Targeting the Nogo Receptor Complex in Diseases of the Central Nervous System

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Abstract: After injury to the central nervous system intrinsic factors such as myelin associated inhibitory factors inhibit cellular and axonal regeneration resulting in permanent disability. Three of these factors (Nogo-A, oligodendrocyte myelin glycoprotein, myelin-associated glycoprotein) bind to a common receptor: the Nogo-66 receptor (NgR1). NgR1 is expressed mainly on neurons and is usually associated in a trimolecular complex. The second member of the complex, LINGO-1, is often connected to NgR1 function and is further found to function independently as a negative regulator of oligodendrocyte proliferation and differentiation. The third member of the NgR complex is either the p75 neurotrophin receptor, TROY, or an as yet unidentified co-receptor. Targeting of factors contained in this complex has been described to lead to the promotion of neurite outgrowth, oligodendrocyte proliferation and differentiation and inhibition of cell death. In the current review, we aim to describe the mechanisms of action of the chemical and biological compounds used in targeting NgR1 and LINGO-1. This will be achieved using three examples: blocking of ligand binding to NgR1 in treatment of spinal cord injury, antibody-mediated inhibition of LINGO-1 to promote oligodendrocyte differentiation in multiple sclerosis, and the use of soluble NgR1 to sequester Abeta peptide in the periphery in Alzheimer's disease.

Keywords: Alzheimer's disease, LINGO-1, multiple sclerosis, myelin associated inhibitory factors, nogo receptor, spinal cord injury.

INTRODUCTION

A major problem facing clinical neurobiologists for the past 100 years is the lack of regeneration of the central nervous system (CNS) after injury or disease. For a long time, this has been explained by the 'post-mitotic milieu' of the CNS, in conjunction with the growth inhibitory environment after injury. In recent years, a clearer picture of CNS biology has been elucidated. This includes the finding of neural progenitor cells in specific regions of the CNS as well as identification of the proteins responsible for inhibiting nerve outgrowth. Now that we have a clearer understanding of regenerative possibilities in the adult CNS, an important next step is to harness this knowledge and use it to treat the large variety of CNS injuries and disorders that exist. In this review, we will focus on three examples, discussing how inhibition of elements of the Nogo receptor complex can result in promotion of nerve outgrowth after spinal cord injury, reducing plaque formation in Alzheimer's disease and boosting oligodendrocyte differentiation in multiple sclerosis.

Myelin-Associated Inhibitory Factors

A major inhibitor of successful recovery after injury to the CNS is the unfavorable environment for axonal regeneration. This has been explained by both physical and molecular factors contained in the glial scar. The meshwork of reactive astrocytes, oligodendrocyte precursors, and microglia create a physical barrier to regeneration. In addition, molecular components such as chondroitin sulphate proteoglycans, ephrins, EphA4, Sema3A, Slit proteins and myelin-associated inhibitory factors (MAIFs) contribute to the molecular inhibition of axonal regeneration. The latter factors and their receptors will be the focus of this review.

With the finding of an antibody capable of blocking the inhibitory action of myelin on axonal outgrowth [1], the antigen was subsequently identified to be Nogo-A [2]. Nogo-A is one of four Nogo isoforms that are members of the reticulon family of proteins [3]. Nogo-A is mostly situated in the endoplasmic reticulum, however a small percentage of protein is found on the cell surface on the inner and outer loops of the myelin sheath [4]. Nogo-A has a short C terminal tail, two transmembrane domains and an extracellular N terminal tail. One of the regions responsible for neurite outgrowth inhibition is called Nogo-66. It is 66 amino acids long, situated between the two transmembrane regions and present in all Nogo isoforms. Adjacent to the N terminal stub, from amino acids 567 to 748 in humans (544 to 725 in rat) there is an additional region termed NiG Δ 20 which is also recognized by a function-blocking antibody [5]. The binding protein specific for the Nogo-66 region of Nogo-A was identified in 2001 and termed Nogo-66 Receptor (NgR) [6]. In the following years, it was found that there are two further myelin-associated ligands for this receptor. Although oligodendrocyte myelin glycoprotein (OMgp) [7] and myelin-associate glycoprotein (MAG) [8] are structurally different from Nogo-A, they bind to and activate NgR in the same way (Fig. **1**). Cleavage of NgR and other GPI-anchored proteins from the cell surface renders axons insensitive to MAG or OMgp. Additionally, introduction of exogenous NgR to

