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Inhibition of LINGO-1 promotes functional recovery after experimental spinal cord demyelination

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ABSTRACT

Blocking LINGO-1 has been shown to enhance remyelination in the rat lysolecithin-induced focal spinal cord demyelination model. We used transcranial magnetic motor-evoked potentials (tcMMEPs) to assess the effect of blocking LINGO-1 on recovery of axonal function in a mouse lysolecithin model at 1, 2 and 4 weeks after injury. The role of LINGO-1 was assessed using LINGO-1 knockout (KO) mice and in wild-type mice after intraperitoneal administration of anti-LINGO-1 antagonist monoclonal antibody (mAb3B5). Response rates (at 2 and 4 weeks) and amplitudes (at 4 weeks) were significantly increased in LINGO-1 KO and mAb3B5-treated mice compared with matched controls. The latency of potentials at 4 weeks was significantly shorter in mAb3B5-treated mice compared with controls. Lesion areas in LINGO-1 KO and mAb3B5-treated mice were reduced significantly compared with matched controls. The number of remyelinated axons within the lesions was increased and the *G*-ratios of the axons were decreased in both LINGO-1 KO and mAb3B5-treated mice compared with matched controls. These data provide morphometric and functional evidence of enhancement of remyelination associated with antagonism of LINGO-1.

Introduction

Demyelinating damage in the central nervous system (CNS) may occur as a result of viral infection, CNS trauma, or demyelinating disease (e.g., multiple sclerosis [MS]). Demyelination is often followed by some spontaneous remyelination but this recovery process eventually fails, thereby leading to progressive and irreversible functional deficits (Miller and Mi, 2007). For example, axonal loss resulting from chronic demyelination in existing MS lesions is thought to be a major contributor to progression of disability in affected subjects (Dutta and Trapp, 2011). Thus, there is an urgent need for therapies that prevent demyelination or facilitate remyelination.

Failure of remyelination has been suggested to involve inhibition of differentiation and maturation of oligodendrocyte precursor cells (OPCs) (Franklin, 2002). Various modulators of OPC differentiation and myelination have been identified, including leucine-rich repeat and immunoglobulin domain-containing, Nogo (neurite outgrowth inhibitor) receptor-interacting protein-1 (LINGO-1), a transmembrane glycoprotein that negatively regulates these processes (Mi et al., 2004; Mi et al., 2005; Mi et al., 2007; Mi et al., 2009). LINGO-1 is expressed exclusively in neurons and oligodendrocytes within the CNS. Antagonism of LINGO-1 function in experimental autoimmune encephalomyelitis (EAE), an animal model of MS, has been shown to improve morphometrically evident remyelination, axonal integrity, clinical scores, and locomotor behavior (Mi et al., 2007; Wang et al., 2014).

Although EAE provides a reasonable model for clinical effects associated with MS, localized injection of a glial toxin such as lysolecithin produces more predictable, localized myelin damage that is amenable to objective assessment (Blakemore and Franklin, 2008). Injection of lysolecithin into the mouse spinal cord produces reliable lesions of similar size and location that undergo partial recovery over 5–6 weeks (Jeffery and Blakemore, 1995). Furthermore, the electrophysiological function of descending tract demyelination/remyelination in the spinal cord can be directly monitored by measuring transcranial magnetic motor-evoked potentials (tcMMEPs), which provide a robust assessment of the integrity of the tract (Linden et al., 1999). In rats

Abbreviations: CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; IgG, immunoglobulin G; KO, knockout; LINGO-1, leucine-rich repeat and immunoglobulin domain-containing Nogo receptor-interacting protein-1; mAb, monoclonal antibody; MS, multiple sclerosis; Nogo, neurite outgrowth inhibitor; OPC, oligodendrocyte precursor cell; RhoA, ras homolog family member A; SD, standard deviation; tcMMEP, transcranial magnetic motor-evoked potential; TROY/Taj, orphan tumor necrosis factor receptor; VLF, ventrolateral funiculus; WT, wild-type.

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and mice, tcMMEPs are only transmitted through the ventrolateral funiculus (VLF) region (Hill et al., 2009; Loy et al., 2002). The integrity of neuroaxonal functioning in the VLF is reflected by the latency and amplitude of recordable tcMMEPs, as demyelination increases their latency and decreases their amplitude. In lysolecithin-induced lesions of the rat VLF, inhibition of LINGO-1 has been shown to improve recovery of tcMMEP amplitude after 4 weeks in parallel with increases in thickness of myelin sheaths (Mi et al., 2009). In the current study, we sought a more detailed characterization of the effects of LINGO-1 inhibition on the recovery of tcMMEPs in lysolecithin-induced lesions of the mouse VLF associated with morphometric evidence of remyelination.

Material and methods

Surgical procedures and animal care were performed according to the Public Health Service Policy on Humane Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals (National Research Council, 1996), and with approval from the Institutional Animal Care and Use Committee and Institutional Biosafety Committee at the University of Louisville, Louisville, KY.

Animals

A total of 40 adult female mice (20–24 g) were used to examine demyelinated lesions, including LINGO-1 knockout mice ($n = 10$) paired with wild-type (WT) mice of the same background (C57BL/6; $n = 10$) (Biogen Idec, Cambridge, MA) (Mi et al., 2005), as well as 2 groups of WT C57BL/6 mice ($n = 10$ in each group) (Jackson Laboratory; Bar Harbor, ME) for experiments involving anti-LINGO-1 antibody (see below). All animals underwent tcMMEP procedures before lysolecithin injections and at weeks 1 and 2 after lysolecithin injections. After the week 2 tcMMEP analysis, 5 animals from each group were sacrificed for morphometric assessment, and the remaining 5 animals in each group underwent tcMMEP and subsequent morphometric assessment at week 4. Each animal had bilateral lesions. No marked differences were observed between the 2 lesions in each animal or between lesions in different animals, and all lesions were analyzed independently. Therefore, data were combined to double the sample size for statistical power.

