Biochemical and Biophysical Research Communications 413 (2011) 92–97

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ganglioside mediate the interaction between Nogo receptor 1 and LINGO-1

Nayanendu Saha *, Momchil V. Kolev, Mariya Semavina, Juha Himanen, Dimitar B. Nikolov *

Structural Biology Program, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, NY 10021, USA

ARTICLE INFO

Article history:
Received 9 August 2011
Available online 22 August 2011

Keywords:
Ganglioside
LINGO-1
Nogo receptor
Nogo-A
p75

ABSTRACT

Upon spinal cord injury, the myelin inhibitors, including the myelin-associated glycoprotein (MAG), Nogo-A and the oligodendrocyte myelin glycoprotein (OMgp), bind to and signal via a single neuronal receptor/co-receptor complex comprising of Nogo receptor 1(NgR1)/LINGO-1 and p75 or TROY, impeding regeneration of injured axons. We employed a cell-free system to study the binding of NgR1 to its co-receptors and the myelin inhibitor Nogo-A, and show that gangliosides mediate the interaction of NgR1 with LINGO-1. Solid phase binding assays demonstrate that the sialic acid moieties of gangliosides and the stalk of NgR1 are the principal determinants of these molecular interactions. Moreover, the tripartite complex comprising of NgR1, LINGO-1 and ganglioside exhibits stronger binding to Nogo-A (Nogo-54) in the presence of p75, suggesting the gangliosides modulate the myelin inhibitor-receptor signaling.

1. Introduction

Following spinal cord injuries, the central nervous system axons are usually unable to regenerate due, in part, to a number of growth inhibitory factors that are present at the site of lesion [1]. The three myelin-specific inhibitors, MAG, OMgp, and Nogo-A, bind to a single receptor/co-receptor complex that includes the Nogo receptor (NgR1), LINGO-1 and either the p75 or TROY co-receptors [2–5]. The signal transducer p75 then undergoes regulated intramembrane proteolysis (RIP) by the combined action of alpha and gamma secretase activities. This releases an intracellular signaling peptide that induces Rho GTPase activation, inhibiting the axonal growth [6]. It is speculated that TROY can function in lieu of p75 but it is unclear whether it can undergo similar RIP. There is a functional redundancy between the inhibitors and the presence of any one inhibitor is sufficient to prevent regeneration by activating the common receptor/co-receptor complex. Interestingly, studies conducted over the past few years have revealed that trisialoganglioside (GT1b) and disialoganglioside (GD1a) are functional binding partners of MAG and may play a role in translocation of p75 to lipid rafts to initiate signal transduction [7]. It has also been shown that NgR1 has the ability to bind GT1b which could be important for its interaction with MAG [8].

NgR1 is a glycophosphatidyl (GPI)-anchored cell-surface receptor. In addition to NgR1, two other receptor isoforms, NgR2 and NgR3, were identified based on sequence similarity and biochemical homology [9–11]. N-terminally, Nogo receptors are comprised of highly conserved eight leucine-rich repeats (LRR) flanked by the N- and C-terminal cap regions (LRRNT and LRRCT, respectively), typical of the LRR family proteins [12]. The LRR domain is connected to the GPI membrane anchor via a "stalk" region. Although the stalk sequence is quite divergent among the NgRs, its importance has been clearly highlighted in the interaction of NgRs with their myelin inhibitors [13]. Cell-based and co-immunoprecipitation assays have pointed to the fact that the entire ectodomain of NgR1 is necessary for interaction with LINGO-1 and TROY, or with LINGO-1 and p75 [4,5]. To better understand the protein–protein interactions regulating these myelin-specific inhibitory signals and the role of gangliosides, we performed and describe here solution-based binding studies with the myelin inhibitor Nogo-A and its neuronal receptor/co-receptor complexes.

2. Materials and methods

Details regarding reagents constructs, cell culture, protein purification are provided in Supplementary data.

2.1. In vitro pull down experiments

In vitro "pull-down" experiments were performed with Protein-A-Sepharose in 10 mM HEPES (pH 7.4), 150 mM NaCl, 0.05% Triton-X100 at 25 °C for 1 h. The Protein-A-Sepharose bound protein mixtures were washed with the binding buffer and analyzed by SDS–PAGE (12.5%). To perform binding in presence of the trisialoganglioside (GT1b Na salt), the Fc-tagged proteins were preincubated with the glycolipid at a molar ratio of 1:10. For pull down with GST-Nogo-54, GST was used as a control.

* Corresponding authors. Fax: +1 212 7173135.
E-mail addresses: sahan@mskcc.org (N. Saha), nikolovd@mskcc.org (D.B. Nikolov).