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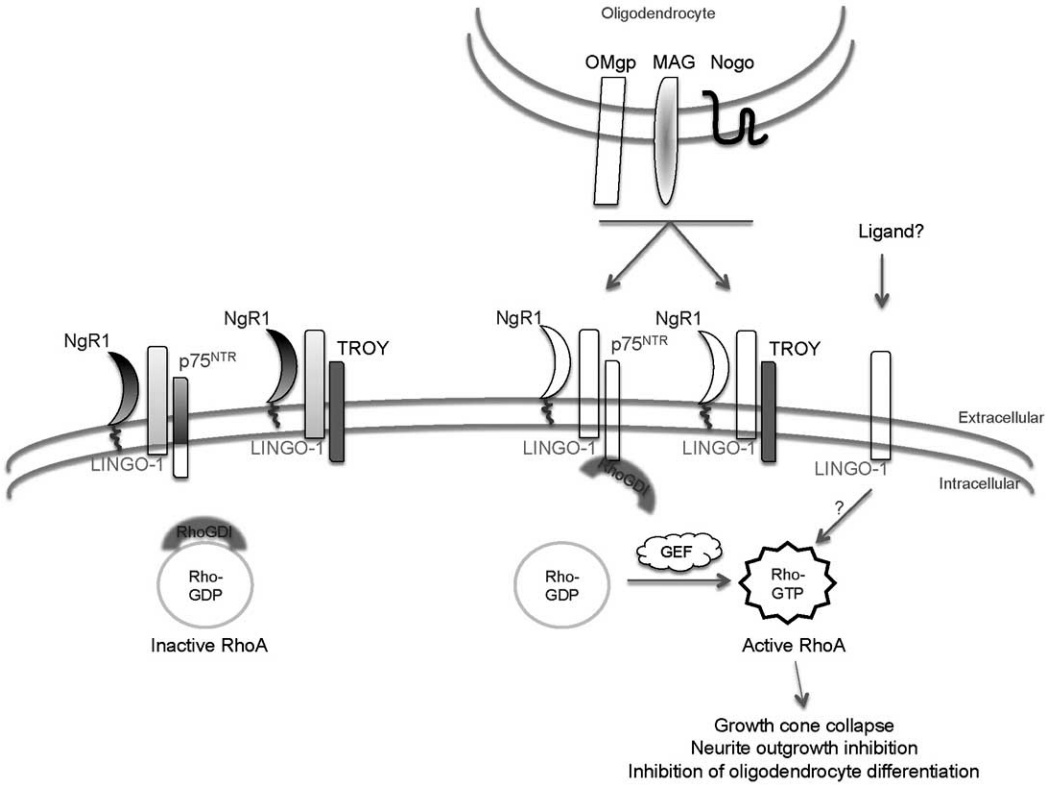

Fig. (1). Signaling mechanisms of the NgR1 complex

NgR1 is mostly found in a tripartite complex, comprised of transmembrane co-receptors LINGO-1 and either $p75^{NTR}$ or TROY. This complex acts as the receptor for three myelin associated inhibitory factors: oligodendrocyte myelin glycoprotein (OMgp), myelin associated glycoprotein (MAG) and Nogo. According to the model proposed by Yamashita *et al.*, RhoA is maintained in the inactive state by Rho guanine dissociation inhibitor (Rho-GDI) [34]. Upon ligand binding to the NgR1 complex, RhoGDI is bound by $p75^{NTR}$, releasing Rho-GDP to be activated to Rho-GTP by an unknown guanine nucleotide exchange factor (GEF). RhoA activation acts as a molecular switch to regulate F-actin dynamics and microtubule assembly leading to cytoskeleton rigidification during cell morphological changes. This ultimately results in growth cone collapse and inhibition of oligodendrocyte differentiation.

otherwise insensitive neurons renders them responsive to the growth inhibitory activity of MAG or OMgp [7, 8].

Since the identification of NgR1, two related proteins (NgR2 and NgR3) showing sequence similarity and overlapping tissue expression patterns have been identified [9-11]. NgR2 is a high affinity binding protein for MAG and was shown to function as a redundant receptor to NgR1 for MAG's inhibition of neurite outgrowth from sensory neuron [12, 13]. So far, no binding partner for NgR3 has been identified. With the discovery of one binding protein for three myelin associated inhibitory factors, there was a flurry of research to inhibit this convergence point for neurite outgrowth inhibition. Although the picture of neurite outgrowth inhibition has proven to be more complex than initially thought, inhibition of elements of the NgR complex are being revealed as promising targets for CNS injury and diseases.

The Nogo Receptor Complex

Nogo-66 Receptor 1

NgR1 is a 473-residue, glycosylphosphatidylinositol (GPI)-anchored protein expressed mainly in the grey matter of the CNS. In situ hybridization identifies expression of NgR1 in cerebral cortical neurons, hippocampal neurons, cerebellar Purkinje cells and pontine neurons [6, 10]. Oligodendrocytes have minimal NgR1 expression. The majority of the globular structure of NgR1 is comprised of a leucine-rich repeat (LRR) domain capped by N-terminal and C-terminal cysteine-rich modules [6]. The cysteine-rich C-terminal region of NgR1 is thought to be necessary for interaction with the co-receptor $p75^{NTR}$ [14]. Protein domains with LRR architecture form curved solenoid structures where the inner concave space is important for ligand binding [15]. The crystal structure of the NgR1 binding domain has been elucidated and indeed suggests a conserved putative ligand-binding site within the concave groove of the LRR [16]. The same study suggests that the promiscuous nature of the receptor is due to the broad region of exposed aromatic residues within the concave belly, expanding its ability to form a variety of unique contacts with different ligands.

In cultured neurons, ectopic expression of NgR1 can induce growth cone collapse and render neurons responsive to myelin inhibition [6]. Neurons cultured from NgR1 knock out (KO) mice show no growth cone collapse reaction when plated on myelin inhibitors [17]. Likewise, physically blocking binding of ligand to NgR1 by addition of ligand-binding soluble NgR1 chimeric protein (NgR310) [18], competitive binding peptide (NEP 1-40) [19] or NgR1 function-blocking antibody to cultured neurons [18] leads to a marked improvement in neurite outgrowth in the presence of myelin-associated inhibitors *in vitro*. Results of the above *in vitro*

experiments have prompted many studies investigating the potential of inhibiting NgR as a therapeutic mechanism to promote axonal outgrowth after injury. The outcomes of these *in vivo* studies will be discussed in further detail below, as well their potential for use in the clinic.

