

## CHAPTER

## 32

# Axonal Growth in the Adult Mammalian Nervous System: Regeneration and Compensatory Plasticity

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## INTRODUCTION

Regeneration and compensatory plasticity are two important structural processes in which the brain and spinal cord regain function after damage (Fig. 32-1). In regeneration, the

cut axon begins to regrow from the damaged end and elongates either through or around damaged tissue to eventually reconnect with de-afferented targets. The second process, termed compensatory plasticity, involves the growth of new axonal connections from the *undamaged* spared neurons to

de-afferented targets, and this source of new axonal growth can be quite distant from the original injury site. Although conceptually different, these two types of recovery mechanisms most likely share a similar molecular basis for promoting axonal regrowth.

## REGENERATION IN THE PERIPHERAL NERVOUS SYSTEM

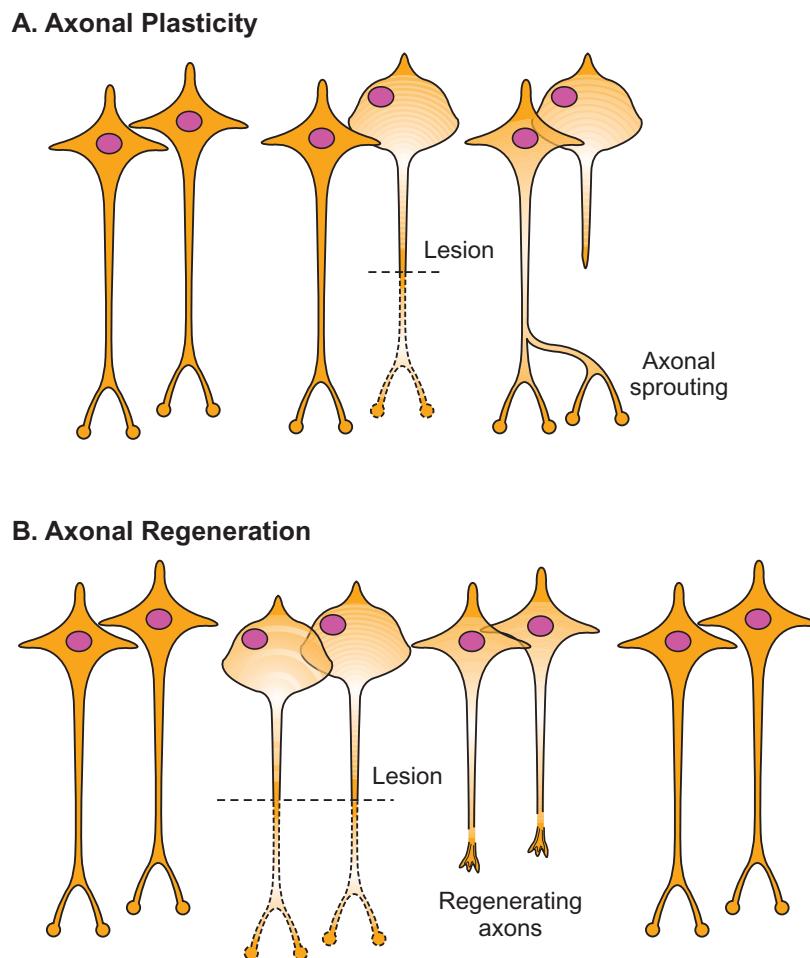
Peripheral nerve injuries are caused by a variety of factors including acute trauma, chronic repetitive insults, and inheritable or acquired metabolic disorders. As opposed to the CNS, which has traditionally been thought to be fixed, axonal injury in the peripheral nervous system (PNS) often results in some degree of spontaneous regeneration, although not always completely successful in terms of targeting or full functional recovery.

**Wallerian degeneration is the secondary disruption of the myelin sheath and axon distal to the injury**

This process was first described in 1850 in frog peripheral nerves by the British physiologist Dr. Augustus Waller, and was subsequently termed Wallerian degeneration. When either central or peripheral axons are damaged, Wallerian degeneration occurs, but with important differences between these two systems. These differences may underlie at least in part their different regenerative potentials (Table 32-1).

**The molecular and cellular events during Wallerian degeneration in the PNS transform the damaged nerve into an environment that supports regeneration**

First, the cut axon degenerates distal to the injury site, and within 18–48 hours, cytoskeletal disintegration of axonal



**FIGURE 32-1** Simplified drawing depicting the difference between axonal plasticity and axonal regeneration. In axonal plasticity (A), following axonal injury the cut axon does not regrow, but other undamaged neurons grow new axons to reinnervate denervated targets. In axonal regeneration (B), cut axons regrow from the damaged sites and reconnect with denervated targets.

neurofilaments and microtubules occurs due to proteases such as calpains that are stimulated by an increase in intra-axonal concentration of calcium ion (Fawcett et al., 2001) (Fig. 32-2). The Schwann cell processes surrounding the axon retract their myelin, but do not die, and are supported by autocrine trophic factors. Axotomy induces the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-1-alpha (IL-1 $\alpha$ ) in Schwann cells almost immediately. These cytokines play a critical role in macrophage recruitment to the injury site through endothelial cell activation and chemokine production (Shamash et al., 2002). After one to two weeks, fairly rapidly as compared to the CNS, degenerating axonal and myelin debris is removed, primarily