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.08.060

2.2. Solid-phase binding assays

2.2.1. Binding experiments

Wells of a 96-well microtiter plate were coated in sextuplicate overnight at 4 °C with 0.1 ml of proteins, glycoproteins (2.5 μg/0.1 ml of HEPES buffer pH 7.4) or glycolipids/phospholipids (1 μg/0.1 ml in methanol). GT1b-Na (referred to as GT1b in the text) was coated in HEPES buffer. Unbound proteins and glycoconjugates were decanted and the wells, after blocking with non-fat dry milk, were incubated with various proteins at 25 °C for 1 h. Finally the color was developed (Supplementary data) and the readings were measured at A_{450}. Purified Fc fragment was used as a control and the A_{450} values were normalized with respect to Fc. The binding to GST-Nogo-54 was normalized with respect to GST.

2.3. Size exclusion chromatography

To measure the stoichiometry of the NgR1-Fc/LINGO-1/GT1b complex, we mixed NgR1-Fc, LINGO-1 and GT1b and subjected to gel filtration chromatography on a SD-200 (10/30 column, GE Healthcare) equilibrated with the same buffer. The column was pre-calibrated with a set of protein markers to evaluate the precise molecular weight and the stoichiometry of the complexes.

3. Results and discussion

3.1. Ganglioside mediate the interaction of NgR1 with LINGO-1

The Fc-fused ectodomains of NgR1 and the NgR2 are referred to as NgR1-Fc and NgR2-Fc, while the truncated versions comprising of the LRR repeats, as NgR1LRR or NgR2LRR, respectively. The ectodomain of LINGO-1 (35-549) comprises of LRRNT, LRR and LRRCT followed by a compact Ig module. The proteins were expressed and purified from the culture supernatants of HEK93 or Hi-five cells (Supplementary data). The proteins migrated at higher molecular weight than that predicted from their amino acid compositions due to glycosylation (Fig. 1A). We employed ELISA-based assays to study the interaction of NgR1 with LINGO-1 and p75 both in the presence and absence of ganglioside, using a ten fold molar excess of the glycolipid. The concentrations of Fc tagged proteins and anti-Fc antibody used for the binding assays were chosen from a predetermined antigen/antibody titration (Supplementary data). As observed in previous studies [4,14], we detected a binary interaction between the ectodomains of NgR1 and LINGO-1, NgR1 and p75 as well as LINGO-1 and p75 (Fig. 1B and C). However in the presence of GT1b, the interaction between LINGO-1 and NgR1 was significantly (three fold) stronger than that without the glycoplipid. The GT1b did not affect the binding of LINGO-1 to p75 or NgR1 to p75 (Fig. 1B and C). The results corroborate previous studies, but they also show for the first time that GT1b directly influences the interaction of NgR1 with LINGO-1.

3.2. The entire NgR1 ectodomain is required for the formation of a 2:1 complex with LINGO-1 in presence of ganglioside

Our biochemical pull down and ELISA-based experiments confirmed that NgR1 binds to LINGO-1 in the presence of GT1b (Fig. 2A and B). For the ELISA-based experiments we used microtiter plates pre-coated with GT1b. The homolog NgR2, which differs from NgR1 mostly in the stalk region, did not interact with LINGO-1 in presence or absence of GT1b (Fig. 2A, right panel). The deletion mutant NgR1 LRR also did not bind to LINGO-1 in presence or absence of GT1b indicating the stalk was necessary for complex formation (Fig. 2A). The results were further corroborated by