Since NgR1 contains a GPI anchor, the entire protein mass is located on the extracellular side of the membrane, meaning it requires transmembrane co-receptors in order to transmit its signal inside the cell. LRR and Ig domain–containing, nogo receptor–interacting protein (LINGO-1) and either p75 neurotrophin receptor ($p75^{NTR}$) [20] or TNF-Receptor Super family member 19 (TROY) [21] are thought to play this role (Fig. **1**). Each protein of the NgR1 complex, as well as what is known about the downstream signaling cascade, will be explained in more detail here.

LINGO-1

LINGO-1 is a CNS-specific trans-membrane glycoprotein identified as interacting with NgR1 [22]. The expression pattern is found to show a gradient from the cortex to spinal cord [22], which is similar to the expression pattern for NgR1 [23]. Using *in situ* hybridization, immunohistochemistry and real time (RT)-PCR studies, LINGO-1 was found to be expressed in neurons and oligodendrocytes, with higher levels found in oligodendrocyte precursor cells (OPCs) [24, 25]. LINGO-1 mRNA was also detected in cultured ciliary ganglion neurons and oligodendrocytes but not in astrocytes by RT-PCR [22].

LINGO-1 belongs to a large family of LRR-Ig-containing proteins involved in CNS development and axonal growth [26]. LINGO-1 contains 12 LRR motifs flanked by N- and C-terminal capping domains, one Ig domain, a transmembrane domain, and a short cytoplasmic tail. The cytoplasmic tail contains a canonical epidermal growth factor receptor-like tyrosine phosphorylation site (residue 591). The crystal structure of LINGO-1 has been identified and demonstrates that it has a bimodular, kinked structure composed of the LRR and Ig-like modules [27]. Both in crystal form and in solution, LINGO-1 consistently associates with itself to form a ring-like tetramer that may serve as a scaffold to facilitate the assembly of the NgR1/LINGO-1/$p75^{NTR}$ receptor complex [27].

LINGO-1 has been shown to bind to both NgR1 and $p75^{NTR}$ in direct binding assays as well as in immunoprecipitation experiments [22]. Triple transfection of non-neuronal COS-7 cells with NgR1, LINGO-1 and $p75^{NTR}$ caused an activation of RhoA when the cells were in contact with OMgp [22]. This effect was not seen when cells were transfected with only one or two members of the complex. Intrathecal administration of the soluble ectodomain of LINGO-1 (LINGO-1-Fc) after dorsal hemisection resulted in enhanced CST axon sprouting and functional recovery [28]. It is thought that soluble LINGO-1-Fc acts as an antagonist to myelin inhibition by competing out the interaction of LINGO-1 with its co-receptors [22].

In addition to functioning as a co-receptor for NgR1, LINGO-1 has also been identified as playing an important role in the inhibition of oligodendrocyte differentiation and myelination. It is expressed to a high extent in oligodendrocyte progenitor cells (OPC) and LINGO-1 mRNA is up-regulated 14 days after spinal cord injury (SCI). In an elegant sequence of experiments, Mi et al. demonstrated that inhibition of LINGO-1 led to increased process length, branching, myelin sheet formation and myelination [24]. They showed that attenuation of LINGO-1 function by dominant-negative LINGO-1, LINGO-1 RNA-mediated interference, or LINGO-1-Fc led to oligodendrocyte differentiation and increased myelination competence in culture [24]. These effects were associated with down regulation of RhoA activity, which has been implicated in oligodendrocyte differentiation. Conversely, over expression of LINGO-1 led to RhoA activation, and inhibition of oligodendrocyte differentiation and myelination [24]. In further *in vitro* experiments, Mi and colleagues demonstrated that treatment of oligodendrocyte and neuron co-cultures with LINGO-1-Fc resulted in highly developed myelinated axons with internodes and well-defined nodes of Ranvier [24]. The importance of LINGO-1 to myelination *in vivo* was confirmed in LINGO-1 KO mice. More myelinated axonal fibers were observed at postnatal day 1 (P1) than in wild-type littermates, where onset of myelination normally occurs at P5 [24].

The identification of LINGO-1 as an endogenous negative regulator of myelination, as well as the promising results of inhibition of its function to promote remyelination has opened a number of possibilities for regenerative therapies after neurodegenerative or demyelinating events. We aim to discuss these in further detail in this review.

$P75^{NTR}$/TROY

The third element of the NgR complex was initially identified to be the common, low-affinity neurotrophin receptor $p75^{NTR}$ [20], and many publications have supported its role as the signal-transducing element of the complex (Fig. **1**) [14, 29]. $P75^{NTR}$ has been shown to bind to full-length NgR1 [30] and mediates the outgrowth inhibition and signaling cascades triggered by myelin inhibitors on cultured neurons. However, $p75^{NTR}$ is not ubiquitously expressed in the adult CNS and is absent in some neuronal cell types that react to myelin inhibition [22, 31-33]. Thus, the search began for other possible components of the receptor complex. To this point, TROY is the most promising co-receptor [21, 30]. It is broadly expressed in postnatal and adult neurons and alkaline phosphatase (AP)-conjugated TROY was found to bind with a higher affinity to full-length NgR1 than AP-$p75^{NTR}$ [30]. The same publication demonstrates that a complex of TROY/NgR1/LINGO-1 activates RhoA in the presence of myelin inhibitors and exogenous TROY reverses myelin-induced neurite outgrowth inhibition *in vitro* [30]. However, its expression also does not always overlap with that of NgR, leaving the question open for the identification of further proteins involved in this inhibitory complex.