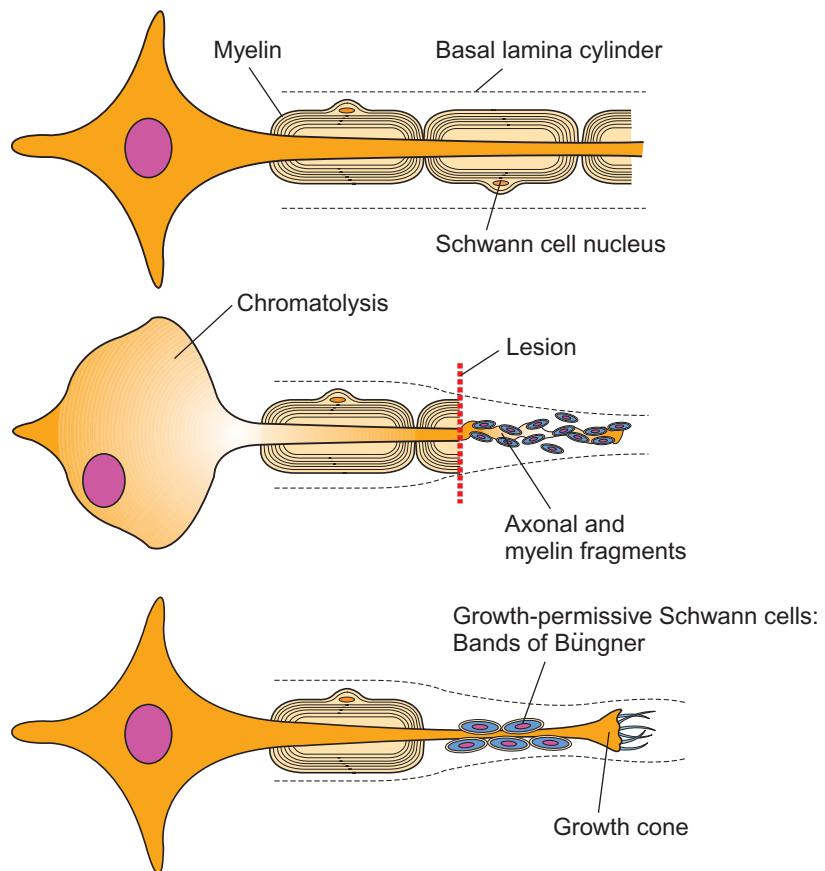
by macrophages migrating in from the bloodstream, and partly by endogenous endoneurial macrophages and Schwann cells. Invading macrophages also serve a key role by secreting a variety of cytokines such as interleukin-1 (IL-1) and platelet-derived growth factor (PDGF) that stimulate Schwann cells to divide, de-differentiate and proliferate distal to the injury. These stimulated Schwann cells are induced to secrete growth factors such as nerve growth factor (NGF), insulin-like growth factor-1 (IGF-1) and ciliary neurotrophic factor (CNTF), which increase the chance that neurons will survive the trauma of axotomy and stimulate axons to regenerate.

### Both Schwann cells and basal lamina are required for axonal regeneration to proceed

Each axon–Schwann cell unit in a peripheral nerve is surrounded by a basal lamina sheath of collagen, laminin and fibronectin. Following injury, this entire basal lamina/Schwann cell cylinder is known as the Band of Büngner, and this unit forms pathways to guide regenerating nerves back to their appropriate target structures. In a localized crush injury, the basal lamina sheaths surrounding the nerves are usually left intact, and therefore axonal regrowth and functional recovery are much better than in a transection injury, where the lamina sheath is cut and guidance mechanisms therefore are lost.

**TABLE 32-1** Major Differences in Wallerian Degeneration

PNS	CNS
Schwann cell survival and differentiation	Apoptotic death of oligodendrocytes
Rapid phagocytosis of injured axons and myelin sheaths by invading macrophages	Slow removal of injured axons, myelin sheaths and persistence of some myelin inhibitory factors
Formation of new myelin sheaths	No new myelin reformation



**FIGURE 32-2** Wallerian degeneration in the PNS. After an axon is injured, resulting chromatolysis—i.e., stress reaction and increased protein synthesis—occurs in the neuronal cell body, with axonal and myelin degeneration distal to the injury. Growth-permissive Schwann cells secrete growth factors that stimulate axons to regenerate.

## Cell surface adhesion molecules, which promote regeneration, are expressed on plasmalemma of both Schwann cells and regenerating peripheral axons

Adhesion molecules such as L1, neural cell adhesion molecule (N-CAM) and N-cadherin promote axonal regeneration by homophilic interactions between axons and Schwann cell surfaces (see Ch. 9). The expression of p75 (low affinity NGF receptor; see Ch. 29) is also increased at the Schwann cell surface after injury. Extracellular matrix (ECM) molecules, such as tenascin and proteoglycans, increase the regenerative potential of damaged peripheral nerves by binding to integrins on the axonal surface.

## Structural and biochemical changes occur after axotomy

At the site of axonal damage, within approximately one hour the cut end of the axon seals off and a motile growth-cone-like structure is formed. Within one to two days, depending upon how far from the cell body the cut is, major changes in gene expression and protein production occur within the cell body and new proteins are transported to the growing axon tip. These include structural proteins such as tubulin alpha 1, pro-regenerative proteins such as Reg-2 (Livesey et al., 1997) and the growth-associated protein GAP-43. This phosphoprotein (also known as B-50, F1, pp46, 48K 4.5) is present on the inside of the growth-cone membrane, where it interacts with various kinases and plays an important role in communication between the growth cone and its microenvironment (Benowitz & Routtenberg, 1997; Meiri et al., 1998). Following axotomy, massive upregulation of GAP-43 occurs in both sensory and motor neurons, and levels remain high until axons reconnect with their targets, at which time expression returns to baseline levels.

## REGENERATION IN THE CENTRAL NERVOUS SYSTEM

For regeneration in the mammalian CNS to occur, the injured neuron must first survive, and then its damaged axon must regrow, sometimes long distances through or around the injury site, eventually connecting with appropriate targets. As in the PNS, the axon is driven forward by the growth cone, a dynamic motile structure at the distal tip of regenerating axons, which contains the molecular machinery for growth and guidance to navigate through the intact and injured CNS. Once contact is made, the axon needs to be remyelinated and functional synapses formed on target cells.

After a CNS axon is injured, Wallerian degeneration distal to the cut end does occur, as in the PNS, but with important differences (Table 32-1). The degenerating myelin and axonal debris persist much longer, with less recruitment of macrophages from the peripheral blood to eliminate the debris; thus myelin inhibitory factors are present much longer than in the injured PNS. Microglia are the first glial cells to react, within a

few hours after injury. Astrocytes and meningeal cells are activated and collaborate in forming the glial scar, with inhibitory molecules such as chondroitin sulfate proteoglycans remaining for several weeks to months. Oligodendrocytes persist or die slowly.

As opposed to the PNS, where growth-associated molecules such as GAP-43 are uniformly upregulated by the lesioned neurons after axotomy, CNS neurons vary greatly in their responses to injury. The pattern of expression of GAP-43 in the CNS during development is ubiquitous, but after synaptogenesis is completed and critical periods for activity dependent plasticity are over, GAP-43 is downregulated in most adult CNS neurons. However, some neurons continue to express high levels of GAP-43, including the dopaminergic neurons of the substantia nigra, and neurons associated with adult plasticity such as hippocampal cells and cells within the olfactory bulb, where axons continue to grow into adulthood. After injury, dopaminergic neurons do not increase their expression of GAP-43, although most central neurons do have some transitory increased response, with greater upregulation of proteins, the closer the injury is to the neuronal cell body.

Despite extensive study, the role of Wallerian degeneration in nerve degeneration and regeneration is not completely understood. However, the discovery of a mutant mouse strain has the potential to add some insight into this important reparative process. The Wld<sup>s</sup> (Wallerian degenerate, slow) mouse is a spontaneously occurring mutant strain with an interesting phenotype of slow Wallerian degeneration and prolonged axonal survival after both CNS and PNS nerve injury (Perry et al., 1990). The Wld<sup>s</sup> mutation is made by the splicing of fragments of two genes within an 85 kb triplication on chromosome 4. This splice results in a new open reading frame and codes for a 42 kDa chimeric protein unique to the Wld<sup>s</sup> mouse, with an as-yet-unknown function. However, introduction of the Wld<sup>s</sup> gene into cultured rat DRG neurons made these previously susceptible neurons resistant to axonal degeneration in a model of toxic neuropathy. Further studies are needed to fully elucidate the potential therapeutic role of the Wld<sup>s</sup> gene in axonal survival and repair (Wang et al., 2001).

