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Myelin-Associated Glycoprotein-Mediated Signaling in Central Nervous System Pathophysiology

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Abstract

The myelin-associated glycoprotein (MAG) is a type I membrane-spanning protein expressed exclusively in oligodendrocytes and Schwann cells. It has two generally known pathophysiological roles in the central nervous system (CNS): maintenance of myelin integrity and inhibition of CNS axonal regeneration. The subtle CNS phenotype resulting from genetic ablation of MAG expression has made mechanistic analysis of its functional role in these difficult. However, the past few years have brought some major revelations, particularly in terms of mechanisms of MAG signaling through the Nogo-66 receptor (NgR) complex. Although apparently converging through NgR, a readily noticeable fact is that the neuronal growth inhibitory effect of MAG differs from that of Nogo-66. This may result from the influence of coreceptors in the form of gangliosides or from MAG-specific neuronal receptors such as NgR2. MAG has several other neuronal binding partners, and some of these may modulate its interaction with the NgR complex or downstream signaling. This article discusses new findings in MAG-forward and -reverse signaling and its role in CNS pathophysiology.

Index Entries: Axonal regeneration; myelin; myelin-associated glycoprotein; Nogo-66 receptor (NgR); $p75^{NTR}$.

Introduction

The myelin-associated glycoprotein (MAG) is a type 1 transmembrane protein found in the peri-axonal membrane of both the central nervous system (CNS) and peripheral nervous system (PNS) myelin sheaths. Alternatively, splicing results in two isoforms: small (S-; 582 residues) and large (L-; 626 residues) MAG *(1)*. In rodents, L-MAG levels peak during CNS development and decline in adulthood, but S-MAG is the predominant form in both adult PNS and CNS *(2)*. Conversely to rodents, the human L-MAG splice variant predominates in adult human brain, whereas human S-MAG is

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most abundant in the PNS *(3)*. Both forms differ only in the cytoplasmic C-terminus but have the same N-terminal extracellular domain, which contains five immunoglobulin-like motifs. MAG is a member of the sialic acid-dependent immunoglobulin-like family member lectins (siglec) family *(4)*. Because MAG is designated siglec-4a, much, but not all, of its interaction with neurons occurs in a sialic-acid-dependent manner *(5)*.

Despite being initially described as a protein that confers neural adhesion and neurite outgrowth function *(6)*, it soon became apparent that MAG is a myelin-associated inhibitor of neurite outgrowth and axonal regeneration *(5,7–9)*, at least in vitro and when examined using adult neurons. Recombinant MAG coated onto beads induces hippocampal neuron growth cone collapse *(9)*. Proteolytically generated, soluble MAG consisting of the extracellular domain is also a potent inhibitor of neurite outgrowth *(10,11)*. However, analysis using MAG knockout mice *(12)* has not provided strong support to the notion of MAG as a major CNS neurite growth inhibitor. There was no apparently significant difference in cell spreading, neurite elongation, or growth cone collapse of several cell types when myelin preparations from either MAG-deficient or wild-type mice were used as a substrate. Furthermore, the extent of axonal regrowth in optic nerve and corticospinal tract lesions in vivo was equally poor in MAG-deficient and wild-type mice. Because axonal regrowth in MAG-deficient mice could be similarly enhanced in wild-type mice by the Nogo-targeting antibody IN-1, it appears that MAG does not carry the majority of the neuronal growth inhibitory activity of myelin.

Earlier investigations using oligodendrocyte neuron cocultures and the analysis of MAG-deficient mice also suggested a role for MAG in the initiation of CNS myelination as well as the long-term preservation of myelin sheaths (extensively reviewed in ref. *13*). Again, however, the subtlety of the phenotypes observed made it difficult to view MAG as a major functional component of myelin. It is obvious that other oligodendroglia components could functionally cover for the complete absence of MAG during development. MAG maybe involved in reverse signaling processes in oligodendrocytes, because crosslinking of MAG with antibodies activates Fyn tyrosine kinase *(14,15)*. The latter phosphorylates the cytoplasmic domain of L-MAG, which is the longer splice isoform specifically involved in CNS myelination *(16)*, principally at tyr 620 *(17)*. Mild hypomyelination was observed in optic nerves of MAG-deficient mice. This is worse in Fyn-deficient mutants, and the severity of the defects were additive in MAG/Fyn double mutants *(18)*, suggesting that although both MAG and Fyn are important for the initiation of myelination, they may nonetheless act independently.