NgR-mediated Signaling

As mentioned above, NgR-mediated signaling involves activation of the small RhoA guanosine triphosphatase (GTPase). RhoA activation is the major converging point for inhibition of regeneration, growth cone collapse, and inhibition of differentiation. In the inactive state, Rho-GDP is bound to the Rho guanine dissociation inhibitor (Rho-GDI, Fig. **1**). Upon binding of ligand to the NgR complex, it is thought that $p75^{NTR}$ or TROY binds RhoGDI, releasing Rho-

GDP to be activated to RhoGTP by guanine nucleotide exchange factors (GEFs) [34]. It is possible to neutralize myelin-mediated inhibition by addition of a peptide that prevents the interaction between Rho-GDI and $p75^{NTR}$ [34]. RhoA activation acts as a molecular switch to regulate filamentous actin dynamics and microtubule assembly leading to cytoskeleton rigidification during cell morphological changes. This ultimately results in growth cone collapse and inhibition of oligodendrocyte differentiation.

Nogo Receptor and Spinal Cord Injury

Spinal Cord Injury

Spinal cord injury (SCI) affects an estimated 2.5 million people worldwide. SCI can lead to paraplegia or quadriplegia and reversal of these effects is an important challenge facing researchers in clinical neuroscience. Many experimental therapies for SCI aim to promote axonal regeneration and recovery of limb function. Here we describe the possible positive effects of promoting axonal regeneration and sprouting by inhibiting interaction of Nogo-A with NgR.

The pathophysiology of SCI is described as biphasic (for review, see Rowland *et al.* [35]). The primary injury phase occurs within two hours of initial mechanical injury and results in fracture and/or dislocation of the spinal column disrupting axons, blood vessels and cell membranes [36]. The secondary injury phase occurs between 2 hours and 2 years after initial injury. It is defined by vascular dysfunction, edema, ischemia, excitotoxicity, electrolyte shift, free radical production, inflammation and delayed apoptotic cell death. In addition to the neurological dysfunction initiated by the primary physical injury, the secondary injury phase results in a protracted period of tissue destruction. In the first hours after injury, tissue damage results from release of reactive oxygen species, processes of lipid peroxidation, glutamate-mediated excitotoxicity, necrosis, neuronal and axonal swelling and peak blood-brain barrier (BBB) permeability resulting in neutrophil invasion. Up to 14 days after injury, the BBB begins to repair and edema decreases. In the months after SCI, there is continued formation of the glial scar and lesion stabilization as well as prolonged Wallerian degeneration. In brief, the secondary injury phase progresses from processes that result in direct neuronal cell death, to more protracted axonal degeneration and demyelination leading to cell death (Wallerian degeneration), and formation of an environment that is unsupportive for neuronal regeneration. Because most therapies cannot be applied until hours or days following injury, the most important targets for promotion of regeneration are those described to occur in the secondary phase.

Current therapies for SCI are mainly concerned with minimizing movement of the patient in the hours following injury and improving functional outcome with physical therapy. As there are currently no functionally restorative therapies for SCI, many potential cures are being tested in animal models and in phase I/II clinical trials (for review, see Thuret et al. [37]). Potential treatments can be separated into cell-based therapies and molecular therapeutic interventions. Some examples of cell-based therapies are peripheral nerve grafts, Schwann cell grafts, olfactory nervous system cell injection, embryonic CNS tissue grafts, embryonic stem/progenitor cell injection, adult stem/progenitor cell injection, engineered stem/progenitor cell injection and activated macrophage application. Potential molecular therapeutic interventions include neuroprotective therapies (such as erythropoietin, minocyclin or intravenous steroids), enhancing conduction by blocking potassium channels, administration of growth factors, delivery of cAMP or small GTPases, extracellular matrix modifiers and modulation of interaction with myelin inhibitors. Many of these proposed interventions have reached phase I/II clinical trials, however successful animal studies have not yet translated into consistently efficacious patient treatment. Thus, the discovery of a single binding protein for a number of myelin-associated inhibitory factors [6-8] was met with great optimism. This has been followed by extensive work demonstrating that inhibition of the receptor can indeed promote nerve outgrowth, and is being translated to patient trials to induce axonal regeneration after SCI. Furthermore, the nature of spinal cord lesions provides a promising substrate for promoting regeneration. This is because primary injury rarely results in full transection of the cord. Axons are commonly found to traverse the lesion site, often occupying a 'subpial rim' of spared yet demyelinated or dysmyelinated long-tract axons [38, 39]. This can be functionally strengthened with rehabilitation therapy for neurological recovery or remyelination and can provide a substrate to guide regeneration of axons. As such, several therapeutic strategies aim to promote re-growth of axons along this tract by blocking specific myelin inhibitory factors or their receptor.

Blocking Nogo/Nogo Receptor in Spinal Cord Injury

As described above, NgR1 is the common binding protein for Nogo-A, OMgp and MAG, with NgR2 being a high-affinity binding protein for MAG [12, 40]. Extensive *in vitro* studies have shown that binding of ligand to NgR1 on neurons results in growth cone collapse and inhibition of neurite outgrowth. Numerous studies have also shown that blocking Nogo-A or NgR leads to growth cone formation and promotion of neurite outgrowth on a substrate of myelin inhibitory proteins [1, 41].

