Journal Club

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Refining Our Understanding of NgR1 Function during Myelin Inhibition

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Review of Chivatakarn et al. (http://www.jneurosci.org/cgi/content/full/27/27/7117)

Although neurons have the inherent potential to regenerate their neurites, the mature CNS environment is not permissive for neurite outgrowth after injury. Both myelin debris and the astroglial scar actively inhibit regeneration by challenging the severed axon with inhibitory proteins that signal to the axon and prevent growth. Several inhibitory proteins have been identified in inhibitory fractions of myelin including Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte myelin glycoprotein (OMgp). Interestingly, these myelin-associated inhibitors (MAIs) can signal through a common receptor present on neurons, the Nogo-66 receptor 1 (NgR1). Thus, NgR1 has become an attractive target to promote CNS regeneration.

NgR1 activity has been neutralized by several approaches. Experiments with peptide antagonists of NgR1 and a dominant-negative mutant of NgR1 demonstrated promising results in reversing myelin inhibition *in vitro* and *in vivo* (Table 1). However, subsequent studies examining CNS repair in two independent NgR1-null mice strains failed to fully support the expectations raised by the molecular antagonists to NgR1. Kim et al. (2004) demonstrated that NgR1 ablation reversed growth cone collapse of dorsal root ganglion (DRG) neurons *in vitro*, but they did not assess the effect of NgR1 ablation on neurite outgrowth. They also observed enhanced regeneration of raphespinal and rubrospinal tracts and locomotor recovery in NgR1-null mice after spinal cord injury. Zheng et al. (2005) did not observe an effect of NgR1 ablation on neurite outgrowth of cerebellar or DRG neurons, but they did not assess growth cone collapse. Neither group detected long-distance regeneration of corticospinal tract (CST) axons in a dorsal hemisection model raising some controversy regarding the *in vivo* role of NgR1 in myelin inhibition and global CNS regeneration. One plausible explanation for this discrepancy is that NgR1 activation is essential for growth cone (GC) collapse, but not long distance growth inhibition. However, this explanation would require cell type-specific effects because raphespinal and rubrospinal tract regeneration was improved in NgR1-null mice.

In a recent paper in *The Journal of Neuroscience*, Chivatakarn et al. (2007) tackled this issue using two knockdown techniques to address whether NgR1 signaling is necessary for MAI-dependent growth cone collapse and neurite growth inhibition. In outgrowth assays using Chinese hamster ovary cells expressing MAG and substrate-bound OMgp, axons from cerebellar, DRG, and cortical neurons of NgR1-null mice did not show enhanced growth [Chivatakarn et al. (2007), their Figs. 1, (http://www.jneurosci.org/cgi/content/full/27/27/7117/F1), 2 http://www.jneurosci.org/cgi/content/full/27/

7117/F2), 3D (http://www.jneurosci.org/cgi/content/full/27/27/7117/F3) and supplemental Fig. 1 (http://www.jneurosci.org/cgi/content/full/27/27/7117/DC1)]. Notably, the authors restricted their analysis to neurons with processes equal or longer than one cell body diameter. This differs from several studies that assess the percentage of cells with neurites greater than one or more cell body diameter or total neurite outgrowth per cell (Grand-Pre et al., 2002; Vyas et al., 2002; Wang et al., 2002; Winton et al., 2002). Although one may argue that outgrowth of <1 cell body diameter is not "long distance" outgrowth, it is difficult to extrapolate the outgrowth from an overnight *in vitro* assay to longer term *in vivo* studies. Substrate-bound MAIs can reduce and/or completely inhibit neurite outgrowth, thus reanalyzing the data using a different methodology might unmask a subtle effect of NgR1 removal. Although the developmental stage, substrate, and the type of neurons used in these assays varied, the same phenotype was observed. These results suggest that NgR1 is not required for MAI-dependent inhibition of cerebellar, DRG, and cortical neurite growth.

To address the possibility of compensatory changes in the NgR1-null mouse, Chivatakarn et al. (2007) used shRNA-mediated knockdown of NgR1. NgR1 knockdown failed to reverse MAG-dependent inhibition [Chivatakarn et al. (2007), their Fig. 3 (http://www.jneurosci.org/cgi/content/full/27/27/7117/F3)]. Although this result seems to differ from previous observations demonstrating that

