell survival within the lesion and this can be potentiated via genetic manipulation. Second, transplants provide a permissive growth matrix on which severed nerve fibers can regrow. Third, transplanted glial cells have the potential to myelinate newly generated axons to support rapid electrical conduction. Fourth, transplants of undifferentiated neural stem cells or fetal neurons supply new neurons within the lesion site, which in turn may relay synaptic information across the lesion.

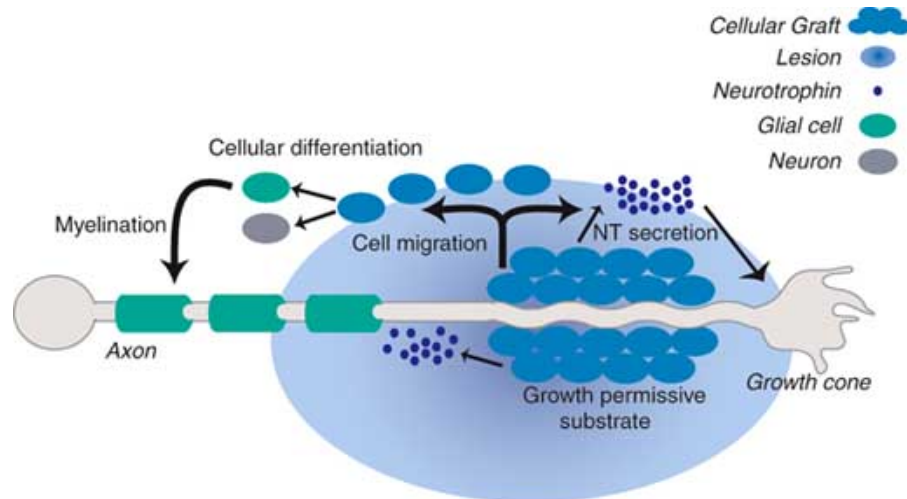


Fig. (2). Schematic representation of the growth-promoting characteristics of cellular transplants. Attempts to bridge the inhibitory gap at a CNS lesion site include transplanting cellular grafts. Many properties of cellular grafts including their ability to provide a permissive growth matrix, to secrete neurotrophins, to differentiate into myelinating glia and to differentiate into neurons contribute to their positive effects on regeneration.

Fetal tissue transplants promote both the survival and axon elongation of host neurons (Bernstein-Goral and Bregman, 1997; Bregman *et al.*, 1998; Diener and Bregman, 1998a; Bamber *et al.*, 1999; Walsh *et al.*, 1999). These effects are translated into improvements in functional recovery in transection, hemisection, and contusion models of injury (Reier *et al.*, 1992; Stokes and Reier, 1992; Tessler *et al.*, 1997; Diener and Bregman, 1998b; Slawinska *et al.*, 2000). While these experimental results are promising, to become a viable option for treatment of spinal cord injury, fetal transplants will have to overcome the ethical controversy associated with their use and issues of limited availability. NSCs offer many of the same advantages of fetal tissue transplants on survival and outgrowth with fewer ethical constraints. While these cells have been harvested from embryonic tissue, it is now clear that NSCs are present in the mature CNS (Reynolds and Weiss, 1992; Weiss *et al.*, 1996) and that NSC cell lines can be harvested from the fetal or adult CNS tissue (Ray *et al.*, 1993; Gage *et al.*, 1995). The human umbilical cord also offers an alternative source of embryonic stem cells (Saporta *et al.*, 2003). NSCs cultured *in vitro* can be differentiated into neurons or glia (Reynolds and Weiss, 1992; Palmer *et al.*, 1997; Whittemore, 1999). Furthermore these progenitors may be enticed to proliferate, migrate and differentiate *in vivo* (Fallon *et al.*, 2000; Kojima and Tator, 2000). Following a contusion injury in rats, NSC transplants differentiate into neurons, oligodendrocytes and astrocytes, and mediate improvements in hindlimb motor function (McDonald *et al.*, 1999). Further, NSCs grafted into demyelinated spinal cord are capable of remyelinating host axons once differentiated into oligodendrocytes (Brustle *et al.*, 1999; Liu *et al.*, 2000). Human umbilical cord blood (hUCB) cells in mouse experimental SCI also improve functional recovery (Saporta *et al.*, 2003). Interestingly, the hUCB stem cells migrate to the injured areas and not to the uninjured areas. The production of neurotrophic factors, such

as NGF, BDNF, GDNF and NT-3 by the NSCs contributes to their effects on regeneration (Lu *et al.*, 2003). Overall, stem cells represent a promising avenue to enhance CNS recovery. Not only can they differentiate into all three major CNS cell types but they also create a favorable environment for axon regrowth of surviving neurons.