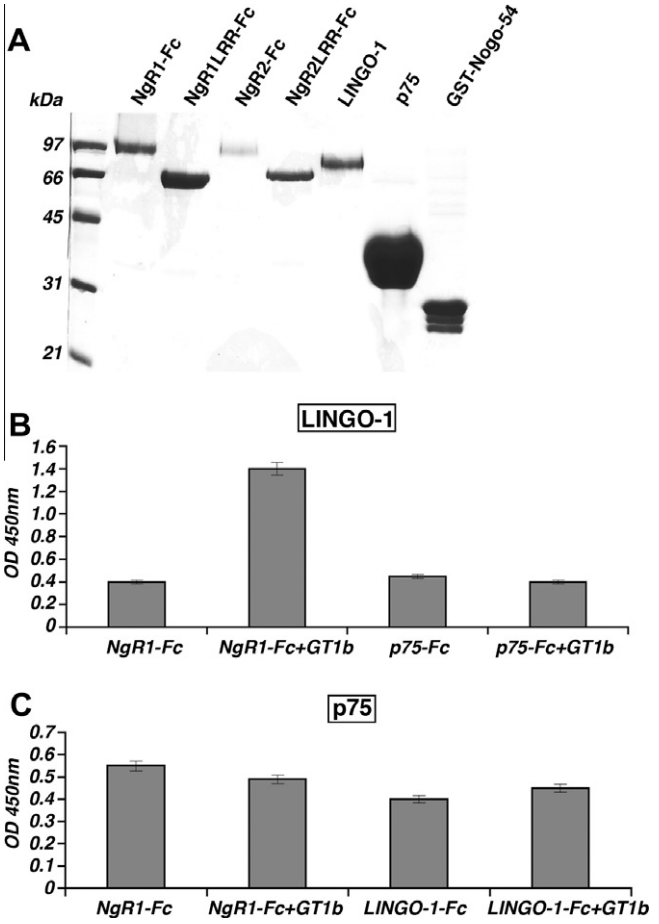

Fig. 1. Interaction of NgR1 with LINGO-1 and p75 in presence and absence of ganglioside (A) SDS–PAGE analysis of the purified proteins with or without the Fc tags. A standard set of protein markers were used to compare the subunit molecular weights (kDa) of the components. (B) Binding of NgR1-Fc and p75-Fc to LINGO-1 immobilized on microtiter wells in the presence and absence of GT1b. Briefly 4 μg of the Fc tagged proteins were used for assay. To monitor binding in the presence of GT1b, the proteins were mixed with the glycolipid at a molar ratio of 1:10. (C) Binding of NgR1-Fc and LINGO-1-Fc to p75 immobilized on microtiter wells in the presence and absence of GT1b. As above 4 μg of the Fc-tagged proteins were used for the assays.

biochemical pull down experiments (Fig. 2B). It is to be noted that in the pull-down experiments, unlike in the ELISA-based ones, we did not observe direct interaction between the ectodomains LINGO-1 and NgR1 in the absence of ganglioside. This is likely due to the fact that the pull-down experiments are less sensitive than the ELISA-based ones and detect only more stable complexes. We observed weak binding of NgR1 to pre-coated GT1b but the binding was significantly enhanced (fivefold) in the presence of LINGO-1. LINGO-1-Fc alone exhibited little or no binding to GT1b (Fig. 2A, left panel). The NgR2-Fc and its truncated version NgR2 LRR bind GT1b weakly (Fig. 2A right panel), almost to the same extent as its homolog NgR1-Fc but clearly showed no evidence of complex formation with LINGO-1. The truncated NgR1 LRR binds GT1b to the same extent as NgR1 ectodomain (Fig. 2A, left panel) suggesting that the FRG motifs in the LRR domains of NgRs might be responsible for the interaction [8]. The results clearly demonstrate that the principal determinants of NgR1/LINGO-1 complex formation are (i) GT1b and (ii) the stalk of NgR1 comprising of residues 312-460.

We next used size exclusion chromatography to study the NgR1/LINGO-1/GT1b complex formation. We mixed GT1b with NgR1 (30-460)-Fc and LINGO-1 and applied to a Superdex-200