That the regenerative ability of adult mammalian neurons can be altered by their environment was first suggested by Ramon y Cajal, and later demonstrated in the now classic experiment by David and Aguayo (David & Aguayo, 1981) (Fig. 32-3). When sciatic nerve segments taken from rats were used as “bridges” between the medulla oblongata and spinal cord, damaged CNS axons at both of these levels grew into the peripheral nerve grafts for long distances, i.e., several centimeters. Importantly, the suggestion that the peripheral nerve *glial* environment was permissive to central axonal regrowth led the way for experiments investigating the differences between central oligodendrocytes and peripheral Schwann cells and their products, i.e. myelin.

## Central nervous system myelin contains molecules that inhibit neurite growth

One of the major obstacles to new neurite outgrowth in the adult CNS is the presence of specific neurite growth

inhibitory molecules, particularly those in CNS myelin. Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp) are proteins synthesized by oligodendrocytes and become enriched in the myelin membrane. Even though these proteins are structurally unrelated, they bind to a common neuronal receptor complex to inhibit neurite outgrowth (Schwab, 2004; Gonzenbach & Schwab, 2007; Walmsley & Mir, 2007). Other inhibitors present

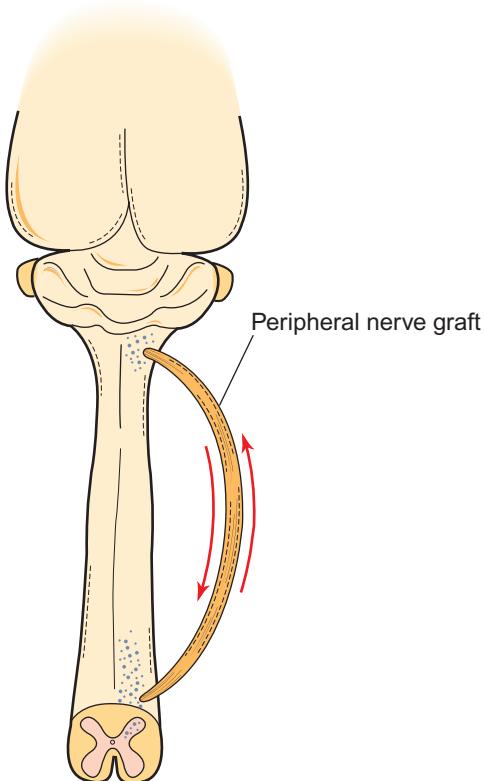
in myelin include the chondroitin sulfate proteoglycans (CSPG) versican V2 and brevican, and the regulators of axonal pathfinding ephrin B3 and semaphorin 4D.

### Nogo-A is a potent inhibitor of neurite growth and blocks axonal regeneration in the central nervous system

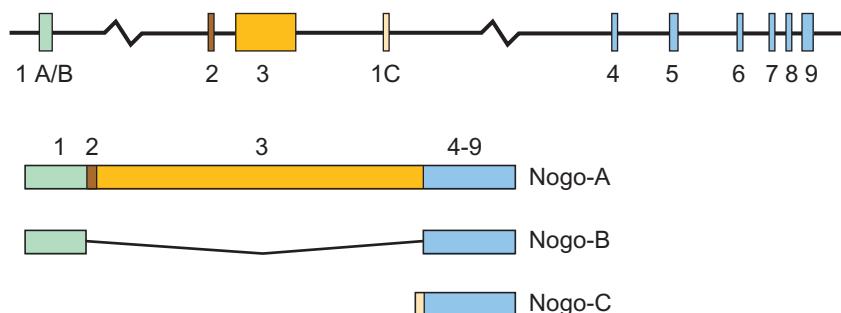
Early *in vitro* experiments showed that neurite outgrowth was impeded across a culture dish coated with CNS myelin, whereas neurites would actively grow on a dish coated with PNS myelin. Biochemical fractionation of rat brain myelin led to the identification of two membrane proteins of about 250 kDa and 35 kDa with strong inhibitory properties when added to growing neurites. Further purification of the corresponding bovine 220 kDa protein enabled sequencing of 6 peptides, which ultimately led to the cloning of the cDNA of a gene that was aptly named *Nogo* (Chen et al., 2000). So far, three distinct isoforms of Nogo have been identified, Nogo-A, B and C, with Nogo-A being the isoform most studied (Fig. 32-4).

Nogo-A is a membrane protein expressed predominantly in the adult mammalian CNS, primarily by oligodendrocytes (Huber et al., 2002). Neuronal expression is also seen in the developing nervous system, e.g., the dorsal root ganglia, sympathetic ganglia, motor neurons, hippocampal pyramidal cells and cerebellar Purkinje cells. Nogo-B is found in many tissues and cell types, including adult neurons and oligodendrocytes, and Nogo-C is most highly expressed outside of the CNS in skeletal muscle. While the functions of Nogo-B and Nogo-C are not entirely known, Nogo-B is found highly expressed in cultured endothelial and smooth muscle cells, as well as in intact blood vessels, and it may be a regulator of vascular homeostasis and remodeling (Acevedo et al., 2004).

Peptide fragment analysis of Nogo-A has shown that inhibition of neurite growth, collapse of growth cones and inhibition of fibroblast spreading are associated with two or three distinct regions of the molecule (Oertle et al., 2003). A principal inhibitory region, termed "Nogo-Δ20" or "Amino-Nogo", is found in the middle of the Nogo-A-specific sequence. Another region able to inhibit neurite growth and induce growth cone collapse is near the C-terminus and consists of a 66-amino-acid residue loop ("Nogo-66"), positioned between two hydrophobic sequences (Fournier et al., 2001) (Fig. 32-5).



**FIGURE 32-3** Diagram of the dorsal surface of the rat CNS, showing a peripheral nerve "bridge" linking the medulla oblongata and the thoracic spinal cord. As indicated by the arrows, damaged CNS axons from both sites of the nerve implantation grew into the graft and continued for long distances, demonstrating the growth potential of CNS neurons when given the microenvironment of the PNS (Modified from (David & Aguayo, 1981), with permission).



**FIGURE 32-4** Isoforms of Nogo. Nogo-A, -B, and -C have a common carboxy terminus of about 180 amino acids (the common domain (blue) contains Nogo-66). Nogo-A and -B share an amino terminus of about 172 amino acids (green).

