HHS Public Access

Author manuscript

Glia. Author manuscript; available in PMC 2023 April 01.

Published in final edited form as:

Glia. 2022 April ; 70(4): 634–649. doi:10.1002/glia.24130.

Activation of microglial GPR109A alleviates thermal hyperalgesia in female lupus mice by suppressing IL-18 and glutamatergic synaptic activity

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Abstract

Many patients with systemic lupus erythematosus (SLE) live with chronic pain despite advances in medical management in reducing mortality related to SLE. Few animal studies have addressed mechanisms and treatment for chronic pain caused by SLE. In this study, we provide the first evidence for the analgesic effects of a GPR109A specific agonist (MK1903) and its action mechanisms in thermal hyperalgesia in female MRL/lpr mice, an SLE mouse model. Specifically, we show that MRL/lpr mice had a higher sensitivity to thermal stimuli at age 11–16 weeks, which was accompanied with significantly microglial and astrocytic activation, increases in p38 MAPK and glutamatergic synaptic activities in the spinal dorsal horn. We demonstrate that thermal hyperalgesia in MRL/lpr mice was significantly attenuated by intrathecal injection of MK1903. GPR109A was expressed in spinal microglia but not astrocytes or neurons. Its expression was significantly increased in MRL/lpr mice with thermal hyperalgesia. Activation of GPR109A receptors in microglia attenuated glutamatergic synaptic activity via suppressing production of interleukin-18 (IL-18). We provide evidence that activation of GPR109A attenuated thermal hyperalgesia in the SLE animal model via suppressing p38 MAPK activity and production of IL-18. Our study suggests that targeting the microglial GPR109A is a potent approach for reversing spinal neuroinflammation, abnormal excitatory synaptic activity, and management of thermal hyperalgesia caused by SLE.

Keywords

EPSC; glial neuronal interaction; HCAR2; neuroinflammation; nociception; synaptic

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AUTHOR CONTRIBUTIONS

Viacheslav Viatchenko-Karpinski, Lingwei Kong participated in designing and performing the experiments, and data analyses. Han-Rong Weng conceived, designed, analyzed the data, and wrote the manuscript. All authors approved the final version of the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is a multiorgan disease of unknown etiology in which the immune responses are directed against the body's own healthy tissues, resulting in inflammation and damage in diverse tissues and organs of the body. It is estimated that there are 241 SLE patients per 100,000 people in North America, with females having a higher incidence than males (Rees et al., 2017). Although advances in medical management have greatly reduced mortality associated with SLE, 90% of SLE patients still live with chronic pain (Lai et al., 2016), which severely impairs the quality of their life (Grigor et al., 1978; Hochberg & Sutton, 1988; Lai et al., 2016; Waldheim et al., 2013). Furthermore, severe pain is a significant symptom not just during the active stage of SLE but also in patients in which other disease symptoms are managed (Kumazawa & Perl, 1977; Kumazawa & Perl, 1978). Current treatment of chronic pain in SLE patients is mainly dependent on nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids. However, their therapeutic efficacy and safety profiles are limited (Grigor et al., 1978; Hochberg & Sutton, 1988; Lai et al., 2016; Waldheim et al., 2013). Developing novel analgesics for the treatment of chronic pain in patients with SLE is highly demanded.