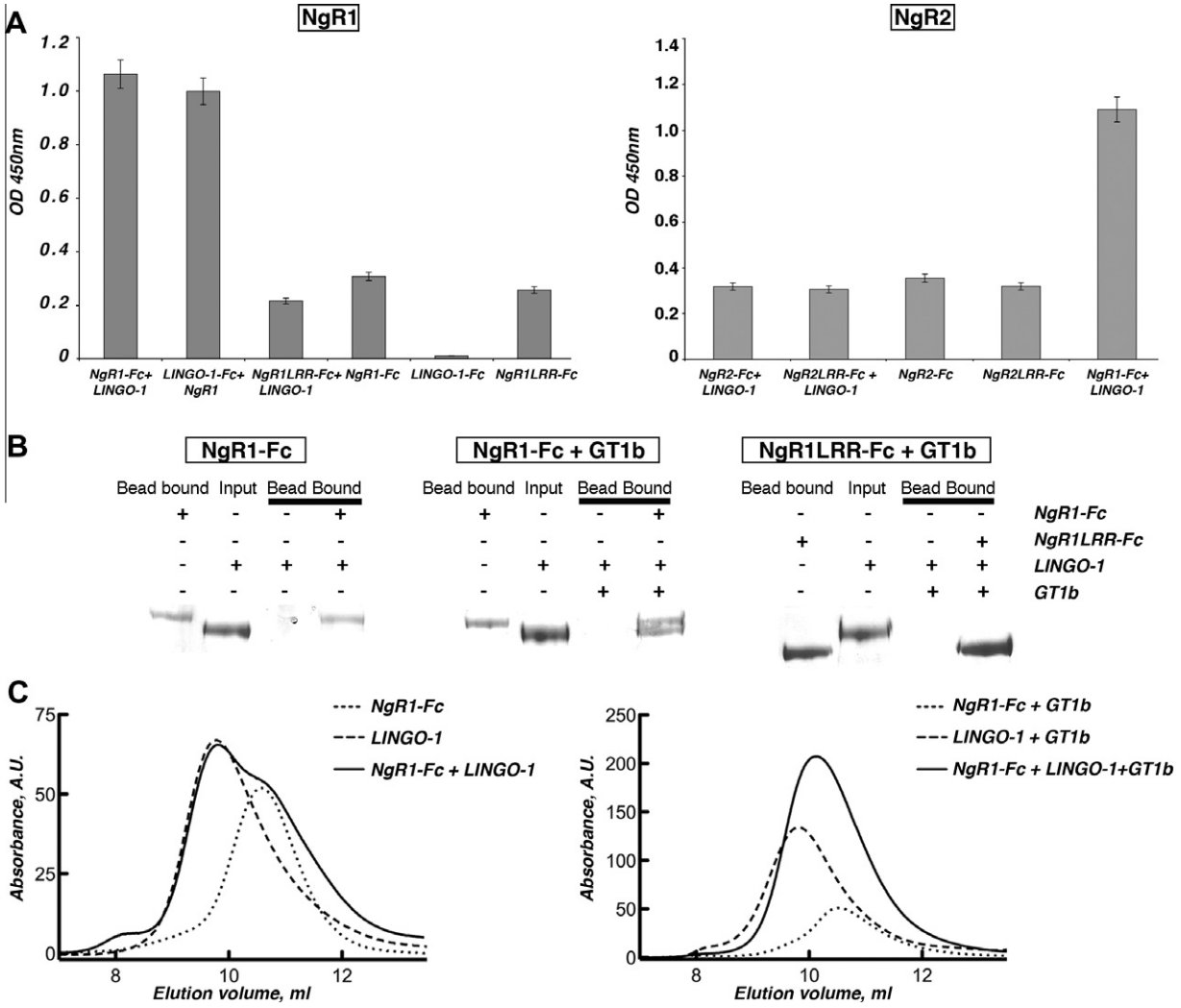

Fig. 2. Ganglioside mediated interaction of LINGO-1 with NgR1 A. (left panel) Binding of NgR1-Fc, NgR1LRR-Fc and LINGO-1 and (right panel) NgR2-Fc, NgR2LRR-Fc and LINGO-1 to precoated GT1b ELISA plates. Briefly, the Fc tagged NgRs were mixed with LINGO-1 at 1:1 M ratio and added to wells of microtiter plates precoated with GT1b. The data represent average of sextuplicate determination. The experiment was repeated twice and the maximum dispersion was within 10% of the mean value B. LINGO-1 binds to NgR1 (30-460)-Fc, but not to NgR1LRR (30-311)-Fc, in the presence of GT1b. Before addition to Protein-A beads, 1 ml fractions of NgR1-Fc (2 μg) or NgR1LRR-Fc (5 μg) were incubated with GT1b at a molar ratio of 1:10. The protein–ganglioside mixture was then incubated with LINGO-1 (4 μg, input), before addition to Protein-A Sepharose beads. The Protein-A bound (bead bound) and input fractions were analyzed on SDS–PAGE. LINGO-1 incubated with GT1b alone was used as a control. C. The elution profiles of NgR1 (30-460)-Fc, LINGO-1, and their GT1b-mediated complex, resolved on a Superdex-200 10/30-gel filtration column. Briefly, NgR1 (30-460)-Fc (100 μg), LINGO-1 (200 μg), GT1b were mixed and resolved on the column as indicated on the figure. The molar concentration of GT1b was 10:1 with respect to NgR1-Fc and 4:1 with respect to LINGO-1 in the protein–glycolipid mixtures.

(SD-200; GE Healthcare) gel-filtration column. Separately, we performed gel-filtrations with mixtures of GT1b/NgR1 (30-460)-Fc and GT1b/LINGO-1 at the same molar ratio. As evidenced from the overlay of the gel-filtration profiles (Fig. 2C, right panel), NgR1-Fc, LINGO-1 and GT1b form a stable ternary complex in solution migrating as a single, distinct peak. Based on the estimated molecular weights, the complex was essentially a 1:2 heterodimer of LINGO-1 and NgR1-Fc. Uncomplexed NgR1-Fc elutes as a dimer while uncomplexed LINGO-1 migrates as a tetramer. The fact that in the absence of GT1b, a mixture of NgR1 and LINGO-1 migrates as two separate peaks (Fig. 2C, left panel) points to the fact that stable complex formation occurs only in the presence of GT1b.