These factors have been tested in animal models of SCI to determine their efficacy for promoting regeneration *in vivo*. Intrathecal application of hybridoma cells secreting a function blocking anti-Nogo antibody to spinal cord transected or to sensory motor cortex lesioned rats leads to significantly positive results (Fig. **2**). After treatment of spinal cord transection with Nogo antibody, various degrees of significant axonal growth of corticospinal tract and improved functional recovery were observed [42, 43]. Furthermore, after sensory motor cortex lesion, Nogo antibody enhanced functional reorganization of intact motor cortex contralateral to the lesion site [44]. Studies with Nogo antibody have even been extended to non-human primates. In four out of five spinal cord-transected Marmoset monkeys treated with Nogo antibody, fine-labeled neurites had grown into, around, and through the lesion site [45]. This study is supported by further experiments showing recovery of manual dexterity and enhanced sprouting of corticospinal axons in macaque monkeys subjected to cervical lesion and treated with Nogo-A-specific antibody as compared to control antibody [46]. The function-blocking antibody is not the only example of at-

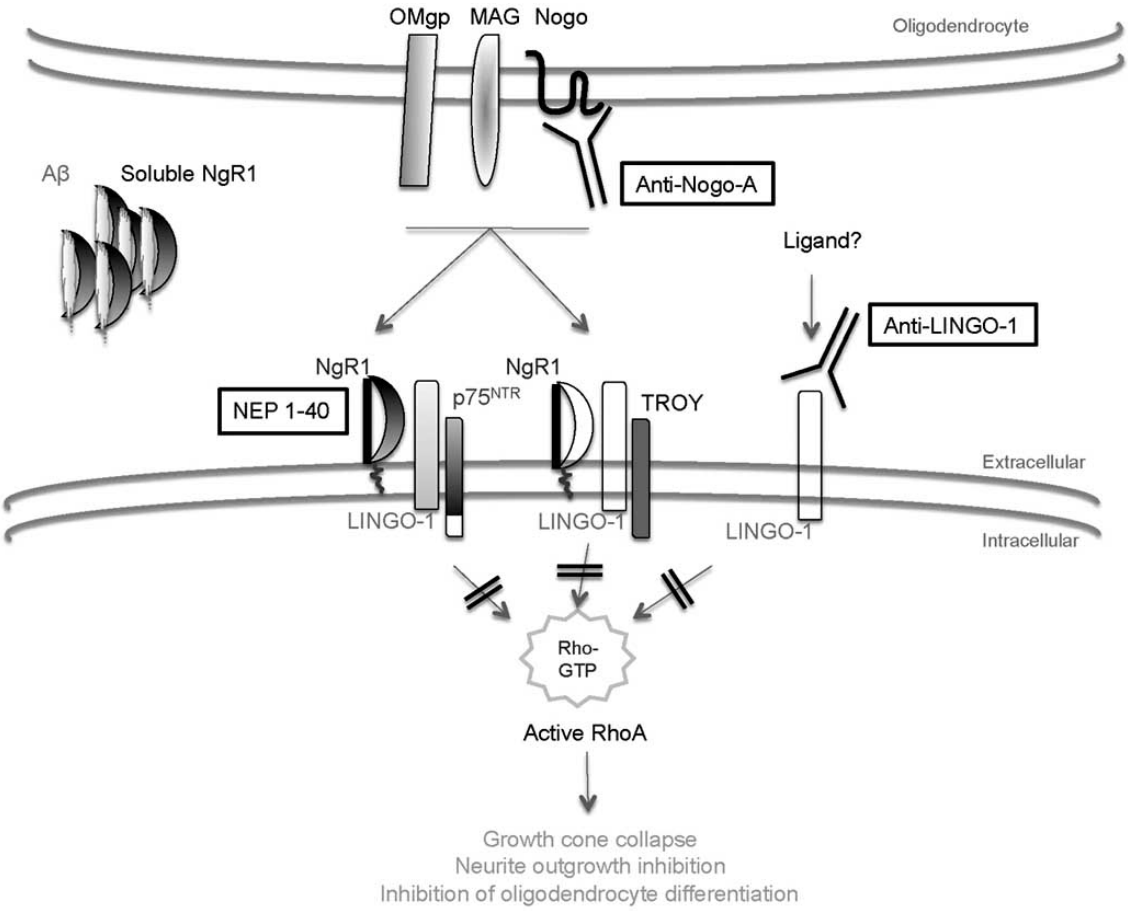

Fig. (2). Mechanisms of targeting elements of the NgR1 complex

Due to the inhibitory nature of the NgR1 complex, several therapeutic methods of targeting it have been developed. Subcutaneous administration of soluble NgR1 is found to sequester Aß in the periphery, preventing its accumulation in the CNS and resulting in improved behavioral outcome in an animal model of Alzheimer's disease. Antibodies directed against Nogo-A promote axonal outgrowth and improved functional recovery after spinal cord injury. NEP 1-40 is an antagonist peptide that binds to NgR1 and prevents activation of the receptor complex by its ligands. This results in promotion of axonal outgrowth and improved functional recovery after spinal cord injury. Blocking LINGO-1 with a function-blocking antibody is found to lead to an increase in oligodendrocyte proliferation and differentiation. Administration of anti-LINGO-1 to mice with EAE resulted in a less severe disease course accompanied by enhanced and new myelination.

tempts to block myelin-associated inhibitory factors. Another method of blocking the inhibitory action of Nogo signaling is with the antagonistic peptide NEP 1-40. NEP 1-40 is a fragment of the Nogo-66 region that occupies the ligand-binding site of NgR, without activating the receptor complex [19]. NEP 1-40 was delivered to spinal cord transected rats via an intrathecally-implanted osmotic mini pump [19] and to spinal cord transected mice with subcutaneous injections [47]. In both cases, significant growth of corticospinal tract axons and improved functional recovery were observed.