An additional 10 mice, 5 each from the matched LINGO-1 KO and WT control groups, were sacrificed without prior demyelinated lesions to assess baseline *G*-ratios (see below).

Spinal cord VLF demyelination

Lysolecithin-induced VLF demyelination was performed as previously described (Blakemore and Franklin, 2008). Briefly, mice were anesthetized with an intraperitoneal injection of 0.1 mL xylazine (10 mg/kg) and ketamine (100 mg/kg) and stabilized in a prone position. The dura was exposed between interlaminar spaces at T8–9 and T9–T10. Using glass micropipettes (outer diameter 40 μm, beveled tip) powered by Nanoject II (Drummond Scientific Co; Broomall, PA), 1 μL of 1% lysolecithin (Sigma; St. Louis, MO) solution in sterile phosphate-buffered saline (pH 7.4) was injected bilaterally into the VLF (0.6 mm lateral to midline at a depth of 1.0 and 1.2 mm) to create 2 focal lesions. To enhance absorption and prevent leakage, the micropipette was left in place for 2 min. The wound was sutured with a 7–0 silk (Ethicon; Somerville, NJ), and the skin was closed with wound clips (Becton Dickinson; Sparks, MD). Animals were given 1 mL of sterile saline subcutaneously after surgery and returned to their cages. Body temperature was maintained at 36–37 °C until the animals fully recovered from the anesthesia.

Anti-LINGO-1 antibody mAb3B5 treatment

Experiments using anti-LINGO-1 antibody to antagonize LINGO-1 function were performed with monoclonal antibody mAb3B5, a murine antibody with high affinity for mouse LINGO-1, or a mouse IgG isotype control (Biogen Idec) (Mi et al., 2007). C57BL/6 mice received an intraperitoneal injection of mAb3B5 (5 mg/kg) or IgG control immediately after the lysolecithin injection and 3 times per week for 4 weeks. The mAb3B5 was generated in LINGO-1 KO mice that were immunized with mLINGO-1 (Biogen Idec) that has been used for chronic studies in mice (Pepinsky et al., 2011) and is a surrogate antibody for the human Li81 mAb used in clinical trials (Tran et al., 2014).

tcMMEP

The method for tcMMEP analysis has been described previously (Hill et al., 2009; Zhang et al., 2007). Briefly, non-sedated mice were restrained and tcMMEP responses were elicited by brain activation using magnetic stimulation (100% output from a Cadwell MES-10 stimulator [Cadwell Laboratories; Kennewick, WA]). The edge of the 5-cm diameter electromagnetic coil was positioned over the inion, hindlimbs were exposed, and recording needle electrodes were placed into the gastrocnemius muscles. The active electrode was inserted into the muscle, the reference electrode was inserted at the tendon, and the ground electrode was placed at the base of the tail. Inter-electrode impedances were maintained below 5 KΩ, the amplifier gain ranged from 1000 to 2000 μV, and the band-pass filter was set at 10–3000 Hz. Evoked responses were displayed on a Cadwell Excel monitor (Cadwell Laboratories). Data from 3 responses measured 1 min apart were averaged. The recordable response rate, onset latency, and amplitude of tcMMEPs were documented before treatment and at 1, 2, and 4 weeks after lysolecithin injection. Weeks 1 and 2 data were obtained from 10 mice (one recording from left and right hindlimb for each mouse; 20 recordings in total) per group; week 4 data were obtained from 5 mice (10 recordings in total) per group.

Tissue preparation and morphometric assessment

Within each group, 5 animals each were sacrificed after tcMMEP analyses at 2 and 4 weeks after lysolecithin injection. Data were obtained from 5 mice (bilateral lesions for each mouse; 10 lesions in total) per group. Animals were anesthetized and perfused intracardially with 30 mL of 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2). Spinal cord samples of the lesion epicenter were dissected and post-fixed in the same solution for 8 h at 4 °C. Trimmed sections of spinal cord containing the VLF lesion were rinsed in sodium cacodylate buffer and placed into 1% osmium tetroxide for 1 h. The samples were dehydrated in graded ethanol and propylene oxide and embedded in Embed812 epoxy resin (Electron Microscopy Sciences; Fort Washington, PA).

For histological analysis of demyelination and remyelination, semi-thin cross-sections (2 μm) were stained with 5% toluidine blue in a 1% Borax solution. Lesion areas were traced and measured using a Nikon Eclipse E800 light microscope with Neurolucida software (MicroBrightField, Inc.; Williston, VT). Lesion size was expressed as a percentage of the entire spinal cord cross-sectional area. The number of remyelinated axons per mm^2 in the lesion site was also counted on toluidine blue-stained sections at the T10 level of the spinal cord. Ten lesions from 5 animals per group per time point were measured.

For electron microscopy, ultra-thin sections (70–90 nm) were placed on 200-mesh grids and stained with uranyl acetate/lead citrate. The ultra-structure of spinal cord was assessed with a transmission electron microscope (Philips CM12; Eindhoven, Netherlands). The *G*-ratio was measured to assess thickness of myelin sheaths on electron micrographs using Stereo Investigator 7 software (MicroBrightField, Inc.; Williston, VT) (lesioned animals, 250–300 axons from 5 animals per