3.3. Sialic acid moieties mediate the interaction of NgR1 and LINGO-1

It is evident from the results reported above that the interaction of LINGO-1 and NgR1 is mediated by ganglioside GT1b. Gangliosides, such as GT1b, GD1b (disialoganglioside) and GM1 (monosialoganglioside), are glycosphingolipids consisting of two parts: a hydrophobic ceramide unit and a hydrophilic oligosaccharide chain with sialic acids as the terminal sugar residues. The ceramide moiety roots the ganglioside to the plasma membrane. To estimate the relative contributions of the ceramide and the oligosaccharide moieties in the complex formation, we used three different gangliosides (GT1b, GD1b, and GM1) and the glycoproteins fetuin and ovalbumin. The gangliosides differ only in the number of their terminal sialic acids [15]. The glycoproteins fetuin and ovalbumin with their polypeptide backbone are chemically different class of molecules. Fetuin has three *N*-linked complex-type and three serine/threonine- or *O*-linked oligosaccharide units, while ovalbumin contains a single Asparagine- or *N*-linked oligomannosidic unit [15]. In ELISA-based binding experiments NgR1-Fc/LINGO-1 bound fairly strongly to GT1b, GD1b, and fetuin but relatively weakly to GM1. We did not detect any binding to ovalbumin (Fig. 3A). This