Treatment with Nogo antibody and NEP 1-40 are just two examples of an extensive body of work demonstrating neurite growth and improved functional outcome after treatment of animal models of SCI with Nogo/NgR blocking agents. Examples of these include: soluble NgR [48], immunization with myelin [49], immunization with nogo-A derived peptide [50], vaccination with spinal cord homogenate [51], immunization with recombinant Nogo-66 and MAG [52], and recombinant DNA vaccine [53]. Thus, attempts to translate this pre-clinical research into therapies for human SCI have already been put into motion. In May 2006, a human Phase I clinical trial was initiated in Europe by Novartis to assess the safety, feasibility and pharmacokinetics of the humanized anti-Nogo antibody in patients with incomplete SCI who are 4 – 14 days post injury. The agent is being administered via continuous intrathecal infusion. To date, the treatment is reported to be well tolerated, with no adverse side effects being observed [54]. NEP 1-40 is in the preclinical study phase for acute spinal cord injury.

Studies with inhibitory factors provide a promising basis for treating devastating and incurable spinal cord injury. In order to better characterize the mode of action of these inhibitory factors, regenerative capacity in mice genetically lacking the various isoforms of Nogo or NgR has been determined. Surprisingly, these approaches have given rise to conflicting results. For example, Cafferty et al. report that after hemilesion of thoracic spinal cord in mice lacking Nogo, MAG and OMgp, there was significant regeneration of both serotonergic and cortical spinal tract axons through and well caudal to the lesion site [55]. These findings were correlated with improved behavioral recovery in the triple KO mice. In gross contrast, Lee et al. conducted a similar

experiment in which they studied the dorsal hemisection and complete transection models in mice lacking Nogo-A, -B, -C, as well as MAG and OMgp [56]. They report no significant corticospinal tract or serotonergic axon regeneration through or beyond the lesion in both wild type and triple null mice. As would be expected, there was also no behavioral improvement in the KO mice. Additionally, in NgR null mice, Kim et al. report enhanced regeneration of raphespinal and rubrospinal fibers, but not corticospinal fibers, as well as improved locomotor function after spinal cord transection [17]. This is in contrast to Zheng et al. who demonstrate that there was no difference in corticospinal tract neuron regeneration after spinal cord dorsal hemisection in NgR null mutants compared to wild type mice [57]. These examples of conflicting results are supported by a number of other publications demonstrating very low to modest regenerative capacity in KO mice [58-61] when compared with treatment with Nogo antibody or NEP 1-40 [62]. This could be due to variations in genetic backgrounds of the mice used for KO experiments, disparity in injury models used or up-regulation of compensatory molecules. The reason for the continued success of the function-blocking approaches could be that the factors used also affect other mediators of inhibition that have not yet been identified, or these studies imply that an acute interference is necessary and the variations in the outcome may depend on the method of application, or on the pharmacokinetic properties of the compounds used. A further obscuring factor that arises from transferring animal model data to humans is the vast difference between the highly-controlled method of inducing spinal cord injury in animals and the highly heterogeneous reality of human SCI, which can include shearing, laceration, acute stretching, and sudden acceleration-deceleration injuries [63]. Thus it will be important to conduct further experiments elucidating all targets and biological effects of the blocking reagents, as well as how they act in a more heterogeneous injury setting.

Nogo Receptor and Alzheimer's Disease

Alzheimer's Disease

Alzheimer's disease (AD) is the most common form of dementia and affects more than 35 million people worldwide. It arises due to an accumulation of misfolded proteins in the aging brain, resulting in oxidative and inflammatory damage, which in turn leads to energy failure and synaptic dysfunction [64]. Important pathological features of AD are cerebral plaques laden with β-amyloid peptide (Aβ) and dystrophic neurites in neocortical terminal fields as well as prominent neurofibrillary tangles in medial temporal-lobe structures [64].

β-amyloid peptides arise from sequential cleavage of amyloid precursor protein (APP) by BACE-1 (beta-site APP-cleaving enzyme 1) and γ-secretase. Aβ can either self-aggregate into oligomers of 2 to 6 peptides which coalesce into intermediate assemblies [65, 66] or into fibrils which arrange themselves into β-pleated sheets. An imbalance between production and clearance of peptides followed by Aβ aggregation causes it to accumulate in insoluble plaques. This is termed the 'amyloid hypothesis' and is widely thought to be the initiating factor in AD [64]. Application of dimers and trimers of Aβ to brain-slice preparations is toxic to synapses [67, 68] and the severity of cognitive defect in AD correlates with levels of oligomers in the brain [69]. In addition to Aβ accumulation, neurofibrillary tangles of filamentous inclusions in pyramidal neurons occur in AD as well as in other neurodegenerative diseases termed tauopathies [70]. The major component of these tangles is an abnormally hyper-phosphorylated and aggregated form of tau. Hyper-phosphorylated tau self-associates into paired helical filament structures that are cytotoxic [71] and impair cognition [72]. Experimental evidence indicates that Aβ accumulation precedes and drives tau aggregation [73-75], supporting the argument for the 'amyloid hypothesis'.

