TARGETING THE NOGO RECEPTOR TO TREAT CENTRAL NERVOUS SYSTEM INJURIES

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Axonal damage is a key pathology in many injuries of the central nervous system (CNS), such as spinal cord injury, traumatic brain injury and stroke, as well as in multiple sclerosis. An attractive drug discovery strategy to treat such conditions is to search for agents that promote CNS axonal regeneration. Historically, limited knowledge concerning the basis of poor CNS regeneration has precluded a rational drug discovery approach for promoting axonal regeneration. The recent identification of the Nogo receptor, which interacts with inhibitory myelin protein, established the crucial role of this molecular pathway in mediating the inhibitory effects of CNS myelin. This provides an unprecedented opportunity to manipulate adult CNS axonal regeneration. The development of therapeutics targeting the Nogo receptor has the potential to promote functional recovery and reverse the devastating consequences of CNS injuries.

MYELIN SHEATH
The sheets of membranes derived from oligodendrocytes wrapping around nerve fibres in the central nervous system that provide trophic support and facilitate nerve impulse transmission. Detergent extraction of these membranes yields a protein mixture named myelin that inhibits neurite outgrowth and induces growth cone collapse.

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Each year in the United States, ~10,000 people become handicapped as a result of spinal cord injury[1] and a further 250,000 from traumatic brain injury[2], and out of the 11 million people that experienced a stroke in 1998, about 770,000 individuals became symptomatic[3]. Although new treatment regimes, primarily based on physical therapy, have slightly improved the recovery of these patients, there are no drugs available to repair the damage to the spinal cord that results from mechanical injury, and the brain tissue damage that results from mechanical injury or ischaemia. So, there is a huge unmet medical need for treating CNS injuries such as spinal cord injury, traumatic head injury and stroke. In multiple sclerosis, axonal transection might be an important pathology contributing to disability[4], and axonal repair might therefore represent an important therapeutic strategy for this disease. One of the obvious physiological hurdles for promoting axon regrowth has been the inability of CNS neurons to regenerate axons after injury, which is generally attributed to the presence of growth inhibitors present in CNS MYELIN SHEATH[5] (FIG. 1). After decades of research, the molecular identities of several of these myelin inhibitors have been discovered. These myelin inhibitors include the IN-1 antigen[6], NogoA (also known as reticulon 4)[7–9], myelin-associated glycoprotein (MAG)[10,11] and oligodendrocyte myelin glycoprotein (OMgp)[12–14]. These myelin proteins induce GROWTH CONE COLLAPSE and inhibit neurite outgrowth, as expected for inhibitory components of CNS myelin.

This myelin inhibitor biology leads to the hypothesis that modulation of the interactions of these myelin proteins with their axonal receptor(s) would overcome the inhibitory effects of CNS myelin and promote axon regeneration, leading to improved functional recovery after CNS injury. Indeed, antagonists of these myelin proteins have been developed and tested for their ability to promote CNS axonal regeneration in animal models. The versatile anti-NogoA antibody IN-1 (REF. 15) was generated by Schwab *et al.* using the rat CNS myelin NI250 protein, a fragment of NogoA, as the immunogen. Grafting of IN-1 hybridomas[16,17], or direct administration of the IN-1 Fab fragment[18] or humanized IN-1 antibody[19], improved functional recovery as measured by BBB SCORING[20] in rats that had undergone spinal cord transection. Another NogoA-based reagent of interest has recently been discovered; out of the 1192 amino-acid