Currently, our understanding about the synaptic and molecular mechanisms underlying chronic pain induced by SLE is largely limited. MRL lupus-prone (MRL/lpr) mice represent a well-established mouse model of human SLE (Kattah et al., 2015; Larson et al., 2012; Perez de Lema et al., 2001; Reilly & Gilkeson, 2002; Theofilopoulos & Dixon, 1985), being widely used for the study of SLE in the field (Giles et al., 2015; Kattah et al., 2015; King et al., 2015; Ramirez-Ortiz et al., 2015). MRL/lpr mice, which carry a mutation in the apoptosis-related Fas gene, spontaneously develop a severe autoimmune disease with many features like those in SLE patients. These include immune abnormalities affecting T and B cells, autoantibody production, immune complex formation, and systemic inflammation (Reilly & Gilkeson, 2002; Theofilopoulos & Dixon, 1985). As manifested in SLE patients, MRL/lpr mice exhibit inflammation in multiple tissues (organs) such as joints (Cox et al., 2010; Greco et al., 2003; Grossman, 2009; Yoneda et al., 2004), muscles (Edwards et al., 1986), vasculatures (Yamada et al., 2003), and kidneys (Andrews et al., 1978; Yen et al., 2013). Each inflammatory site may be a culprit for causing chronic pain in MRL/lpr mice. The spinal dorsal horn is the first station in the CNS that receives and processes sensory inputs from all peripheral sites except those from cranial areas (Kumazawa & Perl, 1977; Kumazawa & Perl, 1978). In this study, we focused on spinal mechanisms in MRL/lpr mice with chronic pain to investigate the convergent effects on the CNS induced by inflammation in the diverse tissues.

Recently, we reported that female MRL/lpr mice spontaneously develop chronic pain (Yan et al., 2017). This was accompanied with neuroinflammation in the spinal dorsal horn, featured by activation of microglia and astrocytes and over production of pro-inflammatory cytokines. Thus, targeting spinal neuroinflammation may be effective for the management of chronic pain caused by SLE. Extensive efforts have been made toward understanding microglial receptors that cause activation of microglia in the spinal dorsal horn. For example, microglial cells are activated upon activation of a host of pro-inflammatory receptors, including chemokine receptors (e.g., CX3CR1), purinergic receptors (P2X4R,

P2X7R, P2Y12, and P2Y13), toll like receptor 4 (LTR4) (Grace et al., 2014; Kobayashi et al., 2008; Taves et al., 2013; Tsuda et al., 2013), and colony-stimulating factor 1 (CSF1) receptors (Guan et al., 2016; Yan et al., 2017). Much less is known about anti-inflammatory receptors expressed in microglial cells and their role in the genesis of pathological pain. Here, we investigated the role of a Gi protein coupled receptor (GPCR), GPR109A, in the regulation of thermal hyperalgesia in MRL/lpr mice. GPR109A was originally discovered in adipocytes, where its activation by niacin suppresses release of free fatty acids and ameliorates dyslipidemia (Li et al., 2010; Tunaru et al., 2003; Wise et al., 2003). Recent studies have identified GPR109A as an important receptor modulating inflammatory responses in multiple immune cell types (Knowles et al., 2006; Kostylina et al., 2008; Maciejewski-Lenoir et al., 2006), including microglia (Fu et al., 2014) and macrophage. Activation of GPR109A in macrophage and microglia cell cultures reduces chemokine and pro-inflammatory cytokine production induced by activation of toll like receptor 4 (TLR4) through suppressing NF-κB signaling (Digby et al., 2012; Fu et al., 2014; Fu et al., 2015; Zandi-Nejad et al., 2013). Currently, it is unknown whether and how spinal neuroinflammation and chronic pain in SLE is regulated by GPR109A receptors.

In this study, we found that GPR109A was expressed in spinal microglia, and activation of GPR109A reduced thermal hyperalgesia in the SLE mouse model. The spinal molecular and synaptic mechanisms used by a specific GPR109A agonist to produce analgesic effects were also revealed.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult female and male MRL/MpJ-fas^{lpr} (MRL/lpr) and MRL/MpJ (MRL control) mice were purchased from Jackson Laboratories (Bar Harbor, ME). Three animals were housed per cage in isolated rooms with a 12-h light cycle. All experiments were approved by the Institutional Animal Care and Use Committee at Mercer University and were fully compliant with the National Institutes of Health Guidelines for the Use and Care of Laboratory Animals. Given that incidence of SLE in females is nine times higher than males (Weckerle & Niewold, 2011), all the experimental data shown in this paper except Supplement Figure 2 were collected from female mice.

