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Full-length Article

Spinal activity of interleukin 6 mediates myelin basic protein-induced allodynia

Justin S. Ko [a,c], Kelly A. Eddinger [a], Mila Angert [a,b], Andrei V. Chernov [d], Jennifer Dolkas [a,b], Alex Y. Strongin [d], Tony L. Yaksh [a], Veronica I. Shubayev [a,b,*]

[a] Department of Anesthesiology, University of California, San Diego, La Jolla, CA, USA
[b] VA San Diego Healthcare System, La Jolla, CA, USA
[c] Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, South Korea
[d] Sanford-Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

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ABSTRACT

Mechanosensory fibers are enveloped by myelin, a unique multilamellar membrane permitting saltatory neuronal conduction. Damage to myelin is thought to contribute to severe pain evoked by innocuous tactile stimulation (i.e., mechanical allodynia). Our earlier (Liu et al., 2012) and present data demonstrate that a single injection of a myelin basic protein-derived peptide (MBP84-104) into an intact sciatic nerve produces a robust and long-lasting (>30 days) mechanical allodynia in female rats. The MBP84-104 peptide represents the immunodominant epitope and requires T cells to maintain allodynia. Surprisingly, only systemic gabapentin (a ligand of voltage-gated calcium channel α2δ1), but not ketorolac (COX inhibitor), lidocaine (sodium channel blocker) or MK801 (NMDA antagonist) reverse allodynia induced by the intrasciatic MBP84-104. The genome-wide transcriptional profiling of the sciatic nerve followed by the bioinformatics analyses of the expression changes identified interleukin (IL)-6 as the major cytokine induced by MBP84-104 in both the control and athymic T cell-deficient nude rats. The intrasciatic MBP84-104 injection resulted in both unilateral allodynia and unilateral IL-6 increase the segmental spinal cord (neurons and astrocytes). An intrathecal delivery of a function-blocking IL-6 antibody reduced the allodynia in part by the transcriptional effects in large-diameter primary afferents in DRG. Our data suggest that MBP regulates IL-6 expression in the nervous system and that the spinal IL-6 activity mediates nociceptive processing stimulated by the MBP epitopes released after damage or disease of the somatosensory nervous system.

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1. Introduction

After injury to the peripheral nervous system (PNS), disruption of the myelin sheath and in electrical insulation of mechanosensory Aβ afferents contribute to the pain behavior phenotype induced by innocuous stimuli (allodynia) (Wu et al., 2002; Devor, 2009; Zhu et al., 2012). The molecular events that engage nonnociceptive Aβ afferents in pain processing are just beginning to unfold. Antigen-specific adaptive immunity and T-helper (Th)1 and Th17 cell polarization promote neuropathic pain via a release of pro-inflammatory, algesic cytokines, such as interleukin (IL)-1β and IL-17, respectively (Moalem et al., 2004; Costigan et al., 2009; Cao and DeLeo, 2008; Sorge et al., 2015; Kim and Moalem-Taylor, 2011a; Kleinschnitz et al., 2006). Our recent findings implicate the proteolytic release of the cryptic Th cell epitopes of myelin basic protein (MBP) as a major event in the pathophysiology of mechanical allodynia after traumatic PNS injury (Liu et al., 2012).

Myelin basic protein (MBP), a major protein of the myelin sheath, is encoded by the *Golli* (*genes of oligodendrocyte lineage*)-*MBP* gene in myelinating glia and immune cells (Boggs, 2006). As an intrinsically unstructured and positively charged protein with the isoelectric point at ~pH 10, MBP interacts with the acidic head groups of the lipid bilayer and a variety of polyanionic proteins, including actin, tubulin and Ca^{2+}-calmodulin. These interactions regulate multiple functions of the axon-glia unit, including cytoskeletal assembly, Ca^{2+} homeostasis and a protein:lipid ratio

* Corresponding author at: Department of Anesthesiology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0629, USA.
E-mail address: vshubayev@ucsd.edu (V.I. Shubayev).