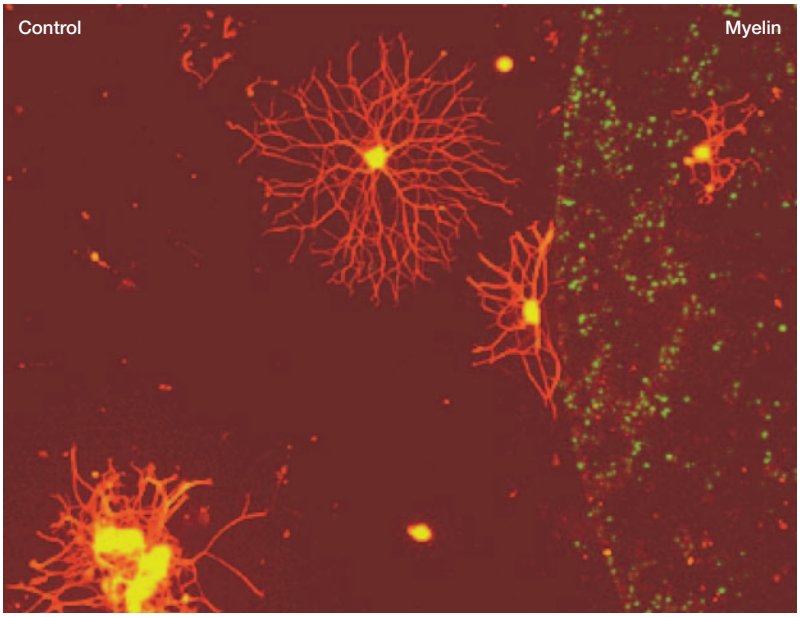

Figure 1 | **CNS myelin inhibits neurite extension *in vitro*.** Neurons do not regenerate axons or extend neurites readily in the central nervous system (CNS) because of the presence of growth inhibitors in the myelin environment. Under favourable culture conditions, neonatal rat dorsal root ganglion neurons extend neurites, as shown by the neuron stained with anti-β-III-tubulin antibody (red) on the left. In the same culture well, neurons cultured on CNS myelin (marked by the green dots) exhibit impaired neurite outgrowth and distorted neurite morphology, as shown by the neuron on the right. The inhibitory effects of CNS myelin on neurite outgrowth is exemplified by a neuron located on the border (centre neuron) that extends neurites on the control side but not on the CNS myelin side. Image courtesy of Adrienna Jirk and Sylvia Rabacchi, Biogen Inc., Cambridge, Massachusetts, USA.

residues in NogoA, a 66-amino-acid peptide that underlies the inhibitory activity of NogoA on neurite outgrowth and growth cone collapse was identified and termed Nogo-66 (REF. 21). Further molecular analyses led to a 40-residue peptide (NEP1-40) that behaves as an antagonist of NogoA[22], attenuating the effects of myelin or Nogo-66 on growth cone collapse and neurite outgrowth, and improving outcome *in vivo* following spinal cord injury[22]. At least two other NogoA-specific domains, separate from Nogo-66, seem to be inhibitory for many cell types through a different mechanism[21,23,24]. These include an amino-terminal domain that inhibits fibroblast spreading, and a domain encoded by the NogoA-specific exon that restricts neurite outgrowth and cell spreading, and induces growth cone collapse[24]. It is possible that NogoA might interact with more than one receptor on different cell types to mediate different cellular events.

Although these inhibitory proteins in CNS myelin had been demonstrated to play a role in limiting anatomical and behavioural recovery following CNS injury, the identification of the receptor for these ligands remained a crucial missing link in the molecular pathway. In 2001, this link — the Nogo receptor (NgR, now also termed NgR1; see REF. 25) — was identified[21]. In this review, we discuss recent advances in the understanding of the biological role of the NgR, and the relevance of these findings to a novel drug discovery strategy of promoting CNS axonal regrowth for treating CNS injuries.

GROWTH CONE COLLAPSE
The tips of the growing axons and neurites are characterized by a fan-shaped structure (laminopodia) accompanied by several outgrowths (filapodia). Together, these structures form the growth cone of an axon or neurite. When a growing axon comes into contact with an unfavourable environment, the growth cone changes in morphology, shrinking to form a stump. This phenomenon is generally described as growth cone collapse, and is indicative of halted neurite growth.

BBB SCORE
A 21-point scale of behavioural scoring for assessment of open-field locomotion abilities.

LEUCINE-RICH REPEATS
Small protein domains comprising ~23 amino-acid residues that are characterized by the dominant presence of leucine residues. Examples include NgR, NgRh1, NgRh2 and OMgp.

NgR and related proteins: structure and function

The Nogo receptor, NgR, was identified using an expression cloning strategy[21]. Not only does NgR bind with high affinity to Nogo-66, but it also exhibits an expression pattern consistent with a role in CNS axonal regeneration. NgR expression is largely confined to neurons of the adult nervous system, and the protein is found on axons[21,26]. By contrast, early embryonic neurons do not express NgR and are insensitive to Nogo-66, but become sensitive to Nogo-66 after transfection with NgR[21]. So, NgR is thought to be the primary mediator of Nogo-66 action, although additional receptor molecules might interact with the other amino NogoA domains. Subsequent studies demonstrated that NgR is also a functional receptor for two other known myelin-derived axonal outgrowth inhibitors, MAG and OMgp[27–29]. Therefore, NgR is a focal point for the convergence of three myelin inhibitors[30].