Schwann cells were originally identified as transplant candidates because they account for the majority of the growth promoting activity of peripheral nerve grafts (Smith and Stevenson, 1988; Montgomery and Robson, 1993). Highly purified populations of peripheral Schwann cells are obtainable from samples of human phrenic nerve (Morrissey *et al.*, 1991; Casella *et al.*, 1996) and harvested Schwann cells can be expanded in culture. Schwann cell transplants promote regeneration and functional recovery in multiple CNS regions including the injured spinal cord, the adult rat forebrain and the optic nerve (Martin *et al.*, 1991; Paino and Bunge, 1991; Kromer and Cornbrooks, 1985; Montgomery and Robson, 1993; Harvey and Plant, 1995; Plant *et al.*, 1995). In addition to promoting growth of injured neurons, Schwann cells underlie the formation of a peripheral nerve-like structure that can myelinate regenerating axons and promote signal conduction (Montgomery and Robson, 1993; Imaizumi *et al.*, 2000).

To further direct regenerative growth through Schwann cells, chemically engineered biodegradable polymers with sophisticated channels are being developed as supports for Schwann cells (Friedman *et al.*, 2002). However even with such supports, fibers enticed to enter the Schwann cell grafts often fail to exit the caudal limit (Montgomery and Robson, 1993). Re-entry of regenerating axons into the host tissue may need to be stimulated by additional administration of neurotrophins or by the neutralization of myelin-associated inhibitors.

The olfactory bulb (OB) represents an exceptional region in the CNS due to its ability to regenerate axons. Olfactory epithelial neurons undergo constant neurogenesis and regrowth. The cellular environment surrounding axons in this region has therefore been a subject of intense study. The OB contains a unique type of macroglia called olfactory ensheathing cells (OECs). OECs isolate olfactory axons from other resident glia thus providing a permissive environment for growth. OECs share both astrocyte and Schwann cell-like characteristics (Wewetzer *et al.*, 2002). An accessible source of OECs resides in the nasal olfactory tissue (Lu *et al.*, 2001a). However, a recent study has also shown that enteric glia, which share many properties with CNS astrocytes and OECs, can be isolated from the rat small intestine and can promote regeneration and re-entry of dorsal root axons into the spinal cord (Jiang *et al.*, 2003). Transplantation of OECs into the injured spinal cord promotes the regeneration of CST axons and entices dorsal root axons to re-enter the spinal cord (Li *et al.*, 1997; Ramon-Cueto *et al.*, 1998). On a functional level, OECs promote partial recovery of voluntary motor function, spinal reflexes and breathing (Ramon-Cueto *et al.*, 2000; Guntinas-Lichius *et al.*, 2002; Lu *et al.*, 2002; Li *et al.*, 2003; Verdu *et al.*, 2003). OECs mediate their positive effects by myelinating regenerating axons, counteracting the inhibitory glial scar environment and by secreting neurotrophic factors. Myelination of regenerating axons is dependent on the remarkable capacity of transplanted OECs to migrate for long-distances throughout the injured CNS (Li *et al.*, 1998; Ramon-Cueto *et al.*, 1998; Imaizumi *et al.*, 2000). Furthermore, transplantation of OECs reduces the degree of astrogliosis and CSPG expression by surrounding astrocytes (Lakatos *et al.*, 2000; Verdu *et al.*, 2001). OECs also secrete several neurotrophic factors including NGF, BDNF, GDNF that confer regeneration promoting properties to these cells (Ramon-Cueto and Valverde, 1995; Woodhall *et al.*, 2001; Lipson *et al.*, 2003). The ability of OECs to migrate into the CNS and to reduce the effects of the glial scar is a major advantage compared to Schwann cell transplants for spinal cord recovery. However, Schwann cells still play a crucial role as growth promoting substrates. A combination of these glial cells may offer a valid approach to enhance long-distance regeneration of CNS axons (Ramon-Cueto *et al.*, 1998).