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in the myelin membranes (Boggs, 2006; Harauz and Boggs, 2013). However, the centrally located cryptic MBP epitopes (e.g., MBP84-104), when released by proteolysis, are encephalitogenic in patients with multiple sclerosis and experimental autoimmune encephalomyelitis (EAE) animals (Boggs, 2006). According to our observations, a localized injection of the MBP84-104 peptide into the intact PNS (sciatic nerve) is sufficient to initiate a molecular cascade leading to robust mechanical allodynia in rats (Liu et al., 2012). Because T cell activity is required mainly for the maintenance of MBP84-104-induced allodynia, as athymic nude rats initially develop mild mechanical hypersensitivity after MBP84-104 injection (Liu et al., 2012), and T cells are among the last immune cell type to infiltrate the PNS injury (Kim and Moalem-Taylor, 2011b), the early algesic mechanisms of the MBP84-104 action, preceding or independent of T cell recruitment, remain obscure.

IL-6 (or interferon β2) is a pleiotropic cytokine with a plethora of regulatory functions (Spooren et al., 2011; Taga and Kishimoto, 1997; Van Snick, 1990), including the transition of innate to adaptive immunity (Jones, 2005). In the nervous system, immune cells, glia and neurons produce IL-6 to regulate a wide range of physiological and pathological events (Spooren et al., 2011; Gadient and Otten, 1994; Gijbels et al., 1990). In EAE, IL-6 mediates T cell recruitment and subsequent Th17 polarization (Eugster et al., 1998; Serada et al., 2008), suggesting that IL-6 activity may also precede and facilitate the algesic T cell activity induced by MBP epitope in the PNS. Accordingly, IL-6 causes robust mechanical allodynia (Jongh et al., 2003) following intraplantar (Cunha et al., 1992), intrathecal (DeLeo et al., 1996) or intracerebroventricular (Oka et al., 1995) injections, and increase in the IL-6 expression after PNS injury has been implicated in the pathogenesis of experimental neuropathic pain (DeLeo et al., 1996; Arruda et al., 1998, 2000; Bourde et al., 1996; Kurek et al., 1996; Murphy et al., 1995; Chernov et al., 2015; Lee et al., 2009; Wei et al., 2013). Consequently, a function-blocking IL-6 antibody delivered intrathecally, attenuates pain associated with spinal nerve ligation (Arruda et al., 2000), sciatic nerve constriction (Lee et al., 2009) and ventral root transection (Wei et al., 2013).

Herein, we demonstrated that IL-6 at least partly mediated pain induced by MBP84-104 peptide. The bioinformatics analyses of our genome-wide transcriptional profiling of the sciatic nerves injected with MBP84-104 (Liu et al., 2012) identified IL-6 as the top-induced cytokine in both the athymic nude and control rat samples, independent of T cell content. Unilateral allodynia caused by the intrasciatic MBP84-104 injection was concomitant with the unilateral increase in the IL-6 expression in the segmental spinal cord. Interference with spinal IL-6 activity by intrathecally delivered function-blocking antibody reduced MBP84-104-induced allodynia, corroborating spinal IL-6 was positioned downstream of the pro-nociceptive MBP activity in neuropathic pain.