The primary translation product of NgR is a protein comprising 473 amino acids that contains a signal sequence, a 420-amino-acid ectodomain, and a glycosyl phosphatidylinositol (GPI) anchorage site at the carboxyl terminus[21]. As predicted for a GPI-anchored glycoprotein, NgR can be released from the plasma membrane by phosphatidylinositol-specific phospholipase C treatment. The mature human protein comprises two domains: the amino-terminal domain, which makes up two-thirds of the protein (amino acids 27–310), contains a LEUCINE-RICH REPEAT (LRR) structure, and the carboxy-terminal domain (amino acids 311–454) does not have known sequence-related relatives. All known myelin-derived ligands for NgR bind exclusively to the LRR domains of the protein[27,29,31,32]. The carboxy-terminal unique domain (amino acids 311–454) of NgR is heavily glycosylated and is essential for signal transduction[31].

The crystal structure of the LRR domain has been determined and comprises eight typical LRR segments flanked by cysteine-rich LRR amino-terminal (LRRNT) and LRR carboxy-terminal (LRRCT) segments[25,33]. These LRRs assume a structure similar to that observed in other crystallized LRR proteins. β-sheet segments containing the leucine residues that define the LRRs are arranged in a parallel array, creating the concave surface of a banana-shaped structure. The NgR LRR domain is most closely related to that of platelet glycoprotein-1b-α (GP1b-α). However, the LRRNT and LRRCT loops that mediate ligand binding in GP1b-α are absent from NgR, so this provides no clue as to which segments of NgR mediate binding of different NgR ligands[25,33]. Co-crystallization and mutagenesis studies will be required to define the ligand-binding regions of NgR with certainty. It is also clear that NgR has some affinity for itself and might create higher-order aggregates[27,31]. The molecular nature of the NgR–NgR interaction and its regulation by ligand is not fully defined in the two crystal structures[25,33], but might constitute another site for the development of NgR antagonists when more fully understood.

Although there are more than 250 LRR domain proteins in the sequence databanks with 20–30% amino-acid identity in the LRR domains, there are two proteins most closely related to NgR[25,33,34]. These proteins have

been termed NgR2 (also known as NgRh1) and NgR3 (also known as NgRh2). Sequence conservation in the eight LRR domains of each protein is close to 50%, and all three proteins seem to be GPI anchored. NgR2 and NgR3 are both expressed in adult mouse brain, and NgR2 is found in some peripheral tissues as well[34]. Despite the similarity in sequence, NgR2 and NgR3 have little or no affinity for NgR ligands[25]. Although the function of NgR2 and NgR3 might therefore be unrelated to NgR function, a comparison of the sequences could provide important clues as to the residues essential for myelin ligand binding.

NgR as a drug discovery target

Therapeutic candidates for targeting NgR include protein antagonists and small-molecule antagonists. Small molecules have obvious advantages for delivery, particularly for a drug that needs to access the CNS. Although it might be tempting to develop high-throughput screening assays to isolate a small-molecule antagonist of NgR that blocks the binding of all three myelin proteins, several issues need to be considered. First, there is a lack of compelling evidence establishing the existence of a single molecular binding epitope on NgR for all three myelin proteins, except that Nogo-66 was shown to compete with MAG for binding to cells expressing NgR[28]. Second, there is no evidence so far for the presence of an allosteric site that regulates NgR. Third, the molecular contact of NgR with protein ligands might be too widely distributed across NgR to successfully block with a single small molecule. To date, the identification of a small-molecule NgR surface antagonist that blocks all three myelin proteins (NogoA, MAG and OMgp) has not been reported. Similarly, few neutralizing anti-NgR antibodies are available[35–37]. It would be important to characterize the abilities of these anti-NgR antibodies to block either one or all of the three myelin proteins, and to compare the relative efficacies of the different anti-NgRs *in vivo* to help prioritize a candidate antibody molecule.