2.2 | Behavior tests

The animals were placed on a glass surface at 30°C while loosely restrained under a Plexiglass cage (12 × 20 × 15 cm^3) and allowed to acclimate for 1.5 h. To test the thermal sensitivity in the animals, a radiant thermal beam was directed from below to the mid-plantar surface of the left and right hind paw to evoke a withdrawal response. The latency of paw withdrawal responses, that is, the time between the stimulus onset and paw withdrawal responses, was recorded (Hargreaves et al., 1988). A cutoff time of 20 s was used to avoid damage to the skin. Each hind paw was stimulated three times with an interval of at least 3 min and the three latencies obtained from each paw were averaged. The experimenters who conducted the behavioral tests were blind to types of mice and the treatments given to the mice.

2.3 | Intrathecal catheter implantation and drug administration

Intrathecal (i.t.) injection of tested drugs to the spinal enlargement was made through a preimplanted intrathecal catheter. Briefly, a polyethylene (PE-10) catheter that ended at the spinal L4 segment was intrathecally placed as previously described (Yadav et al., 2015; Yaksh & Rudy, 1976). Mice at the age of 16 weeks were anesthetized with 2%–3% isoflurane and a PE-10 catheter was carefully inserted into the lumbar subarachnoid space through the space between the fifth and sixth lumbar vertebrae. The muscles were then sutured in layers and the skin edges were closed with skin stables. Mice with hind limb paresis or paralysis after surgery were excluded. Successful catheter implantation was confirmed by hind limb paralysis after lidocaine (2%, 5 μl) was injected via the implanted catheter. The animals were allowed to recover for 5 days before behavioral tests were conducted. Drugs or vehicles in a volume of 5 μl were injected into the spinal lumbar enlargement region through the intrathecal catheter, followed by 10 μl of saline to flush.

2.4 | Spinal slice preparations

Mice at the age of 16 weeks were used. Transverse mouse spinal cord slices (350 μm) of the L4–5 segment were prepared as we described previously (Nie & Weng, 2009; Weng et al., 2007). Briefly, mice were deeply anesthetized via isoflurane inhalation (2.5%–3%). Surgery was made to expose and remove the spinal lumbar enlargement segment. To make spinal slices, the lumbar spinal cord section was placed in ice-cold sucrose artificial cerebrospinal fluid (aCSF, 300 ml) pre-saturated with 95% O_2 and 5% CO_2 and washed thoroughly to get rid of any blood. The sucrose aCSF contained 234 mM sucrose, 3.6 mM KCl, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 12.0 mM glucose, and 25.0 mM $NaHCO_3$. The pia-arachnoid membrane was removed from the section. The L4–5 spinal segment, identified by the large dorsal roots, was attached with cyanoacrylate glue to a cutting support, which was then glued onto the stage of a vibratome (Series 1000, Technical Products International, St. Louis, MO). Transverse spinal cord slices (350 μm) were cut in the ice-cold sucrose aCSF and then preincubated in Krebs solution oxygenated with 95% O_2 and 5% CO_2 at 35°C. The Krebs solution contained (mM): 117.0 NaCl, 3.6 KCl, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 11.0 glucose, and 25.0 $NaHCO_3$ at 35°C.

2.5 | Patch clamp recording and analysis of miniature excitatory postsynaptic currents (mEPSCs) from neurons in the spinal dorsal horn

Patch clamp recording was performed as we previously described (Yan et al., 2013; Yan et al., 2019; Yan & Weng, 2013). Briefly, following preincubation, a single slice was placed in the recording chamber (volume, 1.5 ml), perfused with Krebs solution at 35°C, and saturated with 95% O_2 and 5% CO_2. Borosilicate glass recording electrodes (resistance, 3–5 MΩ) were pulled and filled with an internal solution containing 135 mM potassium-gluconate, 5.0 mM KCl, 2.0 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5.0 mM HEPES, 5.0 mM EGTA, 5.0 mM ATP-Mg, 0.5 mM Na-GTP, and 10 mM QX-314. Live dorsal horn neurons in the spinal lamina I and outer lamina II (II_o) were visualized using a microscope system and approached using a three-dimensional motorized manipulator (Sutter Instrument, Novato, CA, USA), and whole-cell configurations were established by applying moderate negative pressure after electrode contact (Nakatsuka et al., 2003; Nie & Weng, 2009; Weng et al.,