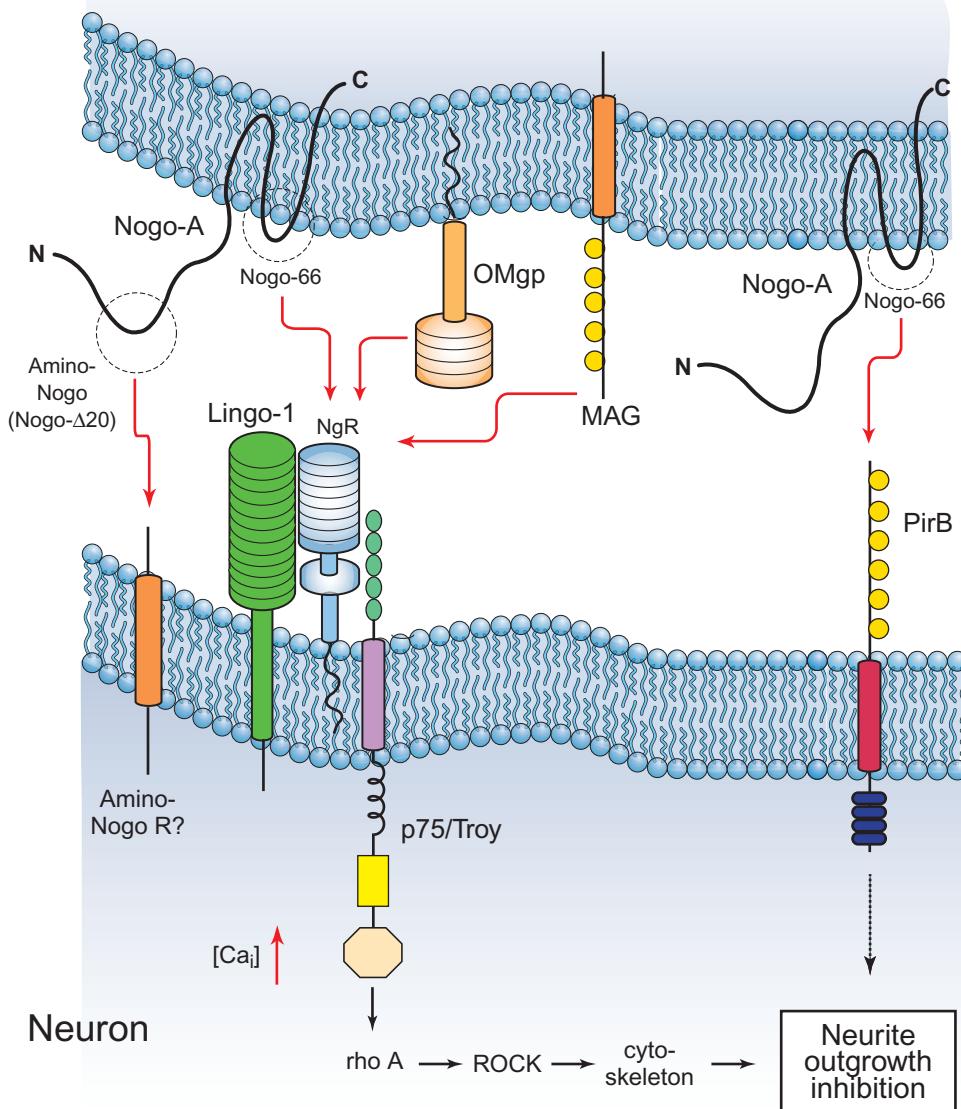
## Nogo gene is a member of the reticulon superfamily

Nogo-A comprises approximately 1,200 amino acids with two long hydrophobic stretches (transmembrane segments or loops) close to the C-terminus. Only about the last 180 amino acids show homologies to a previously known family of three genes, the reticulons (RTN). The RTN proteins are named after the main subcellular localization of RTN-1, which is in the endoplasmic reticulum. Nogo, i.e., RTN-4, not only

co-localizes with the endoplasmic reticulum but is also found on oligodendrocyte plasma membranes. The RTN family is evolutionarily very old and occurs in all eukaryotes including plants and fungi; however, except for Nogo-A, their functions are very poorly understood. The RTN family is distributed widely throughout the CNS and other tissues in animals (Oertle & Schwab, 2003).

Most of the known RTN proteins have a relatively short amino (N)-terminal sequence. The very long N-terminal sequence of Nogo-A, with its potent inhibitory regions, appears

## Oligodendrocyte/Myelin



**FIGURE 32-5** Nogo-A, MAG and OMgp are the principal inhibitors of neurite growth in CNS myelin. A portion of Nogo-A, i.e., Nogo-66, interacts with the same receptor, NgR, as do MAG and OMgp. NgR in the neuronal membrane complexes with p75 (or Troy) and Lingo-1 to activate Rho A and its downstream target, ROCK, and may result in neurite inhibition. Nogo-66 also interacts with the PirB receptor on the neuron to inhibit neurite growth by a mechanism not yet identified. Another major inhibitory area of Nogo-A, known as Amino-Nogo or Nogo $\Delta$ 20, interacts with a receptor that is not yet known. [Ca<sub>i</sub>] = intracellular calcium. (Modified from (Schwab, 2004) with permission).

late in vertebrate evolution, which suggests that Nogo-A is the result of a fusion between an ancient RTN homology domain at the C-terminus and two to three open reading frame sequences at the N-terminus, allowing the evolved protein to adopt a new function, i.e., that of a neurite growth inhibitor. The lack of Nogo-A in fish and salamanders would explain the high regeneration potential of the spinal cord after lesions in the latter animals. Whereas Nogo/RTN-4 has a known neurite-growth-inhibitory function, more generalized cellular functions relating to the endoplasmic reticulum are suggested for the other proteins in the RTN family as well as for Nogo-A, -B and -C, for example, roles in intracellular trafficking or apoptosis.

### Nogo-A function-blocking antibodies and peptides lead to axonal growth and functional recovery *in vivo*

The next step after cell culture experiments was to determine whether the blockade of Nogo-A function *in vivo* would lead to axonal regrowth after spinal cord injury. In one of the early experiments to address this question, Schnell and Schwab (Schnell & Schwab, 1990) used the anti-Nogo-A monoclonal antibody IN-1 raised against myelin fractions enriched for Nogo-A. The antibody was delivered to spinal cord-injured rats by implanting IN-1-producing hybridoma cells into the brain. Control animals received hybridoma cells secreting a nonspecific antibody. Treatment with IN-1 promoted axonal regeneration well beyond the site of injury, correlating, with functional recovery, whereas treatment with the nonspecific antibody resulted in little if any fiber growth. These exciting results have since been confirmed in rats and primates by using local intrathecal pump infusions of recombinant IN-1 Fab fragments or highly purified Nogo-A-specific monoclonal antibodies that recognize various domains in the middle of the Nogo-A protein.