A soluble version of NgR could be one solution to effectively inhibit the action of all three myelin proteins interacting with NgR. Indeed, Liu *et al.*[27] and Fournier *et al.*[31] generated recombinant soluble NgR protein composed of amino-acid residues 27–310 (NgREcto) of rat NgR. This NgREcto protein reduced Nogo–alkaline phosphatase binding to NgR-transfected cells[31] and partially reversed the inhibition of embryonic chick DORSAL ROOT GANGLIA (DRG) neurite outgrowth by Nogo or CNS myelin. Similarly, this monovalent soluble NgREcto and a soluble NgR fused to alkaline phosphatase[28] reversed the MAG-induced inhibition of neurite outgrowth in cultured embryonic chick DRG[27], neonatal rat DRG and rat cerebellar granule cells[28]. Because OMgp also binds NgR[29], it is likely that soluble NgR comprising LRR and LRRCT domains would bind OMgp, thereby inhibiting the interaction of OMgp with NgR. At least in our laboratories, a recombinant rat soluble NgREcto protein (27–310) fused to Fc protein effectively blocked Nogo-66, MAG and OMgp binding to NgR[38] (unpublished data, D. H. S. Lee and S. M. Strittmatter). Taken together, these studies indicate that the soluble NgR protein might be an effective inhibitor of all three myelin proteins, Nogo, MAG and OMgp.

DORSAL ROOT GANGLION
DRG. Groups of sensory neuron cell bodies that correspond to a particular level of the spinal cord. These neurons are frequently used in culture assays to assess neurite outgrowth or growth cone collapse.

Targeting NgR signalling mechanisms

The NgR contains no cytoplasmic domain, so it is not immediately apparent how the protein might signal to the cell interior to limit axonal regeneration[21,30]. On the one hand, the GPI linkage localizes NgR to lipid raft microdomains, and ligand binding might be postulated to alter the signalling of cytoplasmic proteins associated with rafts. However, raft localization does not seem to be essential for NgR signalling[31]. Therefore, a preferred hypothesis is that NgR associates with a transmembrane co-receptor that might in turn transmit a signal from a myelin-derived inhibitor to the axonal cytoplasm[21,30,31]. However, unbiased expression cloning searches for NgR-interacting proteins have yielded only NgR itself and NgR ligands[27].

The low-affinity neurotrophin receptor p75-NTR has been proposed to function as a co-receptor for NgR[32,39]. This hypothesis evolved from observations that the cytoplasmic domain of p75 interacts with RHO[40], and that RHO mediates CNS myelin inhibition of axon growth (see below). The p75–RHO interaction was initially recognized in a yeast two-hybrid screen and later described as occurring indirectly via the RHO–guanine dissociation inhibitor (GDI)[40,41]. Combining the two observations, Yamashita and colleagues considered whether p75 was required for MAG signalling. Neurons from $p75^{-/-}$ mice were unresponsive to MAG, indicating that p75-NTR might mediate MAG signalling, perhaps via gangliosides serving as MAG receptors that in turn linked p75-NTR to RHO[42]. He, Poo and colleagues investigated whether NgR and NgR–ligand complexes might interact with and signal via p75-NTR[32,39]. Co-immunoprecipitation of NgR and p75-NTR, as well as p75-NTR dominant-negative expression studies, supported the conclusion that p75-NTR is an NgR co-receptor mediating signal transduction.

To the extent that p75-NTR mediates myelin-dependent restriction of adult CNS axon regeneration, it is a potential target for the development of regeneration therapeutics, such as p75 antagonists, that block the NgR interaction and/or signalling function. However, several issues remain to be investigated regarding this hypothesis. First, it is still unclear whether p75-NTR is prominently expressed in adult injured neurons. In spinal injury, the predominant site of injury seems to be in glial cells, especially in cells of the oligodendrocyte lineage[43]. At this site, p75-NTR might function most prominently in regulating cell death induced by pro-nerve growth factor (NGF). Second, the relative effects and the potential interactions of neurotrophin/Trk signalling versus myelin/NgR signalling at the p75-NTR have not been determined[44]. Third, because p75-NTR plays a major role in Schwann cell myelination[45], p75-NTR might have a more prominent function in facilitating remyelination than in regenerating axons of the injured adult CNS. Fourth, it remains unclear how p75-NTR agents might differentially regulate cell death