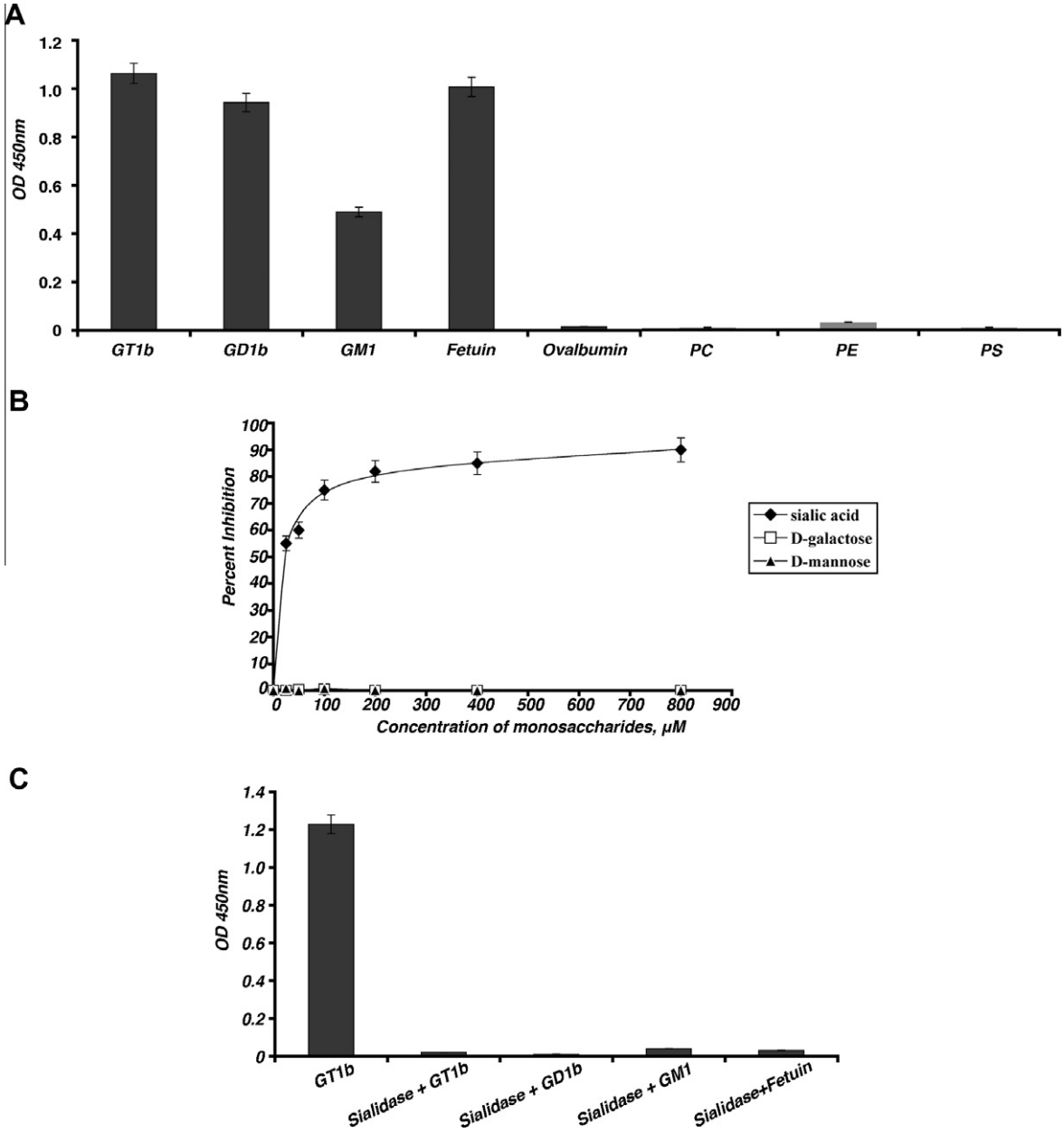

Fig. 3. Sialic acid is the critical determinant of the NgR1, LINGO-1 interaction (A) The wells of ELISA plates were precoated with gangliosides, phopholipids (1 μg/0.1 ml of methanol) or glycoprotein fetuin and ovalbumin (2.5 μg/0.1 ml in HEPES buffer pH 7.4). NgR1-Fc and LINGO-1 (1:1 M ratio) was added to different gangliosides, glycoproteins or phospholipids immobilized on wells of microtiter plates. The data represent average of sextuplicate determinations and the experiment was repeated three times. The maximum dispersion was within 10% of the mean value. (B) The monosaccharide sialic acid inhibits NgR1-Fc + LINGO-1 binding to GT1b. NgR1-Fc and LINGO-1 (1:1 M ratio) was preincubated with various amounts of the monosaccharides for 30 min at 25 °C and then added to wells precoated with GT1b. Percent inhibition was calculated using the data of NgR1-Fc + LINGO-1 binding to GT1b as the positive control. (C) Binding of NgR1-Fc + LINGO-1 to sialidase (neuraminidase) treated fetuin and gangliosides. The data represent average of sextuplicate determinations and the experiment was repeated three times. The maximum dispersion was within 10% of the mean value.

indicated that (i) only the oligosaccharide moieties with terminal sialic acids are capable of binding to NgR1-Fc/LINGO-1 and that (ii) the chemical entity of the backbone does not play a significant role in the binding. To further elucidate this, we next performed binding assays with another class of phospholipids, phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PE). Like ceramide, these phospholipids are constituents of the lipid bilayer. The results show that these phospholipids do not facilitate complex formation (Fig. 3A).

We next examined the ability of simple sugars or monosaccharides, such as sialic acid, galactose, *N*-acetyl glucosamine, to inhibit complex formation. The choice of these sugar residues was largely dictated by the fact that the *N*-linked oligosaccharide chains of GT1b, GD1b, and fetuin are composed of these sugar units. In ELISA-based experiments (Fig. 3B) sialic acid strongly inhibited the binding of NgR1-Fc/LINGO-1 to GT1b while the other monosaccharides, galactose, *N*-acetylglucosmine, and mannose, had no effect. This suggested that the sialic acid moiety was necessary and sufficient for binding and complex formation. This was further confirmed by the fact that sialidase treatment of GT1b, GD1b, GM1 and fetuin completely abrogated the binding (Fig. 3C).

It is interesting to note that when the oligosaccharide chain is extended, the two-carboxyl groups in the sialic residues of GT1b are located about 15–20A° from the membrane surface [16]. The

terminal sialic acid, therefore, can interact in cis with the ectodomain of NgR1 and modulate its interaction with LINGO-1. It is difficult to conclude from our study, however, if the stalk, apart from the three FRG motifs in the leucine rich repeats, directly binds GT1b. It can only be concluded that the tandem sialic acid moieties and the stalk of NgR1 are required for efficient complex formation with LINGO-1. This type of cis interaction between a protein and GT1b has previously been reported for the leukocyte cell surface antigen CD38 [16,17]. However, to our knowledge this is the first demonstration that carbohydrate moieties mediate the interaction between a cell-surface receptor and its co-receptor.

3.4. The transducer p75 influences the binding of the ternary complex NgR1/LINGO-1/GT1b to Nogo-A

Next we investigated whether the ternary complex comprising of NgR1, LINGO-1 and GT1b could interact with Nogo-54 in the presence and absence of p75. Nogo-54, an extracellular fragment of the myelin inhibitor Nogo-A, is functionally similar to the commonly used Nogo-66 fragment but is much more soluble and well structured [18]. The NgR1-Fc/LINGO-1/GT1b used for the study was purified as a complex on a SD-200 column (as mentioned previously). The protein p75 was added at a 1:1 M ratio with respect to the NgR1-Fc/LINGO-1/GT1b complex, or to a mixture of NgR1-Fc and LINGO-1 (1:1). For comparison, we included the binding of NgR1 alone to GST-Nogo-54. The ELISA based assays showed that p75 enhances the binding of the ternary complex (NgR1/LINGO-1/GT1b) to Nogo-54 significantly (4.5-fold) as compared to the complex alone (Fig. 4A). Likewise, the binding was four fold higher as compared to the binding of NgR1 alone to Nogo-54. There was a nominal decrease in the binding of NgR1 to Nogo-54 in the presence of p75 and LINGO-1 (no GT1b).