NEUROTROPHINS

Neurotrophins (NTs) promote the survival, maintenance and outgrowth of neurons in the CNS (Segal, 2003), all characteristics that will promote recovery and regeneration. An additional effect of NTs is that they can also increase the number of oligodendrocytes that remyelinate axons in the CNS (McTigue *et al.*, 1998; Jean *et al.*, 2003). The classic NT family consists of nerve growth factor (NGF), brain derived growth factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5. The NTs bind and signal through specific high-affinity tyrosine kinase receptors (trkA, trkB and trkC) and through a common low affinity receptor (p75^{NTR}). However, a number of other neurotrophic molecules including ciliary neurotrophic factor (CNTF) and glial cell line derived neurotrophic factor (GDNF) have also been implicated in axon outgrowth. In fact, more than thirty neurotrophic factors have been identified and only a small subset of these

factors has been tested in animal models of spinal cord injury (Lacroix and Tuszynski, 2000). The choice of NT for treatment is complicated by the fact that different neuronal types vary in their NT requirement depending on the type of NT receptors they express (Miller and Kaplan, 2001). For example, delivery of NGF, BDNF or GDNF promotes regeneration of local motor axons or sensory axons across the dorsal root entry zone (Jones *et al.*, 2001). Coerulospinal axon outgrowth is promoted by NGF or BDNF treatment while corticospinal axons respond to NT-3 (Tuszynski *et al.*, 2002; Tuszynski *et al.*, 2003). In spite of their widespread effects, application of NTs alone by osmotic minipump or by injection of NT-expressing fibroblasts, supports neuronal survival and local sprouting but fails to promote regeneration (Lu *et al.*, 2001b; Hiebert *et al.*, 2002). However, when NTs are added in combination with cellular transplants, regeneration is significantly enhanced. Genetic manipulation of transplanted cells to overexpress neurotrophins improves the growth of axons into peripheral nerve grafts or fetal transplants (Xu *et al.*, 1995; Bregman *et al.*, 1997; Kobayashi *et al.*, 1997; Ye and Houle, 1997; Coumans *et al.*, 2001; Kwon *et al.*, 2002). In fact, NT addition may be critical since axons fail to grow through fetal transplants and into the host spinal cord in the absence of NTs (Bregman *et al.*, 1997). Transplants alter the host glial environment providing a permissive substrate through which NTs promote outgrowth. Once NT-stimulated axons are primed on the permissive substrate, axons may then be capable of extending beyond the caudal edge of the transplant. This being said, one limitation of NT treatment is their apparent inability to promote axon elongation into the inhibitory white matter caudal to the graft (Ghosh and David, 1997). NT-3 application into lesioned spinal cord promotes axon sprouting and long distance regrowth of CST fibers through gray but not white matter (Schnell *et al.*, 1994; Grill *et al.*, 1997). However, a combination of NT-3 and IN-1 antibody to neutralize the inhibitory myelin environment, can enhance outgrowth through the white matter (Schnell *et al.*, 1994). Overall the data suggests that NTs have positive effects on neuronal survival and outgrowth following CNS injury but that the full potential of NTs may only be realized when they are applied in conjunction with other therapies.

The positive effects of NT treatment are likely mediated in part by their ability to stimulate a robust cell body response. The cell body response of injured CNS neurons differs completely from PNS neurons, which recapitulate a developmental pattern of gene expression following injury. A number of growth-associated proteins (GAPs) whose expression levels are downregulated in the adult such as GAP-43 and T 1 tubulin, are upregulated in the PNS following injury (Bulsara *et al.*, 2002). The expression of major growth cone proteins such as GAP-43 and CAP-23 may therefore render neurons competent for regeneration. Co-expression of GAP-43 and CAP-23 in DRGs is sufficient to elicit a 60-fold increase in fiber outgrowth after SCI (Bomze *et al.*, 2001). The importance of this cell body response was first recognized when examining the capacity of DRG neurons to regenerate. It was shown that the central spinal projection of DRG neurons could regenerate into peripheral nerve transplants if the peripheral projection was previously transected; a manipulation termed a "conditioning

lesion" (Richardson and Issa, 1984). The conditioning lesion induced dramatic changes in gene expression in the neuronal cell body, which were not induced by transection of the central branch.

Treatment of injured retinal ganglion cells with BDNF enhances the expression of some, but not all GAPs (Fournier *et al.*, 1997; Fournier and McKerracher, 1997). Furthermore, the substrate that injured neurons are exposed to may have dramatic effects on the expression of growth-associated proteins. CNS neurons that are enticed to regenerate through peripheral nerve grafts mimic a pattern of expression of GAPs that is seen both during development and during PNS regeneration (Fournier and McKerracher, 1997). There is also evidence that inhibitory molecules in the CNS environment actively inhibit the expression of GAPs. Infusion of a function blocking antibody to Nogo leads to mRNA upregulation of multiple GAPs including actin, myosin and GAP-43 (Bareyre *et al.*, 2002).