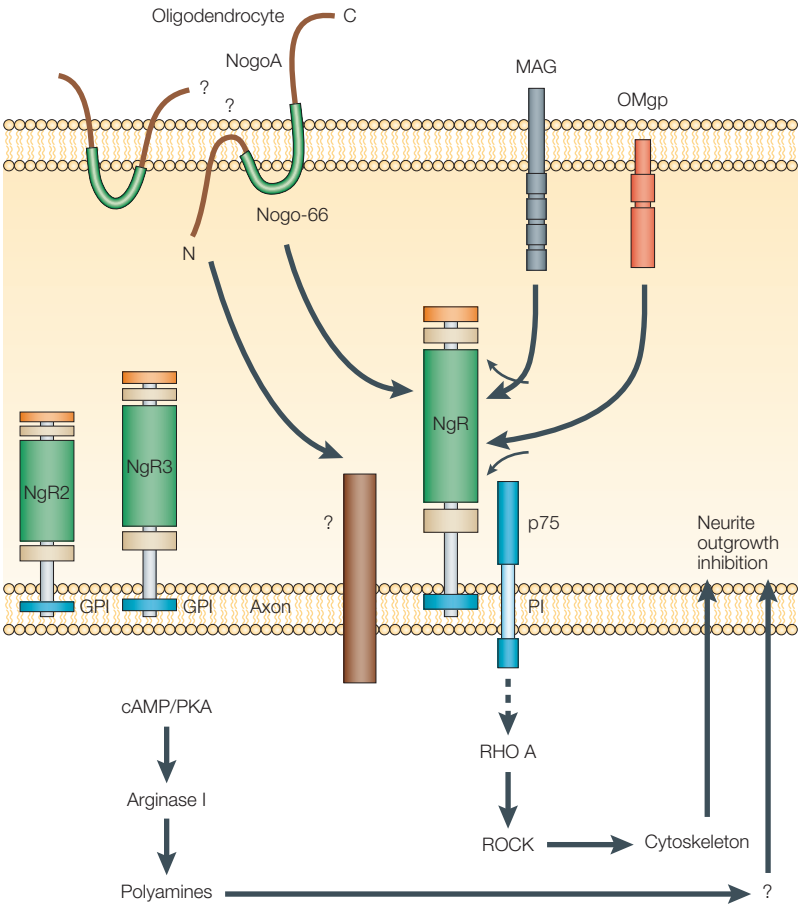

Figure 2 | **The Nogo receptor and inhibition of axon regeneration.** The leucine-rich repeat domains of the NgR are necessary for interaction with Nogo-66, MAG and OMgp. As a GPI-anchored protein, NgR does not transduce signals directly, but instead recruits co-receptor molecules such as p75 or other molecules. Co-receptor activation in turn activates the RHO and ROCK pathway to modulate the cytoskeleton and neurite growth. The amino-terminal domain of NogoA can be present either in the cytosol or in the extracellular space. In the latter position, it can bind to an unknown neuronal protein and thereby inhibit neurite growth. Although structurally similar to NgR, NgR2 and NgR3 show no or little binding to the known myelin-derived NgR ligands, and their functions in axon regeneration are unclear. The NgR-independent cAMP/PKA pathway also affects neurite growth, but the details of the mechanisms are less well understood. GPI, glycosyl phosphatidylinositol; MAG, myelin-associated glycoprotein; NgR, Nogo receptor; OMgp, oligodendrocyte myelin glycoprotein; PI, phosphatidylinositol; ROCK, RHO-associated kinase. PKA, protein kinase A.

versus axonal extension at injury sites *in vivo*[44]. Although these molecular roles have not yet been fully established, p75-NTR and potentially other NgR co-receptors can be considered alternative sites for intervention to promote CNS axonal regeneration.

Downstream of NgR and within the cytoplasm of the neuron, a monomeric GTP-binding (G) protein, RHO, is activated by CNS myelin and limits axonal growth. It was first demonstrated that RHO inhibition by the clostridial protein C3 exoenzyme blocks CNS myelin inhibition of axon growth[46]. Later, individual NgR ligands from CNS myelin were shown to cause RHO activation that could be blocked by C3 exoenzyme treatment[47–49]. However, there are controversial data that indicate that neither NgR nor other GPI-linked proteins are necessary for the regulation of RHO GTPases or inhibition of neurite outgrowth from cerebellar granule cells by MAG expressed by CHO cells or a NiG-Δ20–alkaline phosphatase fragment of NogoA[49]. Some studies have demonstrated that the C3 protein or modified C3 can promote axon regeneration and functional recovery *in vivo*[47,50]. Although the potential therapeutic efficacy of C3 requires further investigation, the RHO protein might prove a difficult molecular target for small-molecule drug development given the experience of parallel efforts with the superfamily member RAS in malignancy. There are many effectors downstream of RHO that are implicated in the regulation of the actin cytoskeleton and its dynamics. However, several studies point to a prominent role for RHO-associated kinase (ROCK) in linking the downstream cytoskeletal action of NgR to RHO signalling. The ROCK inhibitor Y-27632 reverses myelin inhibition of axon outgrowth in culture, and promotes axon regeneration and locomotor recovery in *vivo*[47,50]. A potential benefit of targeting ROCK is that potent small-molecule inhibitors could be optimized following on from the work on Y-27632. Second, the inhibitory glial scar component, chondroitin sulphate proteoglycan (CSPG), also limits axon regeneration and can be blocked by ROCK inhibitors[51]. However, both RHO and ROCK are expressed ubiquitously, in contrast to the neuronal selectivity of NgR. Therefore, therapeutic manipulation of these downstream elements might alter numerous other physiological events and limit their potential value as CNS regeneration therapeutics. A schematic summary of the NgR pathway and inhibition of axon regeneration is presented in FIG. 2.