In addition to antibodies designed to block function by binding to Nogo-A, other approaches have been used that target the Nogo receptor (NgR). NEP1-40 is a competitive antagonist of NgR composed of the first 40 amino acids of the C-terminus extracellular loop of Nogo (Nogo-66). NEP1-40 binds to the Nogo-66 receptor portion of NgR, thus blocking receptor function and allowing neurite growth to occur. Treatment of spinal cord-injured rats with NEP1-40 resulted in enhanced axon growth and improved recovery of function (GrandPré, et al., 2002). A more inclusive antagonist of NgR function, designed to block the action of several CNS myelin-derived NgR ligands, was developed by Li, et al., (2004). These investigators constructed a soluble ectodomain of NgR, which they termed “NgR(310)ecto-Fc” because it contained amino acids 27-310 of NgR fused to the rat IgG1 Fc domain. As with NEP1-40, administration of NgR(310)ecto-Fc to spinal cord-injured rats improved axon growth and recovery of function.

### Lines of knockout mice null for the Nogo genes have been developed

Initial attempts to study the role of Nogo in neurite growth and regeneration *in vivo* using knockout mice yielded conflicting results (Simonen, et al., 2003; Kim, et al., 2003; Zheng, et al.,

2003). Different lines were developed in separate laboratories in which the expression of either Nogo-A, Nogo-A/B or Nogo-A/B/C was deficient. As is often done, two different strains of hybrid mice were used that differed greatly in various relevant respects, such as neuroinflammatory response, cell death at lesion sites, scarring response and overall behavior. After spinal cord lesion, the Nogo-A-specific knockout mice showed a moderate but clearly detectable increase in regenerative sprouting and elongation (Simonen, et al., 2003). The same phenotype was quantitatively enhanced in one of the Nogo-A/-B knockout lines (Kim, et al., 2003). Because Nogo-B was greatly upregulated in the Nogo-A knockout, the Nogo-66 site seemed to compensate partially for the absent Nogo-A-specific active site. At odds with these observations were the results from a different laboratory, in which neither the Nogo-A/-B knockout lines nor the survivors from a single Nogo-A/-B/-C knockout mouse that escaped lethality showed major enhancement of sprouting or regeneration of the lesioned corticospinal tract (Zheng, et al., 2003). Further analysis of the Nogo-A-specific knockout after it was backcrossed into each of the original strains of mice (Dimou, et al., 2006) demonstrated that the dissimilar genetic backgrounds may account for the discrepancies in the extent of regeneration reported by the different studies.

### Additional myelin components have growth-inhibitory activity

In addition to Nogo-A, MAG and OMgp have been characterized as myelin-derived neurite growth inhibitors that transmit their inhibitory signals through the Nogo receptor (NgR, see below). MAG is a member of the immunoglobulin (Ig) superfamily and is a sialic acid-binding transmembrane glycoprotein. In the CNS, MAG is found in the periaxonal myelin membrane, and in the PNS, in the inner and outermost membranes of the myelin sheath (Filbin, 2003). MAG has long been known to have potent neurite inhibitory activity *in vitro*. There is evidence that immunization with myelin or recombinant Nogo-66/MAG promotes axonal regeneration after corticospinal tract lesions in adult mice (Sicotte, et al., 2003). However, MAG knockout mice did not show long-distance regeneration in the lesioned spinal cord (Bartsch, et al., 1995). OMgp, which is a GPI-linked protein expressed by oligodendrocytes (Wang, et al., 2002a) and a relatively minor component of myelin, is believed to be localized to the paranodal loops, next to the node of Ranvier. OMgp contains a leucine-rich repeat (LRR) domain and a C-terminal domain with serine/threonine repeats. Like MAG, OMgp is also found in the PNS. Like Nogo, OMgp is expressed in adult neurons; however, at the present time the function of OMgp found within neurons is not known.

Although *in vitro* studies generally have shown that MAG and OMgp each have neurite growth inhibitory activity, their function *in vivo* is controversial. Recently, two groups have studied the effects of single, double or triple deletions of the three myelin-derived neurite growth inhibitors, Nogo-A, MAG, and OMgp, on axon sprouting and regeneration of injured axons in the CNS (Lee, et al., 2010; Cafferty, et al., 2010). In one report (Lee, et al., 2010) only the Nogo-A deletion had improved axon sprouting in the corticospinal tract after injury,

with no effect on regeneration or behavioral recovery and no synergistic effect in the triple mutants. In contrast, Cafferty et al., (2010) reported that loss of Nogo-A resulted in greater axon growth and behavioral recovery after injury in the triple mutant than in wild type, while deletion of MAG or OMgp had no effect. Interestingly, in the latter study the triple mutant showed axon growth and behavioral recovery greater than in the Nogo-A deletion alone, implying some sort of synergistic action by MAG and OMgp. These types of discrepancies underscore the difficulty of interpretation of knockout studies and the need for complementary approaches to understand protein function.

### Inhibition of neurite growth is mediated through surface receptors and intracellular signaling molecules

To date, two receptors have been identified that bind to the myelin-associated neurite outgrowth inhibitors Nogo-66, MAG and OMgp. The first receptor to be characterized was an 85 kDa glycosylphosphatidylinositol (GPI)-linked, leucine-rich repeat glycoprotein that was designated NgR1 (Nogo receptor) (Fournier et al., 2001). This receptor is expressed on the surface of various neurons and is frequently referred to as the Nogo-66 receptor since it binds to a specific 66-amino-acid region in the C-terminal domain that is common to Nogo-A, -B and -C, but NgR1 also binds to MAG and OMgp. Because NgR1 is a GPI-linked protein, and therefore does not have an intracellular component, it was not surprising to find that NgR1 required at least one other protein to form a functional receptor complex. Originally, the low-affinity p75 nerve growth factor (NGF) receptor was identified as the transmembrane co-receptor capable of transducing the inhibitory signal across the cell membrane (Wang et al., 2002b). However, because some neurons lacked p75, it was later discovered that TROY (also known as TAJ), a member of the TNF receptor family, could function in place of p75 (Park et al., 2005; Shao et al., 2005) (Fig. 32-5). A third important component of the NgR1 complex is the transmembrane protein LINGO-1 (leucine-rich repeat and Ig domain-containing, Nogo-receptor-interacting protein), which interacts with both NgR1 and p75 to form a functional signaling complex (Mi et al., 2004). More recently, a second candidate receptor separate from NgR and capable of binding to Nogo-66 as well as MAG and OMgp was identified as PirB (paired immunoglobulin-like receptor B) (Atwal et al., 2008). The detailed mechanisms by which this receptor transduces inhibitory signals have yet to be established.