2. Methods

2.1. Reagents and antibodies

Routine reagents were purchased from Sigma unless indicated otherwise. MBP84-104 (ENPVVHFFKNIVTPRTPPPSQ) and scrambled (s)MBP84-104 (NKPQTNVVEPFHRTFPIPPVS) peptides, derived from the human MBP sequence (GenBank #AAH08749), were synthesized by GenScript. The peptides were protected from degradation by exoproteinases using N-terminal acetylation and C-terminal amidation. The following primary antibodies were used in our immunofluorescence analyses: goat polyclonal IL-6 [R&D Systems (AF506), 1:100], goat polyclonal IL-6 receptor [IL-6R, R&D Systems (AF1830), 1:100], rabbit polyclonal glial fibrillary acidic protein [GFAP, DAKO (Z0334), 1:500], mouse monoclonal NeuN [EMD Millipore (MAB377), 1:1000], rabbit ionized Ca^{2+}-binding adapter molecule 1 [Iba1, Wako (019-19741), 1:500], mouse neurofilament 200 [NF200, Millipore (MAB5262), 1:200], rabbit polyclonal calcitonin gene-related peptide [CGRP, Abcam (ab47027), 1:400], and rabbit polyclonal activating transcription factor 3 [ATF3, Santa Cruz Biotechnology (SC-188), 1:100].

2.2. Animal models

Female Sprague–Dawley rats (200–225 g), athymic nude rats (Hsd:RH-*Foxn1*^{rnu}, 8-week-old) and their heterozygous controls (Hsd:RH-*Foxn1*^{rnu}/*Foxn1*$^{+}$, 8-week-old, n = 6) were obtained from Harlan Labs and housed in a temperature-controlled room (~22 °C), on a 12-h light/dark cycle with free access to food and water. All the procedures and testing were conducted during the light cycle. Under isoflurane anesthesia, the common sciatic nerve was exposed unilaterally at the mid-thigh level. A single intrasciatic (IS) bolus injection of the MBP84-104 and sMBP84-104 peptides (50 μg in 5 μl PBS each) was performed into the nerve fascicle using a 33-gauge needle on a Hamilton syringe. In a subset of animals, the exposed sciatic nerve received three loosely constrictive chromic gut ligatures to produce chronic constriction injury (CCI) (Bennett and Xie, 1988). Sciatic nerve, lumbar (L)4-5 dorsal root ganglia (DRG) and L1-L6 spinal cords were excised and stored in RNA-later (Ambion) at −20 °C for RNA analyses, or in animals perfused with 4% paraformaldehyde, the excised tissues were post-fixed and cryoprotected in graded sucrose for immunochemistry. Tissues from naïve animals were used as control. Animals were sacrificed using Beuthanasia IP (Schering-Plough Animal Health). All animal procedures were performed according to the PHS Policy on Humane Care and Use of Laboratory Animals with the experimental protocol approved by the Institutional Animal Care and Use Committee at the University of California, San Diego and VA San Diego Healthcare System, and complied with ethical guidelines of the International Association for the Study of Pain.

2.3. Behavior tests

Animals were habituated to the testing environment prior to baseline tests. Testing was performed daily for three consecutive days prior and then up to daily after a single IS MBP84-104 and sMBP84-104 injection. For assessment of *mechanical withdrawal threshold*, rats were placed in individual Plexiglas compartments with wire mesh bottom and von Frey filaments (0.41–15.2 g, Stoelting, Wood Dale, IL, USA) were applied perpendicularly to the mid hind paw and held for 4–6 s. A positive response was noted if the paw was sharply withdrawn. The 50% probability of withdrawal threshold was determined by Dixon's up-down method (Chaplan et al., 1994). To assess *thermal escape latencies*, a modified Hargreaves type device was employed (Hargreaves et al., 1988). Rats were placed individually in Plexiglas cubicles with glass surface and, after habituation, a radiant heat stimulus was applied to each paw and the latency defined as the time (seconds) required for the paw to show a brisk withdrawal.

2.4. Drug delivery

The drugs were delivered at day 7 after a single IS MBP84-104 injection. To allow intrathecal (IT) delivery, chronic lumbar IT catheters, single-lumen polyethylene (OD 0.36 mm, 8.5 cm in length), were implanted under isoflurane anesthesia (Malkmus and Yaksh, 2004). The function-blocking goat antibody against rat IL-6 (R&D Systems, AF506) or normal goat IgG (R&D Systems, AB-108-C) was administered IT (50 ng each in 10 μl saline), followed by 10 μl saline (0.9% NaCl) flush. Gabapentin (Toronto

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