Table 1. Summary of various approaches used to neutralize NgR1

Strategy	In vitro		In vivo	
	GC collapse	Neurite outgrowth	Axon regeneration	Behavioral recovery
NEP1–40[a]	E12 chick DRG with Nogo-66	E12 chick DRG on myelin	Corticospinal tract	Locomotor
NgR(1–310)ecto[b,c]	ND	E13 chick DRGs on Nogo-66 and myelin	Corticospinal and raphespinal tracts	Locomotor
DN-NgR1[d,e]	ND	P7–P9 mouse CGNs on Nogo-66, MAG, OMGp, and myelin	Growth-stimulated retinal ganglion cells	ND
NgR1-null mice[f,g]	P6 DRGs with Nogo-66, MAG, OMGp, and myelin	ND	Raphespinal and rubrospinal tracts[f] and corticospinal fibers[g]	Locomotor[f]
NgR1-null mice[h]	ND	No reversal for P7 CGNs and P10 DRG neurons on Nogo-66 and myelin	No corticospinal tract regeneration	No recovery

To assess the effects of NgR1 on long-distance regeneration and functional recovery in CNS injury models, NgR1 activity has been neutralized by several approaches. The small Nogo-66 (1–40) antagonist peptide (NEP 1–40) is a competitive antagonist of the Nogo-66 binding site of NgR. The soluble ligand-binding domain of NgR1 [NgR(310)ecto] cannot interact with coreceptors. A dominant-negative form of NgR (DN-NgR) lacks the last 136 aa in the C-terminal domain, which prevents NgR1 binding to coreceptors such as p75. Most of the neurons used in these studies are cerebellar granule neurons (CGNs) and DRG neurons. E, Embryonic day; ND, not determined. [a]GrandPre et al., 2002. [b]Fournier et al., 2002. [c]Li et al., 2004. [d]Wang et al., 2002. [e]Fischer et al., 2004. [f]Kim et al., 2004. [g]Cafferty and Strittmatter, 2006. [h]Zheng et al., 2005.

siRNA-mediated knockdown of NgR1 promoted DRG outgrowth on myelin, there are several differences between the two studies (Ahmed et al., 2005). Most notable are differences in inhibitory substrates; Ahmed et al. (2005) applied soluble myelin whereas Chivatakarn et al. (2007) grew neurons in the presence of MAG. This could be important because MAG also signals through NgR2 and MAG accounts for a small proportion of the inhibitory activity of total myelin. NgR1 signaling may be sufficient for MAI-induced inhibition in some neuronal types and not others. Although MAG- and OMgp-dependent neurite outgrowth inhibition was not attenuated in DRGs derived from the NgR1 null mice, the incomplete knockdown achieved with shRNAi complicates the interpretation [Chivatakarn et al. (2007), their Fig. 3B (http://www.jneurosci.org/cgi/content/full/27/27/7117/F3)]. The efficacy of the shRNA-dependent knockdown of NgR1 could be assessed by treating cells with various MAIs and assessing levels of RhoA activation.

Although concluding that NgR1 is not required to inhibit axonal growth in the presence of MAIs, Chivatakarn et al. (2007) also revisited the role of NgR1 during growth-cone collapse. GC collapse was significantly reversed in DRGs of NgR1-null mice when presented with MAG and OMgp, but not Sema3A [Chivatakarn et al. (2007), their Fig. 4 (http://www.jneurosci.org/cgi/content/full/27/27/7117/F4)]. These results support previous observations (GrandPre et al., 2002; Kim et al., 2004). The inhibition obtained in this assay leaves a small window for reversal of inhibition; however, these are difficult experiments to perform because of the high basal GC collapse and poor growth of postnatal day 25 DRG explants. It would be interesting to complement this data with a growth cone collapse assay using CNS neurons. Nevertheless, Chi-

vatakarn et al. (2007) used adult DRGs in the outgrowth and GC collapse assays, demonstrating a distinction between MAI-mediated GC collapse and neurite outgrowth inhibition.

Chivatakarn et al. (2007) have neatly consolidated opposing interpretations of *in vitro* data examining MAI responses of neurons from two strains of NgR1 knock-out mice. Although it is still premature to conclude that NgR1 mediates GC collapse but not neurite outgrowth inhibition in all neurons and in response to each MAI, the idea that collapse and neurite outgrowth inhibition are mechanistically dissociated is intriguing. The data presented here does raise some interesting issues. If NgR1 is not required for neurite outgrowth inhibition, then how does one explain the long-distance regeneration of the raphespinal and rubrospinal tracts seen in the NgR1-null mice (Kim et al., 2004)? Although NgR1 activity is required for MAI-induced inhibition in some neurons, it is plausible that another yet unidentified receptor mediates inhibition in other neurons. Outgrowth assays using neurons from the red and raphe nuclei could test this idea. However, NgR1 may be required for MAI-induced neurite outgrowth inhibition of additional neuronal populations *in vivo* because a pervious study has shown regeneration of the CST axons after unilateral pyramidotomy in NgR1-null mice (Cafferty and Strittmatter, 2006). The beneficial effect of NgR1 ablation in some CNS tracts could be masked by other inhibitory factors such as the glial scar and chondroitin sulfate proteoglycans. These observations are a useful reminder of the limitations of translating *in vitro* assays to the more complex *in vivo* situation.

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