Application of NgR-based reagents *in vivo*

The efficacy of NogoA-based reagents in animal models of CNS injury has been studied extensively in spinal cord injury and to a limited extent in stroke. The anti-NogoA antibody IN-1 and improved versions (in the form of hybridomas and engineered IN-1 Fab fragment) have been delivered to rats with spinal cord injury. Not only was functional recovery improved as measured by BBB scoring, but histological analyses also clearly demonstrated enhanced neurite sprouting and regeneration in the IN-1-treated animals[16–19]. The administration of a NogoA-derived peptide, NEP1-40, either by INTRATHECAL DELIVERY or systemic administration, was equally effective in promoting axon regeneration and functional recovery[22,52]. Similar positive experimental data were obtained when initiation of NEP1-40 peptide administration was delayed to seven days post-injury (FIG. 3)[52]. NEP1-40 peptide provides proof-of-concept that CNS axon regrowth can be promoted by manipulating the inhibitory components of myelin. It would be of great interest to characterize the specific nerve tracts that responded to the NEP1-40 treatment in these experiments, especially if data from electrophysiology end-point measurements were available to document the efficacy of these novel reagents. As the NEP1-40 peptide specifically blocks the Nogo–NgR interaction, NgR-based reagents are likely to have similar efficacy in neurological recovery.

INTRATHECAL DELIVERY
Direct delivery of a molecule via a catheter or needle inserted under the dura of the spinal cord, thereby bypassing the blood–brain barrier. The molecule subsequently becomes distributed via the cerebral spinal fluid to different parts of the spinal cord and can reach the brain.