CONCLUSIONS AND PERSPECTIVES

Current treatments for human SCI focus on limiting secondary damage and minimizing lesion spread (Dumont *et al.*, 2001). Clinical trials have demonstrated a beneficial effect of these treatments on acute SCI; however approaches to promote long distance regeneration may lead to a more complete recovery. Several of the approaches discussed in this review promote axon regeneration in animal models of SCI and suggest avenues for the development of therapeutics. As is evident for several interventions, combining treatments may enhance the positive outcome. A combinatorial approach of tissue transplantation, NT application, and application of an antagonist to multiple inhibitors may offer the most effective strategy. Some combinations have been tested such as treatment with NT-expressing Schwann cells (Xu *et al.*, 1995), or NTs together with CSPG inhibitors (Tropea *et al.*, 2003). Yet the reductionist approach remains a necessary exercise to explore the strengths and weaknesses of each intervention.

As our knowledge of the cellular response to injury increases, a number of new challenges are also being presented. A viable method of delivery for any therapeutic approach must be established. For the application of soluble molecules such as NTs or small molecule antagonists, non-invasive microspheres composed of natural or synthetic biodegradable polymers are being surgically implanted into the adult mammalian brain to explore their utility as a delivery system (Friedman *et al.*, 2002). Ex-vivo gene therapy could accomplish the same objective and can be accomplished by harvesting cells, genetically modifying these cells, and then transplanting them back into the patient (Ruitenberg *et al.*, 2003). Furthermore, while many types of CNS neurons are capable of regenerating into peripheral nerve grafts (Benfey and Aguayo, 1982; So and Aguayo, 1985; Vidal-Sanz *et al.*, 1987), more recent evidence suggests that in certain regions of the CNS regenerative capacity may be restricted to neuronal subpopulations (Goldberg *et al.*, 2002). However, it is also true that regeneration of a subset of neurons may be sufficient to restore a disproportionate amount of function (Becker *et al.*, 2003). While both anatomical and behavioral recovery can

be assessed in animal models of SCI, it is unclear, how this will translate into behavioral recovery in humans. In spite of these challenges, the advances in our understanding of the cellular response to injury and the environmental influences on the severed axon raise exciting possibilities in our efforts to develop a therapeutic treatment for brain and spinal cord injury.

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ABBREVIATIONS

BBB	=	Basso, Beattie, and Bresnahan
BDNF	=	Brain Derived Neurotrophic Factor
CALI	=	Chromophore-assisted laser inactivation
cAMP	=	Cyclic adenosine monophosphate
CAP-23	=	Cortical cytoskeleton-associated protein-23
CNS	=	Central nervous system
CNTF	=	Ciliary neurotrophic factor
CSPG	=	Chondroitin sulfate proteoglycan
CST	=	Corticospinal Tract
DRG	=	Dorsal root ganglion
E15	=	Embryonic day 15
EAE	=	Experimental allergic encephalomyelitis
GAP	=	Growth associated protein
GDNF	=	Glial cell line derived neurotrophic factor
GPI	=	Glycosyl phosphatidyl inositol
GTPase	=	Guanosine triphosphatase
HUCB	=	Human umbilical cord blood
ICAM	=	Intercellular adhesion molecule
IFA	=	Incomplete freund's adjuvant
MAG	=	Myelin associated glycoprotein
MBP	=	Myelin basic protein
MP	=	Methylprednisolone
NEP1-40	=	Nogo-66 (1 40) antagonist peptide
NGF	=	Nerve growth factor
NgR	=	Nogo-66 Receptor
NSC	=	Neural stem cell
NT	=	Neurotrophin
OB	=	Olfactory bulb
OEC	=	Olfactory ensheathing cell
OMgp	=	Oligodendrocyte myelin glycoprotein
p75 ^{NTR}	=	p75 neurotrophin receptor
PKA	=	Protein kinase A

PNS	= Peripheral nervous system
RGC	= Retinal ganglion cell
RhoGDI	= Rho guanine nucleotide dissociation factor
ROK	= Rho-associated kinase
SCI	= Spinal cord injury
TGF	= Transformation growth factor
TNF	= Tumor necrosis factor

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