Several *in vitro* and *in vivo* studies indicate that there is an additional inhibitory domain within the Nogo-A-specific region that does not include Nogo-66 or require NgR for activity. This inhibitory region is referred to as Nogo- $\Delta$ 20 or Amino-Nogo and at the present time its receptor mechanism is unknown. The most compelling evidence for this inhibitory region is derived from work with purified monoclonal antibodies directed against the Nogo-A-specific N-terminal region. For example, subjects in which these antibodies were applied directly into the cerebrospinal fluid via intrathecal catheter after spinal cord injury showed a much greater number of regenerated corticospinal tract fibers and a significantly

better functional recovery than control animals (Liebscher et al., 2005). Similar regenerative effects were observed with Nogo-A-specific antibody treatment after injury of other CNS pathways (Gonzenbach & Schwab, 2007).

Two intracellular signaling components of neurite-growth inhibition have been identified so far, namely, calcium ion and the Rho-A/Rho kinase (ROCK) pathway (Fig. 32-5). The inhibitory effects on neurons mediated by the small GTPase RhoA are thought to be via intracellular regulation of cytoskeletal assembly. Importantly, inhibition of these intracellular pathways by appropriate blockers can prevent myelin- or Nogo-A-induced collapse of growth cones and inhibition of neurite growth *in vitro*, and these blockers can also induce corticospinal tract sprouting after spinal cord lesions *in vivo* (Fournier et al., 2003). Finally, several studies have shown that elevated levels of cAMP can cancel the repulsive and inhibitory effects of several inhibitors, including MAG and Nogo (Filbin, 2003). cAMP may act locally on growth cones, as well as on neuronal cell bodies, to mediate growth-enhancing effects.

A recent study by Joset et al., (2010) has provided *in vitro* evidence supporting an endocytic mechanism in which signaling endosomes containing Nogo-A-specific regions mediate growth cone collapse and inhibit neurite outgrowth. This pathway is capable of triggering the usual signaling molecules associated with Nogo-A inhibition of neurite outgrowth (see above), but requires a transcytotic uptake of Nogo-A containing membrane domains or proteolytically released Nogo-A fragments from the opposing myelin membrane.

### Neuronal expression of Nogo-A regulates neurite outgrowth

Although oligodendrocyte/myelin-associated Nogo-A has received the greatest attention with regard to regulation of neurite outgrowth, it is now becoming clear that Nogo-A expressed by neurons also plays an important role in modulating growth cone motility and the dynamics of neurite formation (Montani et al., 2009; Petrinovic et al., 2010). Neuronal expression of Nogo-A occurs primarily during development but also is evident in certain adult neurons and is upregulated after injury (Cheatwood et al., 2008). Neutralizing Nogo-A in cultured neurons with function-blocking antibodies or using neurons derived from neonatal Nogo-A knockout mice enhanced both the fasciculation and length of the neurites, suggesting a role for neuronal Nogo-A in adhesive and repulsive interaction (Petrinovic et al., 2010).

### Axon growth is inhibited by the glial scar

Another barrier to CNS regeneration is the glial scar, which consists mainly of reactive astrocytes and proteoglycans. As early as Ramon y Cajal (Cajal, 1928) glial scar tissue was implicated as an impediment to adult axonal regeneration after CNS injury. More recent studies since the early 1990s demonstrated that failure of axonal regeneration may be due to the non-permissiveness to neurite growth of ECM molecules, including the CSPGs and keratin sulfate proteoglycans in the scar (Silver & Miller, 2004). *In vitro*, the inhibitory nature of CSPGs has been attributed both to the core protein and to

associated chondroitin sulfate side chains (Ughrin et al., 2003). *In vivo* studies have shown that after CNS injuries, various CSGPs that are normally expressed on immature glial cells are re-expressed in the glial scar. These include NG2, neurocan, brevican, versican, aggrecan and phosphacan. Degradation of associated chondroitin sulfates after spinal cord injury partially enhanced axonal growth and functional recovery, indicating that reduction in selective scar components can increase regenerative growth in the adult CNS (Bradbury et al., 2002). Furthermore, CSGPs appear to have a role in regulating visual cortical synaptic plasticity. After CSPG degradation in adult rats, monocular deprivation caused an ocular dominance shift toward the non-deprived eye, indicating that the mature ECM is inhibitory for experience-dependent plasticity (Pizzorusso et al., 2002).

Astrocytes play a key role in scar formation, but the role of reactive astrocytes in CNS injury is not completely understood. Adult mutant mice with selective and conditionally targeted gene deletions have shown that reactive astrocytes also protected tissue and preserved function after spinal cord injury (Faulkner et al., 2004). This protection was attributed to the action of reactive astrocytes in blood-brain barrier repair and restriction of the inflammatory response. Presently, one is left with the conclusion that inflammation, including the role of astrocytes in CNS injury, is a complex and inadequately understood area warranting further study. In particular, a main problem is to overcome the inhibitory environment of the glial scar, without compromising the positive effects of glial cells.

### Neurotrophic factors promote both cell survival and axon growth after adult CNS injury *in vivo*

Neurotrophic factors comprise several complex families of molecules that support axonal regrowth and neuronal survival in the adult CNS (see detailed discussion in Ch. 29). These molecules include the traditional neurotrophins, the neurokine or CNTF-family, the glial-derived neurotrophic factor (GDNF) family, and factors like the bone morphogenic proteins (BMPs), the IGFs and fibroblast growth factors (FGFs), with each having multiple roles including neurotrophic functions. The trk family of receptor tyrosine kinases is the high-affinity signal-transducing receptor for the NGF family, with NGF the primary ligand for TrkA, BDNF and NT-4/5 for TrkB, and NT-3 for TrkC. NT-3 applied to spinal cord lesion sites enhanced regenerative sprouting (Schnell & Schwab, 1994; Grill et al., 1997). In experiments using neurotrophins to study regenerative properties of adult rubrospinal neurons, Tetzlaff and colleagues found that the application of BDNF or NT-4/5 (but not NGF or NT-3) prevented the atrophy of axotomized rubrospinal neurons, stimulated the expression of GAP-43 and  $\text{T}\alpha\text{l}$ -tubulin in these neurons, and promoted the regeneration of axotomized neurons into grafts of peripheral nerves implanted into spinal cord lesions (Kobayashi et al., 1997). Interestingly, the treatment was most effective when neurotrophic factors were applied in the vicinity of the cell bodies themselves and not at the spinal cord injury site, except for NT-3, which stimulated corticospinal tract regenerative sprouting if applied to the injury site

(Schnell & Schwab, 1994). Surprisingly, it was found that even when animals were treated up to one year after axotomy, local infusion of BDNF was still capable of rescuing these axotomized neurons and of stimulating them to regenerate into peripheral nerve implants (Kwon et al., 2002). These exciting results indicate that the regenerative capabilities of adult CNS neurons are not necessarily lost even after long-standing chronic injury.