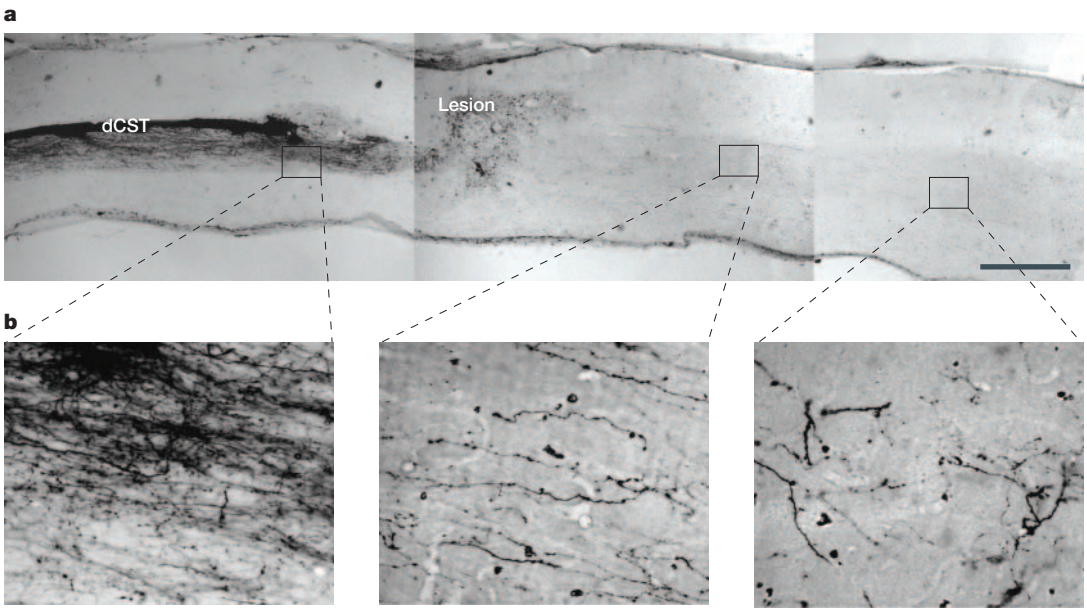

Figure 3 | **Axonal regeneration promoted by delayed systemic treatment with a NgR peptide antagonist.** The NEP1-40 peptide was administered by continuous subcutaneous infusion to mice after mid-thoracic dorsal hemisection injury. Infusion of the NgR antagonist was not initiated until seven days after injury. **a** | Low-magnification view of a parasagittal section from the thoracic spinal cord; rostral is to the left and caudal is to the right. The descending dorsal corticospinal tract (dCST) is traced by biotinylated dextran acetate injection (BDA TRACING) into the cerebral cortex (dark reaction product visible). The details are as in REF. 48. Note the sprouting of corticospinal axons both rostral to the lesion site, and in the caudal spinal cord, visible in the insets at high magnification (**b**). The scale bar is equivalent to 500 μm in the upper panel and 50 μm in the lower panels. Micrographs courtesy of Shuxin Li, Yale University, New York, USA.

The first demonstration of the effects of the anti-NogoA antibody IN-1 in the middle cerebral artery occlusion model of stroke in rats was recently reported[53]. Injection into the cerebral cortex of hybridoma cells producing IN-1 promoted functional recovery in a forelimb-reaching task. Histological analyses indicated that the recovery was probably achieved via enhanced neuronal plasticity and compensatory sprouting that occurred within uninjured areas in the CNS. These results indicate that the NogoA pathway might be important in promoting recovery after stroke. Further analysis in a sensorimotor cortex aspiration lesion model demonstrated that administration of the IN-1 antibody by injection of hybridoma cells into the lesion site promoted ipsilateral movement of the lesion-impaired forelimb in response to intracortical microstimulation[54]. The efficacy of NogoA inhibitors in stroke was further supported by data from another recent study that evaluated a monoclonal anti-NogoA antibody, 7B12. This was administered in two rat stroke models[55]: the photothrombotic cortical injury model and a permanent middle cerebral artery occlusion model in spontaneously hypertensive rats. Intraventricular infusion of the 7B12 antibody was initiated 24 hours post-injury, and the effects of treatment were measured at six to nine weeks or four to twelve weeks. Treatment with 7B12 improved forepaw function and significantly increased midline crossing of corticospinal fibres originating in the unlesioned sensorimotor cortex, with no effect on infarct volume or brain atrophy. Although the effects of NgR-based reagents in animal models of stroke have not yet been reported, these results indicate a potential role of the Nogo–NgR pathway in functional compensatory reorganization of the intact CNS after injury induced by ischaemia or mechanical lesion.

BDA TRACING
Biotinylated dextran acetate (BDA) is a stable, non-metabolized, low-molecular-mass compound that after injection into the motor cortex is transported along the axons. The molecule can be detected in tissue sections using appropriately tagged strepavidin (for example, conjugated with horse radish peroxidase) for labelling nerve fibres.

Alternative targets for CNS axonal regeneration

Other therapeutic candidates and targets have been assessed for a potential role in CNS axonal regeneration, including neurotrophic factors, such as a NGF, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) (REFS 56,57), inosine[58], neuroimmunophilins[59], chondroitinase ABC[60] and cyclic AMP/protein kinase A (PKA)[61].

Neurotrophic factors. Attempts to administer neurotrophic factors for treating spinal cord injury have had limited success. Local injection of NGF into the lesioned spinal cord did not increase sprouting[56]. BDNF that was delivered via grafting of fibroblasts expressing BDNF into the spinal cord lesion site or via local injection into the lesioned spinal cord[55] failed to promote recovery. By contrast, NT-3 delivered to the lesion via grafting of fibroblasts expressing NT-3 led to partial functional recovery and a significant increase in corticospinal tract sprouting at and distal to the lesion[57]. Local injection of NT-3 into the lesioned spinal cord increased sprouting at the lesion site only[56]. These results indicate that NT-3, but not NGF or BDNF, might represent a viable approach to promoting CNS compensatory plasticity after injury.