The combination of the aging brain, extracellular amyloid plaques and neurofibrillary tangles has been found to lead to synaptic failure, oxidative stress, inflammation, loss of calcium regulation, axonal transport deficits, aberrant cell cycle re-entry, depletion of neurotrophins and neurotransmitters, neuritic dystrophy, neuronal loss and severe mitochondrial failure [76, 77]. All of which lead to the energy failure, synaptic dysfunction and neuronal death that cause progressive memory loss and decline in cognitive abilities. Most therapies currently used to treat AD focus on modifying the symptoms of the disease, only targeting the behavioral and cognitive symptoms and not the underlying cause. A number of experimental therapies for AD are currently focused on reducing Aβ load, including decreasing Aβ production with secretase inhibitors [78], increasing Aβ degradation [79-82], and promoting Aβ-specific immunity [83-85]. However, problems with toxicity and clearing the BBB have hampered efforts to treat AD [86, 87]. Furthermore, although active and passive Aβ immunotherapies have been shown to lower cerebral Aβ levels and improve cognition in animal models of AD, dosing of the phase II clinical trial of the AN1792 Aβ vaccine in humans was stopped when approximately 6% of the immunized patients developed meningoencephalomyelitis [87, 88]. Thus, a method for reducing Aβ levels without involving the immune system is desired.

Soluble Nogo Receptor and Alzheimer's Disease

After finding that NgR can bind to APP and Aβ, Park et al. began a sequence of experiments to determine if this affinity could be harnessed to lower cerebral Aβ levels [89, 90], (Fig. **2**). The authors began by determining NgR localization in human AD brain samples and found that neuronal expression was shifted out of the cell soma in these patients and that it was concentrated in amyloid plaques [89]. These findings were confirmed in a transgenic mouse model of AD, APPswe/PSEN-1(ΔE9) [91, 92]. In *in vitro* experiments, they found that APP interacts with NgR on transfected cells and brain and that this interaction occurs via the Aβ domain [89]. They later used mutagenesis to define the amino acid residues required for the Aβ-NgR interaction and found that NgR amino acids 210, 256, 259, and 284 are likely to contribute selectively to Aβ but not Nogo-66 interaction [90]. They demonstrate that the 15-28 central residues of Aβ bind to a pocket on the concave surface of NgR that can be distinguished from the surface required for Nogo-66 binding. The binding sites for the two ligands appear to be closely apposed along the mid-portion of the concave surface [90].

To define the physiological relevance of these binding partners, their interaction was determined in an *in vivo* setting. The authors first found an increased Aβ accumulation in the hippocampal dentate gyrus and cerebral cortex of mice lacking NgR [89]. They also found that intracerebroventricular administration of NgR(310)ecto-Fc to transgenic APPswe/PSEN-1(ΔE9) mice reduces Aβ plaque deposition and there is an inverse relationship between the level of NgR and the level of Aβ, plaque deposits, and neuritic dystrophy [89]. Furthermore, delayed subcutaneous administration of NgR(310)ecto-Fc suppressed histological evidence of Aβ-associated disease in transgenic mice and resulted in improved short-term memory in the radial arm water maze task [90]. Thus, Park et al. demonstrate that peripheral administration of NgR can act as a 'sink' to bind Aβ peptide in the periphery, thereby reducing its accumulation in the CNS, resulting in improved memory outcomes. They also demonstrate that very little NgR(310)ecto-Fc was detected in CNS after subcutaneous administration. Therefore, it cannot pass the BBB in APPswe/PSEN-1ΔE9 transgenic mice, reducing the risk for toxic side effects in the CNS. Moreover, as opposed to active or passive immunological reagents, NgR is an endogenous protein and is not predicted to activate an immune response or to require cell-mediated mechanisms to reduce Aβ levels and Aβ-associated cognitive deficits. Consequently, peripheral NgR(310)ecto-Fc provides a promisingly efficacious and seemingly safe therapy for sequestering Aβ, reducing the formation of plaques, decreasing dystrophic neurites and improving spatial memory and learning.

LINGO-1 and Multiple Sclerosis

Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis

Multiple Sclerosis (MS) is the most frequent neurological disease in young adults, affecting one in 1000 people, with females being twice as susceptible as males. MS is an inflammatory-mediated demyelinating disease of the CNS that causes progressive neurological disability [93] and occurs in genetically susceptible individuals after an environmental trigger, such as exposure to viral pathogens. The current hypothesis of disease pathogenesis is that MS is initiated by autoreactive T lymphocytes specific for oligodendrocytes and myelin, followed by a phase of selective demyelination mediated by microglia, macrophages and other invading peripheral immune cells [94-97]. This leads to a phase of axonal loss thought to arise from a combination of a direct immune-mediated attack against axons and the lack of myelin-provided trophic support.

The most common form of MS begins as a relapsing remitting (RR-MS) disease course. Over time, relapses become more frequent and the disease is described as being secondary progressive (SP-MS). RR-MS is characterized by acute CNS lesions that usually arise in areas of white matter and are accompanied by a disturbance in the BBB, local edema and demyelination. These lesions can spontaneously resolve, even in the absence of clinical attacks. It is thought that inflammatory activity is the main driving force behind RR-MS. As the disease progresses to SP-MS, inflammatory activity is much less conspicuous, despite faster evolution of disability. At present, treatments for MS non-specifically block or modify the pro-inflammatory immune response (for example Interferon-βs, glatiramir acetate and glucocorticosteroids), and most treatments currently in clinical trials are also immunomodulatory [98]. These treatments thus target the initial stage of the disease and are largely successful in modifying inflammation and disease progression; however there is currently no treatment for neuroregeneration or remyelination. There is a large volume of ongoing research dedicated to finding therapies that are more efficacious for MS patients. The aim is to treat both the inflammatory and degenerative sides to the disease, as well as being easy to administer with minimal side effects. There has already been a considerable quantity of possible therapies that were successful in the animal model of MS (EAE, described below) but were either unsuccessful or caused devastating side effects in clinical trials [99-103].