The past 3 yr had provided substantial breakthroughs in our understanding of the forward signaling of MAG. In 2002, MAG was shown to interact with the leucine-rich repeat (LRR)-containing, GPI-linked Nogo-66 receptor (NgR) *(19)*, which transduces its growth inhibitory effect on neurons *(20,21)*. The latter was initially cloned as a neuronal receptor mediating the neurite outgrowth inhibition by the extracellular 66-amino acid loop (termed Nogo-66) common to all major splice isoforms of Nogo *(22)*, the target of the neurite growth-enhancing IN-1 antibody *(23)*. Another myelin-associated protein, oligodendrocyte-myelin glycoprotein (OMgp), also inhibits neurite growth through the NgR. The NgR transduces a growth inhibition signal by engaging the transmembrane $p75^{NTR}$ *(24,25)* or TAJ/TROY *(26,27)* in conjunction with another LRR-containing transmembrane protein known as LINGO-1 *(28)*. Signaling through $p75^{NTR}$ ultimately leads to the activation of Rho and its effectors, resulting in changes of the actin cytoskeleton that underlie growth cone collapse or repulsion *(29)*.

In the midst of these new developments, it should be emphasized that the effect of MAG on neurite growth is qualitatively different from that of Nogo-66. This article reviews new findings on MAG's forward inhibitory signaling to neurons, highlighting the differences

with that of Nogo-66 and citing references to MAG's neuronal binding partners. It then briefly surveys some new implications of MAG in CNS pathology.

MAG's Neuronal Interacting Partners I: Sialoglycolipids

In the CNS, MAG is localized to myelin membranes juxtaposed to axons *(30)*, and purified MAG protein incorporated into liposomes binds neuronal processes *(6)*. Although much of the recent excitement has focused on MAG's interaction and action through the NgR, the latter is not the only neuronal molecule interacting with MAG. A prominent feature of MAG–neuron interaction is dependent on sialic acid, a feature that is not apparent in earlier reports of MAG–NgR interaction *(20,21)*. MAG binds best to 2,3-linked sialic acid on a Gal(β1→3)GalNac core structure *(31,32)*. Interestingly, MAG's sialic acid binding site is actually distinct from its neurite inhibitory activity. This was elegantly illustrated as a truncated form of soluble recombinant MAG (MAG-Fc; containing Ig domains 1, 2, and 3 but missing domains 4 and 5, fused to the immunoglobulin heavy-chain), which was bound to neurons in a sialic-acid-dependent manner but did not inhibit neurite outgrowth like full-length MAG. Mutation of arginine 118 (R118) in MAG to either alanine or aspartate abolished its sialic-acid-dependent binding. However, R118-mutated MAG retained a weakened capacity of inhibiting neurite outgrowth and remained a potent inhibitor when expressed at cell surfaces rather than being added as a soluble protein *(33)*.

The sialic acid moiety alluded to earlier is often found on a class of glycosphingolipids known as gangliosides, and MAG has been shown to bind a limited set of structurally related gangliosides known to be expressed in myelinated neurons. These include the major brain ganglioside GD1a and GT1b, as well as a minor ganglioside GQ1bα, expressed on cholinergic neurons *(34–36)*. Gangliosides mediate MAG's inhibition of neurite outgrowth from primary rat cerebellar granule neurons, because the latter is clearly attenuated by neuraminidase treatment of the neurons, blocking of neuronal ganglioside biosynthesis, or antiganglioside monoclonal antibodies. Furthermore, multivalent clustering of GD1a or GT1b using precomplexed antiganglioside antibodies mimicked MAG's inhibitory effect *(35)*. An earlier study showed that antibody-crosslinking of cell surface GT1b, but not GD1a, mimicked the effect of MAG. Notably, the Rho kinase (ROCK) inhibitor Y27632 blocked inhibition of neurite outgrowth by both MAG and anti-GT1b antibody. Activation of Rho-ROCK is a universal convergent point downstream of neurite outgrowth inhibitors, including all the major neuronal guidance ligand-receptor systems *(37)*. Therefore, the gangliosides appeared to be an authentic neuronal MAG receptor mediating its neurite growth inhibitory function.