The pull-down experiments also showed that the preformed ternary complex, consisting of NgR1-Fc/LINGO-1/GT1b interacts strongly with p75 and Nogo-54 when both molecules were present in the reaction mixture (Fig. 4B). At this point, the precise molecular mechanism by which the ternary complex simultaneously interacts with p75 and Nogo-54 is unclear. However, this network of interactions suggests that there may be two distinct steps involved in the complex formation: (i) GT1b mediated association of NgR1/LINGO-1 to form a functional receptor/co-receptor complex and (ii) simultaneous recruitment of the transducer p75 and the myelin inhibitor Nogo-54. It is also possible that once the GT1b-mediated tripartite receptor/co-receptor complex is formed between NgR1/LINGO-1 and p75, the overall affinity of NgR1 for the Nogo-54 ligand is enhanced (Fig. 4C).

4. Synopsis

We document that gangliosides can facilitate the interactions of the Nogo receptor (NgR1) with its co-receptors LINGO-1, p75 and the myelin inhibitor Nogo-A. The interaction of NgR1 with LINGO-1 is directly mediated by the carbohydrate moiety of GT1b. The reported results also indicate that GT1b is an important constituent of the previously known tripartite receptor/co-receptor complex comprising of NgR1/LINGO-1 and p75. The solution studies with purified components delineate the Nogo receptor-myelin inhibitor interactions, and could be used to design high throughput screens (e.g. using homogeneous time resolved fluorescence spectroscopy) of chemical libraries for antagonists that disrupt these interactions and promote neuronal regeneration following spinal cord injury and paralysis.