The standard animal model for MS is experimental autoimmune encephalomyelitis (EAE) [104]. This is induced in mammals by immunization with myelin peptides such as proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG) or myelin basic protein (MBP) in complete Freund's adjuvant. In order to achieve full induction of EAE in mice, it is necessary to also inject pertussis toxin. EAE is a CD4+ T cell–mediated autoimmune disease characterized by perivascular CD4+ T cell, mononuclear cell inflammation and subsequent primary demyelination of axonal tracts in the CNS, leading to progressive hind-limb paralysis. Due to the complexity of MS, comprising heterogeneous clinical, pathological and immunological phenotypes, the simplistic EAE model is unfortunately not an ideal model for the disorder. However, it is the accepted model of MS and is useful when determining specific components of the disease such as CNS immunity, autoimmunity, and oligodendrocyte degeneration.

Blocking LINGO-1 in Multiple Sclerosis

As mentioned above, LINGO-1 has recently been found to play an important role in negative regulation of oligodendrocyte differentiation and myelination. Due to the oligodendrocyte-targeted pathophysiology of MS, an interesting possibility has arisen to promote remyelination in MS patients by blocking LINGO-1. A pre-clinical study has been completed observing the effects of inhibiting LINGO-1 after MOG-induced EAE in adult rats [25]. In a first experiment, the authors found that inducing EAE in LINGO-1 KO mice resulted in a less severe disease course compared to WT. This was the same when EAE was induced and the animals were then treated with anti-LINGO-1 antibody. EAE was less severe in animals treated with anti-LINGO-1 both intrathecally before disease onset, and systemically by intraperitoneal injection after attaining a disease score of 1 out of 5. Improved disease course was correlated with physiological improvements in axonal integrity as revealed by DTI imaging, and histological staining and electron microscopy revealed enhanced and new myelination respectively. In addition to promoting oligodendrocyte differentiation and myelination, anti-LINGO-1 has also been reported to promote axonal growth *in vitro* [22]. Furthermore, treatment with anti-LINGO-1 antibody after SCI improved hind limb function, and this correlated directly with enhanced axonal regeneration, less axon retraction, reduced RhoA activation,

and increased neuron and oligodendrocyte survival adjacent to the lesion [28], (Fig. **2**).

With the discovery of growth factors, hopes were high that it would finally be possible to promote regeneration in the adult CNS. However, the disappointing outcomes of clinical trials using growth factors such as BDNF, NGF, CNTF, PDGF and GDNF [105-113] suggests the importance of looking for targets of regeneration that are more tissue-specific and less toxic. LINGO-1 is a compelling prospect due to its CNS-specific expression and the above-mentioned negative regulatory functions. Moreover, LINGO-1 is found to be up regulated in human MS and Parkinson's disease [114], suggesting it plays an active role in preventing oligodendrocyte differentiation during these diseases and would be an important target for therapeutic intervention. Since January 2010, Biogen Idec has been recruiting healthy volunteers for an ascending dose study to determine the safety of anti-LINGO-1 in humans. This is a promising avenue to finally be able to modulate the degenerative component of MS. If it is well tolerated, it will most likely be administered in combination with the immunomodulatory drugs that are currently in use. Furthermore, it will also be necessary to fully identify interacting proteins of LINGO-1 as well as the exact down-stream signaling cascade and what possible ligands or signaling mechanisms are involved.

CONCLUSIONS

After many years of thinking that regeneration was impossible in the adult CNS, experimental factors to promote regeneration are finally reaching patients in the first steps of clinical trials. In the current review, we have focused on three examples of such factors targeting the NgR complex. A major benefit of the examples demonstrated here is that the inhibitory factors can also be used in a variety of other CNS injuries and neurodegenerative disorders. For example, blocking LINGO-1 to promote oligodendrocyte proliferation and differentiation could be a possible therapy not only for MS, but also for other demyelinating diseases such as optic neuritis, Devic's disease and transverse myelitis. Furthermore, blocking binding of myelin-associated inhibitory factors to their receptors has already been a subject of interest in animal models of stroke and optic nerve degeneration. In addition, preventing toxic accumulation of protein in the CNS by subcutaneous injection of binding proteins provides a prototype for other disorders involving protein aggregation in the CNS such as Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and prion diseases.

Although these targets appear to be very promising, there is still some controversy regarding the mechanisms of action of the inhibitory factors used. For example, the exact role of Nogo and NgR, as well as other inhibitory factors and receptors are still to be elucidated. This is exemplified in the various KO experiments for Nogo-A, -B, -C, MAG, OMgp, NgR1 and NgR2 [17, 55, 57, 58, 60, 61] as well as the findings that paired immunoglobulin-like receptor B (PirB) is a functional receptor for Nogo, MAG and OMgp [115] and that B lymphocyte stimulator (BLyS) is a functional ligand for NgR [116]. These results suggest that there are perhaps compensatory mechanisms of neurite outgrowth inhibition and the inhibitory factors currently being used may have other functions besides simply blocking outgrowth inhibition. Thus it will be interesting to discover the outcomes of the clinical trials as well as necessary to further characterize the molecular pathways involved in NgR1 and LINGO-1 binding and signaling.

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