Because gangliosides are not membrane-spanning structures with a sizable cytoplasmic domain, they are incapable of relaying a signal to the cytoplasm. The initial picture of MAG signaling through gangliosides is that MAG binding causes ganglioside clustering in lipid rafts, which somehow results in the activation of signaling proteins found in such membrane microdomains. In other words, MAG-induced ganglioside clustering presumably leads to the activation of a coreceptor(s). It is apparent that $p75^{NTR}$ is a promising candidate for such a coreceptor *(38)*. Adult dorsal root ganglion neurons or postnatal cerebellar neurons from mice that are null for functional $p75^{NTR}$ are insensitive to MAG inhibition and Rho activation. MAG does not appear to interact directly with $p75^{NTR}$ but could associate with it through GT1b (which specifically associates with $p75^{NTR}$). Additionally, the newly discovered Nogo–NgR system also searches for a transmembrane signaling coreceptor. Based on these leads, research has demonstrated that MAG, similarly to Nogo-66 (and OMgp), can bind NgR and engages $p75^{NTR}$ in neurite growth inhibitory signaling *(24,25)*.

If we have a direct link between MAG and p75^{NTR} in the form of NgR, are the gangliosides still necessary? The answer appears to be affirmative, and therein is one major difference between MAG and the other NgR ligands. Analysis of mice that were deficient in β1, 4-*N*-acetylgalactosaminyltransferase (ref. *39*; therefore lacking all complex gangliosides in the brain, including GD1a and GT1b) indicated that its neurons were no longer susceptible to the growth inhibitory effect of MAG-Fc but remained sensitive to Nogo-66 peptide *(40)*. On the other hand, mice deficient in GD3 synthase (lacking the b-series gangliosides—that is, those with GD1a but not GT1b; ref. *41*) were still susceptible to both MAG-Fc and Nogo-66. Gangliosides are apparently also necessary for MAG's activation of Rho. Interestingly, both MAG-Fc and Nogo-66 peptide induce clustering of p75^{NTR} into lipid rafts. The latter appears to be required for the growth cone collapsing activity of myelin-associated inhibitors, because this activity is abolished by cholesterol extraction with methyl-β–cyclodextrin *(40)*. It is clear from the aforementioned findings that gangliosides plays a role in modulating spatial, and perhaps temporal, response of axons to MAG in a way Nogo-66 is not subjected to.

How exactly does engagement of NgR and p75^{NTR} by MAG activate Rho? In the cell, Rho is kept inactive in the cytosolic pool by its binding to the Rho GDP dissociation inhibitor (RhoGDI) *(42)*. p75^{NTR}–RhoGDI interaction is apparently strengthened by the binding of MAG or Nogo to the receptor complex, and therefore, p75^{NTR} could activate Rho by somehow displacing it from RhoGDI. A new insight to this theory has been obtained. p75^{NTR} was known to undergo ectodomain shedding and regulated intramembrane proteolysis, an α-secretase-mediated process that generates an extracellular domain and a C-terminal motif. The latter is further cleaved by the γ-secretase complex to generate an intracellular domain *(43,44)*. Filbin et al. *(45)* have shown this PKC-dependent cleavage of p75^{NTR} by to be induced by MAG binding to cerebellar neurons, and it is necessary for Rho activation. As mentioned earlier, p75^{NTR} activates Rho, presumably by acting as a displacement factor that realeases Rho from Rho-GDI *(46)*. This finding has provided some possible resolution to the paradoxical observation that endogenous p75^{NTR} is in complex with Rho–RhoGDI and that MAG/Nogo-66 binding appears to strengthen existing p75^{NTR}–Rho–RhoGDI complexes rather than weaken it. Therefore, upon cleavage the intracellular domain fragment of p75^{NTR}, unlike full-length p75^{NTR}, could perturb RhoGDI's ability to inhibit GTP–GDP exchange on Rho, thus resulting in its dissociation from RhoGDI.

MAG's Neuronal Interacting Partners II: Sialoglycoproteins

Gangliosides are also not the only neuronal molecules that interact with MAG, because MAG-Fc binding to neurons is sensitive to trypsin (*47*), and a pull-down screen revealed several high-molecular-weight proteins interacting specifically with trypsin *(48)*. Two NgR homologs (designated NgR2 and NgR3) were recently identified in the mammalian genome *(49–51)*, but preliminary analyses provided the impression that these did not bind the known NgR ligands.