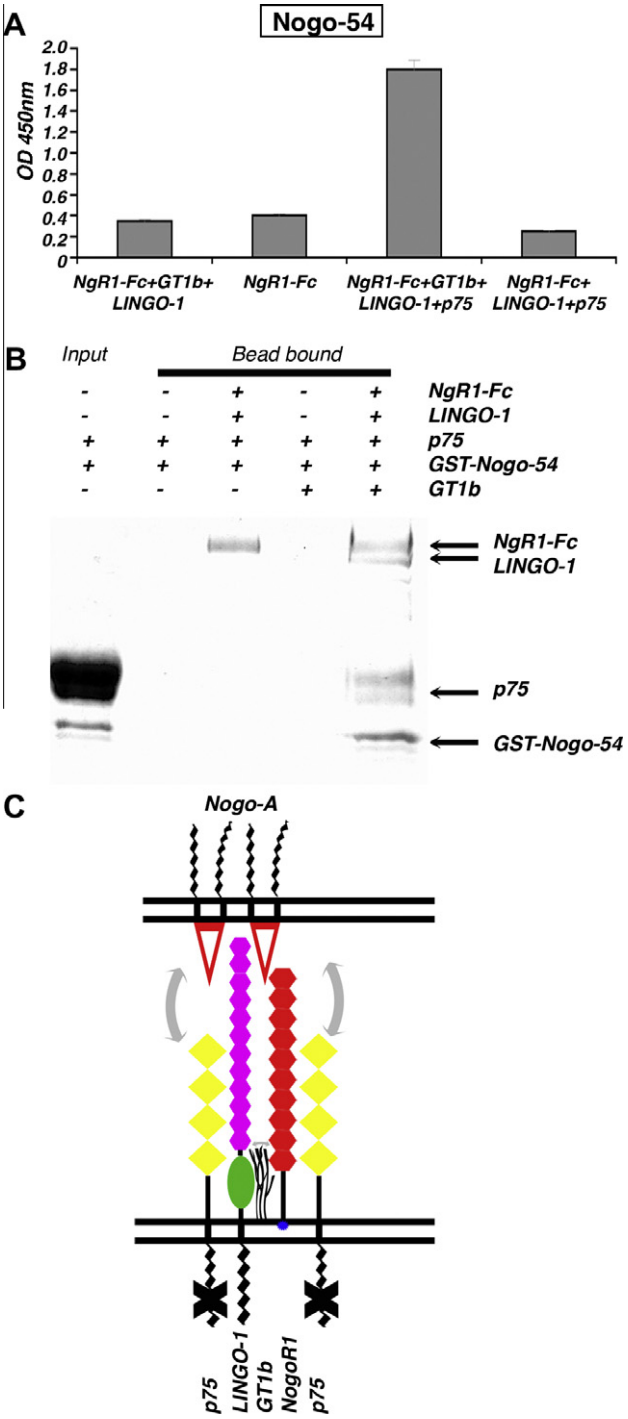

Fig. 4. Interaction of the NgR1/LINGO-1/GT1b complex with Nogo-54 in presence and absence of p75 A. Binding of the ternary complex NgR1-Fc/LINGO-1/GT1b to GST-Nogo-54 immobilized on microtiter beads in the presence and absence of p75. For direct binding of NgR1-Fc to GST-Nogo-54 we used 4 μg of the Fc tagged protein. B. The ternary complex NgR1, LINGO-1, and GT1b bind to Nogo-54 and p75 when they are both present. A mixture of GST-Nogo-54 (6 μg) or p75 (12 μg) does not bind to NgR1-Fc and LINGO-1 in absence of GT1b. Binding occurs when p75 and GST-Nogo-54 are simultaneously added, in presence of GT1b, to the NgR1-Fc/LINGO-1complex pre-bound on Protein-A-Sepharose beads. The Protein-A (bead bound) and input fractions were analyzed on SDS–PAGE. C. Schematic representation of the ganglioside mediated interaction of the myelin inhibitor (Nogo-A) with the receptor/co-receptor complex.

Acknowledgment

The research was supported by a New York State Spinal Cord Injury Research Program Grant (C-022047) to N. Saha. The authors declare no competing interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.08.060.

References

[1] M.T. Filbin, Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS, Nat. Rev. 4 (2003) 703–713.
[2] A.E. Fournier, T. GrandPre, S.M. Strittmatter, Identification of a receptor mediating Nogo-66 inhibition of axonal–regeneration, Nature 409 (2001) 341–346.
[3] M. Domeniconi, Z. Cao, T. Spencer, Myelin-associated glycoprotein interacts with nogo66 receptor to inhibit neurite outgrowth, Neuron 34 (2002) 499–502.
[4] S. Mi, X. Lee, Z. Shao, et al., LINGO-1 is a component of the Nogo-66 receptor/p75-signaling complex, Nat. Neurosci. 7 (2004) 221–228.
[5] J.B. Park, G. Yiu, S. Kaneko, et al., A TNF receptor family member, TROY, is a co-receptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors, Neuron 45 (2005) 345–351.
[6] M. Domeniconi, N. Zampieri, T. Spencer, et al., MAG induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite outgrowth, Neuron 46 (2005) 849–855.
[7] B.E. Collins, L.J. Yang, G. Mukhopadhyay, et al., Sialic acid specificity of myelin-associated glycoprotein binding, J. Biol. Chem. 272 (1997) 1248–1255.
[8] G. Williams, A. Wood, E.J. Williams, et al., Ganglioside inhibition of neurite outgrowth requires Nogo receptor function: identification of interaction sites and development of novel antagonists, J. Biol. Chem. 283 (2008) 16641–16652.
[9] W.A. Barton, B.P. Liu, D. Tzvetkova, et al., Structure and axon outgrowth inhibitor binding of the Nogo-66 receptor and related proteins, Embo. J. 22 (2003) 3291–3302.
[10] X.L. He, J.F. Bazan, G. McDermott, et al., Structure of the Nogo receptor ectodomain: a recognition module implicated in myelin inhibition, Neuron 38 (2003) 177–185.
[11] V. Pignot, A.E. Hein, C. Barske, et al., Characterization of two novel proteins, NgRH1 and NgRH2, structurally and biochemically homologous to the Nogo-66 receptor, J. Neurochem. 8 (2003) 717–728.
[12] B. Kobe, A.V. Kajava, The leucine-rich repeat as a protein recognition motif, Curr. Opin. Struct. Biol. 11 (2001) 725–732.
[13] L.A. Robak, K. Venkatesh, H. Lee, et al., Molecular basis of the interactions of the Nogo-66 receptor and its homolog NgR2 with myelin-associated glycoprotein: development of NgROMNI-Fc, a novel antagonist of CNS myelin inhibition, J. Neurosci. 29 (2009) 5768–5783.
[14] J. Lauren, F. Hu, J. Chin, et al., Characterization of myelin ligand complexes with neuronal Nogo-66 receptor family members, J. Biol. Chem. 282 (2007) 5715–5725.
[15] R.G. Spiro, Glycoproteins, Adv. Protein Chem. 27 (1973) 349–467.
[16] M. Hara-Yokoyama, Y. Nagatsuka, O. Katsumata, et al., Complex gangliosides as cell surface inhibitors for the ecto-NAD+ glycohydrolase of CD38, Biochemistry 40 (2001) 888–895.
[17] M. Kukimoto, O. Nureki, M. Shirouzu, et al., Crystallization and preliminary X-ray diffraction analysis of the extracellular domain of the cell surface antigen CD38 complexed with ganglioside, J. Biochem. 127 (2000) 181–184.
[18] M. Li, Y. Li, X. Liao, et al., Rational design, solution conformation and identification of functional residues of the soluble and structured Nogo-54, which mimics Nogo-66 in inhibiting the CNS neurite outgrowth, Biochem. Biophys. Res. Commun. 373 (2008) 498–503.