Inosine. Inosine is a purine nucleoside that stimulates process outgrowth from cultured neurons. Benowitz *et al.* reported that inosine delivery into the contralateral sensorimotor cortex promoted extensive sprouting of axons from intact pyramidal neurons after corticospinal tract transection[58]. Their results indicate that inosine might enhance compensatory sprouting following CNS injury.

Neuroimmunophilins. The neuroimmunophilin ligands were first discovered because of their ability to protect neurons and to promote axonal outgrowth. Bavetta *et al.* reported that following dorsal column transection and sciatic nerve crush, FK506 treatment promoted sensory axon regrowth and sparing, as well as axonal sprouting[59]. Interestingly, GPI 1046 did not promote axon regrowth or sparing, and stimulated sprouting to a lesser extent. Sciatic nerve crush injury, which is known to enhance the regenerative response of DRG neurons, seemed to be important for sprouting and regeneration induced by FK506. The requirement for sciatic nerve crush injury might be a consideration that limits the use of FK506 or other neuroimmunophilins in the clinic.

Chondroitinase ABC. Chondroitin sulphate proteoglycans comprise one class of extracellular matrix molecules that contribute to the glial scar that forms at the site of CNS injury. These molecules have been reported to inhibit axon growth *in vitro*. Chondroitinase ABC is a bacterial enzyme that cleaves glycosaminoglycans and has been used to block the inhibitory activity of chondroitin sulphate proteoglycans. Bradbury *et al.* used this enzyme to treat rats after lesioning the dorsal columns, and reported that intrathecal administration promoted regeneration of corticospinal tract axons as well as functional recovery of locomotor and proprioceptive function[60]. However, the glial scar forms a local impediment to short-range axonal regeneration, whereas components of CNS myelin form a widespread impediment to long-range axonal regeneration. It remains to be determined which of these inhibitory structures — the glial scar or CNS myelin — is more important for limiting axon regrowth.

cAMP/PKA. Elevation of cAMP by direct injection into DRG overcomes inhibition by MAG and myelin, and results in extensive regeneration of dorsal column axons lesioned one week later[61]. This process initially involves PKA, but eventually becomes PKA-independent. The elevated cAMP upregulates ArginaseI, a key enzyme involved in polyamine synthesis. Both overexpression of ArginaseI and exogenous addition of polyamines can overcome MAG and myelin inhibition[61,62]. Similarly, in a model of optic nerve axotomy, co-injection of 8(4-chlorophenylthio)-cAMP and ciliary neurotrophic factor increased the survival of retinal ganglion cells and axonal regrowth[63]. As such, modulation of cAMP levels might promote axon regeneration.

Summary

Recent advances in the characterization of the inhibitory molecules in CNS myelin have led to novel therapeutic strategies and possible drug candidates. In particular, several of these inhibitory molecules converge on the NgR, rendering NgR the focal point for modulating axonal regrowth. The importance of NgR has been demonstrated by a Nogo/NgR-based reagent, NEP1-40, which successfully promoted functional recovery and unprecedented long-range axonal regeneration in an animal model of spinal cord injury. There are many important issues yet to be addressed, including some of the following questions. What specific types of axons might be repaired by modulating the NgR pathway? For example, *in situ* hybridization expression data[64] show that there are neurons (for example, neostriatal neurons and neurons in the ventral lateral geniculate nucleus) that do not express NgR but still fail to regenerate. Likewise, there are some neurons in the deep cerebellar nuclei that readily regenerate axons into nerve grafts even though they express NgR[64], suggesting the participation of additional pathways for specific subsets of neurons. What characteristics do repaired axons possess? Do regenerated axons re-establish specific synaptic connections and contribute to neuronal circuits that mediate proprioception, locomotion and nociception? Despite these outstanding issues, the NgR provides a rational intervention point for drugs to promote CNS axonal regeneration. The importance of NgR in axon regeneration, and its potential as a therapeutic target, will be further clarified when the phenotypes of genetically modified NgR-deficient animals become available. New therapies targeting this receptor have the potential to stimulate axon regrowth in the injured human CNS, repairing the damage that underlies the devastating consequences of CNS injury. Characterization of other molecules and receptors in this pathway or alternate pathways will add to the repertoire of drug targets for promoting axon regeneration. The coming decade will represent an important and exciting period for drug discovery for spinal cord injury, traumatic brain injury and stroke, as these new therapies reach the clinic for assessment in patients. Furthermore, such therapies could potentially be applied more broadly to the repair of damaged CNS axons, with potential benefit for neurodegenerative diseases such as multiple sclerosis.

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