However, a more recent in-depth analysis revealed that MAG exhibits a sialic-acid-dependent affinity for NgR2 that was several-fold higher than NgR *(52)*. Interestingly, the study also revealed that MAG's interaction with NgR is sialic-acid-dependent, whereas Nogo-66's interaction with NgR was not. Additionally, Nogo-66 does not interact with NgR2. Therefore, in addition to that observed for the sialoglycolipids, MAG and Nogo differ in terms of sialoglycoprotein binding. Pertaining to MAG, NgR2 appears to have a role analogous to NgR because its ectopic overexpression in neurons confers susceptibility to inhibition by MAG. However, it has not been determined whether this inhibition also occurs via the engagement of p75^{NTR}/TAJ (and/or LINGO-1) as in the case of NgR.

MAG Signaling and Axon–Glia Pathology

MAG–ganglioside interaction goes beyond the signaling of neurite growth inhibition. In fact, it has been proposed that at least some of MAG's interaction with its neuronal ganglioside ligands is a lipid raft-to-lipid raft affair occurring on opposing oligodendroglia and axonal membranes *(53)*. This implies a structural organization for bidirectional signaling that may be important for the stabilization of the axon–glia interaction. Mice lacking complex gangliosides as a result of a deletion of GM2/GD2 synthase developed Wallerian degeneration, myelination defects, and, interestingly, a reduction in CNS MAG levels *(54)*. Researchers recently reported that when backcrossed to a more than 99% C57BL/6 strain purity, MAG-deficient mice exhibited marked CNS (as well as PNS) axonal degeneration *(55)* that was qualitatively similar to mice deficient in β1, 4-*N*-acetylgalactosaminyltransferase *(39)*.

As mentioned earlier, MAG is apparently connected to oligodendrocyte differentiation and myelination via its interaction with Fyn kinase. Therefore, a particular recent finding is noteworthy. Mi et al. *(56)* found that LINGO-1 is also expressed in oligodendrocytes and further showed that it appears to regulate myelination. Accordingly, attenuation of LINGO-1 expression or activity in primary oligodendrocytes increases myelination competence, whereas the overexpression of LINGO-1 inhibits oligodendrocyte differentiation and myelination. LINGO-1 affects the aforementioned processes via modulation of the expression and phosphorylation of Fyn, as well as the activation of Rho. The exact mechanism by which this is achieved is unknown, as is how MAG could be involved in this connection. On one hand, LINGO-1 action in oligodendrocytes may occur via pathways and signaling components independent of MAG until their convergence at Fyn and Rho. However, although MAG's interaction with the neuronal NgR complex containing LINGO-1 occurs *in trans*, it is not entirely inconceivable that MAG might interact in *cis* with a similar complex in oligodendrocytes and might modulate its activity somewhat. These possibilities remain to be explored.

MAG and Other CNS Neuropathology

An interesting recent finding implicated MAG's involvement, for the first time, in a human hereditary disorder: familial late-onset orthochromatic leukodystrophy *(57)*. This genetic neurological disease has no clear signs of neuropathy. Analysis of two members of an Italian family with the disease indicated their brain myelin contained a truncated from of L-MAG that is about 5 kDa shorter than wild-type. The defect is not the result of a mutation in either the coding or untranslated region of the *MAG* gene, and the alteration in MAG is likely to be secondary to a yet unknown primary defect that resulted in their production with age. It is unclear how this truncated form of L-MAG affects the CNS white matter. It should be noted that unlike rodents, L-MAG is the predominating form in the adult human CNS. Therefore, any alteration to the protein is likely to result in either a loss- or gain-of-function defect that could lead to oligodendroglial or neuronal pathology.

Another aspect of MAG that has been recently explored involves its use as a therapeutic target in CNS injuries. Preclinical intervention models targeting the NgR with *in situ* delivery of dominant-negative NgR proteins showed significant benefits in optic nerve injury and stroke *(58,59)*. However, a MAG-specific agent for CNS injury has not been developed and explored until recently. There are indications that a MAG-specific agent may be beneficial in brain injuries such as stroke. In the rat, it has been shown that L-MAG is re-expressed in oligodendrocyte cytoplasms in the white matter around experimental cerebral infarcts produced by middle cerebral artery occlusion (MCAO) *(60)*. MAG expression is elevated in cortical lesions, and neuraminidase treatment of axotomized entorhino-hippocampal cultures