Other ways to use neurotrophins in CNS repair paradigms are to combine them with growth-promoting cells or bridging materials, or to transplant them into lesion sites cells that are genetically engineered to produce specific neurotrophins. In one example, engineered Schwann cells that expressed BDNF were used to stimulate growth of supraspinal axons across the transected adult rat spinal cord. A polymer channel was placed in between the two stumps, and engineered Schwann cells expressing BDNF were seeded into the channel. Although BDNF did provide increased selection for TrkB-expressing axons, neurites did not leave the substrate of the guidance channel, once again demonstrating the importance of a permissive environment in order to achieve long-distance functional regeneration (Menei et al., 1998).

Despite the extensive use of neurotrophins to induce adult axonal regrowth and neuronal plasticity, results have been mixed, and many challenges remain. Neurotrophins have roles in the CNS that go beyond cell survival and neurite outgrowth, and include such diverse and basic activities as modulation of membrane excitability, regulation of cell differentiation and intracellular molecular trafficking. A better understanding of the role of neurotrophins in cellular regulation and control would augment their use as important therapeutic agents in the repair of CNS injury.

## CENTRAL NERVOUS SYSTEM INJURY AND COMPENSATORY PLASTICITY

### Neonatal brain damage results in compensatory plasticity

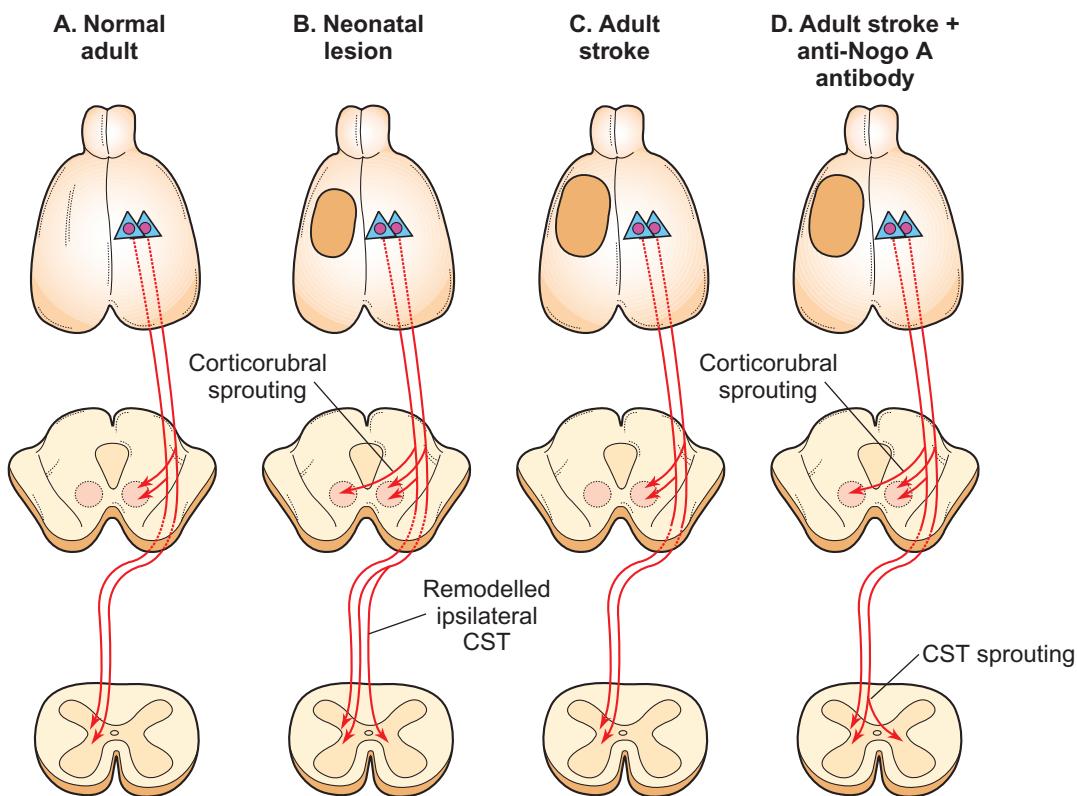
Although, without intervention, axonal growth in the injured adult CNS is limited, the immature CNS responds to injury with a remarkable rerouting of neuronal pathways from undamaged areas to re-innervate denervated areas. This is called *structural compensatory plasticity*. This lesion-induced plasticity following perinatal brain damage occurs in all systems studied, including the visual, auditory, sensorimotor and limbic systems. For example, following lesion to the motor cortex of two-day-old rat pups, the motor cortex from the opposite, unlesioned hemisphere reroutes cortico-efferent connections to bilaterally innervate subcortical areas including the striatum, thalamus, red nucleus, basilar pontine nuclei and spinal cord (Fig. 32-6) (Castro, 1990). This new pathway formation is thought to underlie the better functional outcome often seen after neonatal brain injury, as compared to the same injuries sustained in adulthood (Z'Graggen et al., 2000). Therefore, ways to enhance structural plasticity after adult CNS lesions would greatly improve functional recovery and thereby quality of life.

## Compensatory plasticity and functional recovery can be enhanced in the injured adult central nervous system through blockade of Nogo-A

Interestingly, some of the same molecules that restrict *regeneration* in the adult CNS, e.g., Nogo-A, are also responsible for shaping adult lesion-induced neuronal plasticity. In a series of experiments, adult rats were subjected to unilateral ischemic brain damage through middle cerebral artery occlusion and given antibodies to block Nogo-A. A dramatic recovery in skilled forelimb use was seen which correlated with new cortico-efferent plasticity from the unlesioned sensorimotor cortex to subcortical motor areas such as the red nucleus and the spinal cord (Fig. 32-6) (Papadopoulos et al., 2002; Wiessner et al., 2003). Furthermore, electrophysiological mapping studies of the opposite, unlesioned hemisphere showed a functional reorganization of the motor map, with new areas of the motor cortex now innervating the previously impaired forelimb (Emerick et al., 2003). The specificity of these new pathways indicates that developmental mechanisms of neuronal target recognition and synapse specification probably persist throughout life, thus assuring that under conditions of enhanced axonal plasticity and regeneration, functional networks can be formed (Bareyre et al., 2002; Bareyre et al., 2004).