Fig. 1. A schematic diagram of MAG-associated forward and reverse signaling pathways and components in the axon–glial system. Engagement of neuronal NgR–$p75^{NTR}$ and LINGO-1 by oligodendroglial MAG mediates neurite growth inhibition, as measured by growth inhibition assays, resulting in the activation of RhoA and suppression of Rac 1 (not shown here) *(29)*. The receptor complex presumably activates the trimeric G protein Gi and the downstream phospholipase C–protein kinase C (PKC)/Inositol 1,4,5-triphosphate (IP_3) pathways (as depicted in ref. *67*). PKC is activated by both phospholipase C-generated diacylglycerol and the elevation of growth cone cytoplasmic Ca^{2+} (a result of IP_3-induced Ca^{2+} release from internal stores). PKC appears to be important for Rho activation, because PKC inhibitors attenuate MAG's ability to inhibit neurite growth *(68)* and may, in some cases, enhance neurite growth *(67)*. It is unclear exactly how PKC activity might affect Rho activation (dotted lines), but intramembrane proteolysis of $p75^{NTR}$ by γ-secretase is apparently PKC-dependent *(45)*. Upon cleavage, the intracellular domain of $p75^{NTR}$ displaces Rho-GDI from Rho, leading to its activation by guanine nucleotide exchange factors (not shown here).

In growth cone turning assays, $p75^{NTR}$ and NgR are also responsible for the intracellular activation of Ca^{2+} that resulted in growth cone turning *(25)*. However, the effect of MAG-induced Ca^{2+} on growth cone behavior is complex. It may mediate growth cone repulsion or attraction, depending on the exact concentration of Ca^{2+} elicited *(69)*. This effect is modulated by intracellular cyclic adenosine monophosphate (cAMP) *(69,70)*, which is elevated by neurotrophin (N) signaling through Trk family receptors. The cAMP elevation has been demonstrated to overcome MAG's inhibition of neurite outgrowth *(71–73)*. The modulatory effect of cAMP and protein kinase A in modulating neurite outgrowth has been extensively reviewed. It is not described here, and the reader is referred to the excellent and in-depth discussion by Filbin *(74)* for more details. Note that neurite growth inhibition and growth cone repulsion and attraction are experimental phenomena. Different experimental paradigms have different emphasis regarding parameters measured. On the whole, it appears that the same set of pathways and components are engaged, but there may be subtle differences.

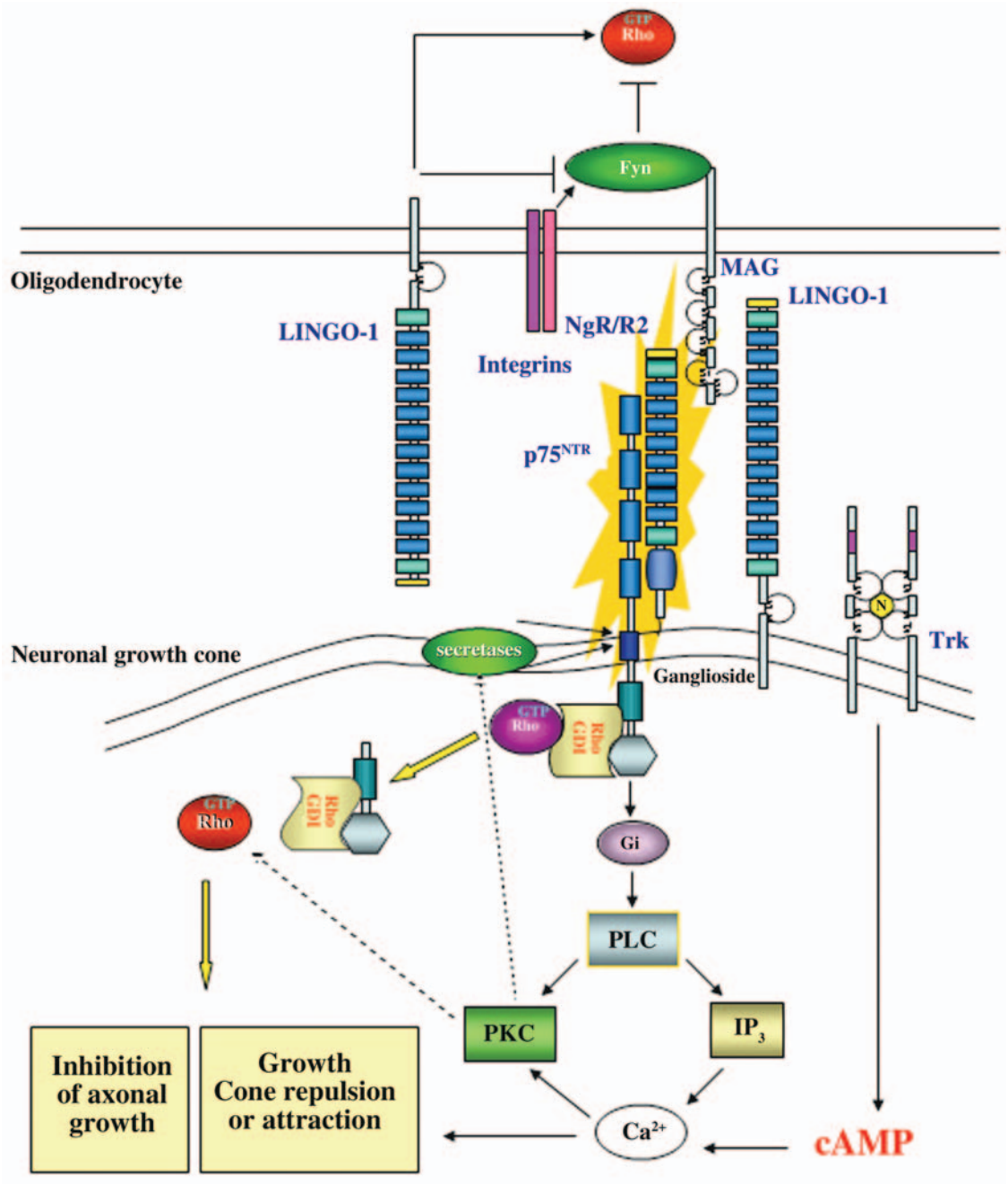
Fyn tyrosine kinase is a key modulator of oligodendrocyte differentiation and interacts with MAG. Crosslinking of oligodendroglial MAG by antibodies could result in Fyn phosphorylation. Differentiation of oligodendrocytes could be triggered by engagement of the extracellular matrix via integrins, whose signaling via Fyn to Rho family GTPases regulates morphological differentiation *(75)*. LINGO-1 on oligodendrocytes modulates its differentiation by activating Rho. It is unclear whether MAG has a direct role in this effect of LINGO-1.

potentiates axonal regeneration *(61)*. Irving and colleagues *(62)* found that an anti-MAG monoclonal antibody that could neutralize the neuronal inhibitory effect of MAG could also protect oligodendrocytes from glutamate-mediated oxidative stress-induced cell death. Importantly, this antibody showed significant beneficial effects in a rat model of MCAO. Central and systemic administration of the antibody 1 h after stroke induction significantly reduced infarct volume at 7 d. Neuroprotection was also associated with significant improvement in motor function. This finding indicates the potential for the use of anti-MAG antibodies as therapeutic agents for the treatment of stroke.

An emerging aspect of myelin-associated proteins in CNS pathology has occurred in the area of schizophrenia and bipolar disorder, which have been associated with oligodendrocyte dysfunction *(63,64)*. MAG has been shown to be downregulated in schizophrenic brains. Genetic studies linking polymorphisms at the MAG locus to schizophrenia have also been reported recently for Chinese family cohorts *(65,66)*. It remains to be seen whether these linkages are borne by more extensive studies, or whether the association between MAG and schizophrenia will be as controversial as that of Nogo.

Conclusion

Although a major portion of MAG's functional effect on neurons in CNS pathophysiology is likely to be mediated by NgR, MAG's actions differ from that of Nogo-66, as shown by the availability of ganglioside coreceptors (*see* Fig. 1 for a detailed review of some of the signaling pathways and components associated with MAG). Neuronal receptors that are



MAG-specific, such as NgR2, also confer a unique neuronal response to MAG. MAG clearly has other neuronal binding partners that have yet to be molecularly identified. Some of these may modulate its interaction with the NgR complex or downstream signaling, whereas others may have completely unrelated effects. MAG's reverse signaling in

oligodendrocytes has remained largely unexplored. The availability of promising new molecular handles may soon change this. Contraindicative to the subtlety of its knockout phenotype, further investigations in MAG–neuron interaction and MAG reverse signaling and continuous exploration of its value as a therapeutic target in CNS pathophysiology now appear to be worthwhile ventures.

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