These findings of enhanced axonal plasticity and functional recovery after stroke and anti-Nogo-A therapy have now been extended to the aged rat. Twenty-four-month-old rats were trained on a reaching task and given anti-Nogo-A antibody treatment via intracerebroventricular (ICV) pump one week after experimental stroke. These aged animals showed dramatic improvement in skilled forelimb function (Markus et al., 2005) and memory performance on the Morris water maze (Gillani et al., 2010), indicating that even the aged CNS retains the proper molecular cues to allow for neuronal plasticity and functional recovery, given the proper conditions. Another important aspect of these studies was that the time window for starting the treatment was extended to two months after stroke (Tsai et al., 2011), demonstrating that anti-Nogo-A therapy is effective even beyond the time of acute brain damage. These findings open the possibility of treatment options for those suffering from chronic brain injuries.

Even in the case of spinal cord injury where application of anti-Nogo antibodies results in regeneration of the cut axons, an additional important element for functional recovery is enhanced fiber growth from the unlesioned fibers, i.e., compensatory plasticity, as discussed above. After high corticospinal tract injury in the rat at the level of the medullary pyramid and treatment with anti-Nogo antibodies,



**FIGURE 32-6** Diagram depicting cortico-efferent projections from the rodent motor cortex to subcortical areas, including the red nucleus and the spinal cord. In the normal adult (A), cortical neurons project to the ipsilateral red nucleus and the contralateral spinal cord grey column. After a neonatal cortical lesion (B), cortico-efferent fibers spontaneously sprout to the contralateral denervated red nucleus and the ipsilateral, denervated spinal cord (gray). After an adult stroke lesion (C), very little spontaneous sprouting is seen. However, after adult stroke and treatment with anti-Nogo-A antibody (D), plasticity to the denervated red nucleus and spinal cord is again seen, correlating with functional recovery.

## ENHANCEMENT OF FIBER GROWTH AND REGENERATION IN THE INJURED SPINAL CORD

### *Antibody-Mediated Neutralization of the Neurite Growth-Inhibitory Myelin Protein Nogo-A as a Novel Therapy for CNS Injuries*

Martin E. Schwab

A few seconds of inattention on the road or in sports and you can end up wheelchair bound for the rest of your life. Since the early days of neurology, it has been recognized that permanent functional deficits produced by large spinal cord and brain lesions are linked to the absence of regeneration of injured fiber tracts in the CNS. In the late 1980s the presence of specific neurite growth inhibitory factors in the adult CNS, particularly in CNS white matter, was recognized. Ablation of myelin, antisera against CNS myelin, or use of specific function-blocking antibodies against one of the most potent neurite growth inhibitory constituents of CNS myelin, Nogo-A, rapidly showed that regeneration of lesioned fiber tracts in injured rat or chicken spinal cord occurred under these conditions over long distances (Schwab, 2004). The initial anatomical experiments were complemented by the demonstration of functional recovery, as shown in a variety of locomotor and skilled movement tests. Importantly, the results with function-blocking antibodies against the neurite growth inhibitor Nogo-A were confirmed by experiments that utilized other biochemical approaches to block the Nogo–Nogo receptor signaling pathway, including peptides blocking the Nogo receptor (NgR1), truncated receptor fragments that block the NgR1 ligands, and pharmacological blockers of the downstream signaling pathway involving Rho and ROCK (Schwab, 2004). In contrast to these acute interventions, which all produced a similar enhancement of regeneration, compensatory fiber sprouting, and functional recovery, Nogo-A knockout mice had weaker and more variable outcomes, probable due to functional compensation by other inhibitory factors (Dimou et al., 2006). As an important proof of concept step towards the human situation, macaque monkeys with defined cervical spinal cord lesions producing a unilateral hand paralysis were shown to regain almost full hand and finger dexterity along with regenerative fiber growth of the transected corticospinal tract following anti-Nogo-A antibody treatment.

In collaboration with an industrial partner, we produced a human IgG-antibody with high binding affinity and function-blocking properties against human Nogo-A. Efficacy for

regeneration was shown in macaques, and the absence of neurological as well as general side effects was tested extensively in the required two distant species, i.e., rodents and primates. Due to the impermeability of the blood–brain barrier to antibodies, the application route has to be directly into the CNS compartment. Lumbar intrathecal pumps, such as are in routine clinical use (e.g., for the application of the antispastic drug baclofen) and injections into the lumbar subarachnoid space were used. As this was the first antibody applied directly into the CNS compartment, a very careful in-human dosage and toxicology phase, Phase I of the clinical trial, was required. The trial is currently (2010) being conducted by Novartis in a network of leading spinal cord injury centers in Europe and North America. To show the efficacy of this growth- and regeneration-enhancing antibody, the availability of standardized, sensitive, functionally meaningful read-outs for the recovery of lost functions is crucial. Major efforts are devoted to the improvement of currently available assessment scales for locomotion, hand use, autonomic functions, spasticity, pain and daily life activities (Alexander et al., 2009).

A clinical demonstration of enhanced functional recovery after spinal cord or brain trauma by a novel therapeutic approach based on an understanding of the underlying molecular and cell biological mechanisms would be extremely encouraging. However, the complexity of CNS injuries will probably require combined treatments at least for the repair of the most extensive lesions. Stimulation of the neuronal growth program, minimizing the barrier function of scars at lesions sites, combined suppression of several growth and inhibitory factors, and, ideally, bridging of large lesions by implants or cell grafts are approaches that are currently tested in animal models. Before they can be applied in combination to patients with spinal cord or brain injuries, however, safety and efficacy of each individual treatment need to be shown. Although the way to these novel therapies for CNS lesions seems still to be long, the door is wide open now, and research is pursued with great effort in many basic science and clinical laboratories worldwide.

## SUMMARY

The adult mammalian brain and spinal cord have limited capacity for spontaneous functional and structural recovery after injury. However, over the past 25 years a virtual explosion of scientific evidence has shown us that damaged adult neurons are indeed capable of regrowth given the proper conditions, and a number of strategies in which adult central nervous system (CNS) neurons can be influenced to regrow following injury have been found. These strategies include suppression of action of growth inhibitory molecules, which are

rubrospinal pathways were shown to sprout into deafferented areas of the spinal cord, resulting in high levels of functional recovery, i.e., a “functional switch” in the remodeled pathway (Raineteau & Schwab, 2001). Furthermore, various studies suggest that neutralizing Nogo antibodies can induce a transient growth response in the intact adult CNS (Bareyre et al., 2002; Papadopoulos et al., 2006). Interestingly, while Purkinje cells of the cerebellum do not upregulate GAP-43 at all after axotomy, increased expression of GAP-43 as well as regeneration are seen after application of anti-Nogo antibodies to the intact cerebellum (Buffo et al., 2000).

primarily found in myelin; reduction of scar barriers; enhancement of the neuronal response to axotomy through supplementation with growth factors; and enhancement of the growth potential of the injured neuron. These investigations, discussed in this chapter, lay the foundation for understanding CNS repair mechanisms and provide a direction towards developing effective therapies for spinal cord injuries and other CNS injuries that previously were thought to be hopeless.

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