2007). Recordings of mEPSCs were made from neurons receiving monosynaptic input from the primary afferents using the criteria established previously (Weng et al., 2006; Yoshimura & Jessell, 1989). mEPSCs were recorded in the presence of tetrodotoxin (TTX, 1 μM), bicuculline (10 μM), and strychnine (5 μM) in the external solution to block voltage-gated sodium channels, $GABA_A$, and glycine receptors respectively at a membrane potential at −60 mV. mEPSCs were recorded using Axopatch 700B amplifiers, digitized at 10 kHz, and analyzed off-line. Access resistance within the range of 10–20 MΩ was monitored continuously throughout the experiments. The recording was abandoned if the access resistance changed by more than 20%. The frequency and amplitude of mEPSCs in the 2 min before, during, and after washout of the perfusion of tested drugs were analyzed and averaged using a peak detection program (MiniAnalysis; Synaptosoft Inc., Decatur, GA). All the drugs were applied through bath-perfusion unless indicated.

2.6 | Western blot experiments

Tissues used for western blotting were either from intact animals at the age of 16 weeks or spinal slices. For extracting tissues from intact animals, animals were deeply anesthetized with urethane (1.3–1.5 g/kg, i.p.). The L4 and L5 spinal segments were exposed by surgery and removed from the mice. To determine the effects induced by activation of GPR109A on related molecular protein levels, mouse spinal slices of the spinal L4–5 segment were obtained in the same fashion as those used for patch clamp experiments described above. Spinal slices were incubated with the plain aCSF or aCSF plus MK1903 (10 nM) bubbled with 95% O_2 and 5% CO_2 at 35°C for 1.5 h. The dorsal halves of the spinal cord tissues from intact animals or spinal slices were isolated and quickly frozen in liquid nitrogen and stored at −80°C. The frozen tissues were homogenized with a hand-held pellet pestle in lysis buffer for 0.5 h at 37°C. Homogenates were then centrifuged at 14,000g for 20 min at 4°C and the supernatant were collected. Protein concentrations were determined using Nanodrop 1000. Protein samples (30 μg) were electrophoresed in 10%–13% SDS polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked with 5% milk and incubated overnight at 4°C with primary antibodies against cathepsin B (1:50, Santa Cruz, Dallas, Texas), GAPDH (1:5000, Proteintech, Rosemont, IL), GPR109A (1:500, Cell Signaling, Danvers, MA), Iba1 (1:100, Santa Cruz, Dallas, Texas), IL-1β (1:500, Cell Signaling, Danvers, MA), IL-18 (1:500, Biovision, Milpitas, CA), p38 MAPK (1:100, Santa Cruz, Dallas, Texas), and phospho-p38 MAPK (1:100, Santa Cruz, Dallas, Texas). Then the blots were incubated for 1 h at room temperature with a corresponding HRP-conjugated secondary antibody (1:5000; Santa Cruz Biotechnology, CA, USA), visualized in ECL solution (SuperSignal West Pico Chemiluminescent Substrate, Pierce, Rockford, IL, USA) for 1 min, and exposed to the FluorChem HD2 System. The intensity of immunoreactive bands was quantified using ImageJ 1.46 software (NIH). The ratio of each protein expression over the loading control protein GAPDH was calculated.

2.7 | Immunohistochemical studies

Immunocytochemistry was used to determine which cell types in the spinal dorsal horn express GPR109A. Mice at the age of 16 weeks were deeply anesthetized with urethane (1.3–1.5 g/kg, i.p.) and perfused intracardially with heparinized phosphate-buffered saline

solution (PBS, pH = 7.35) followed by a solution of 4% formaldehyde in 0.1 M phosphate-buffered saline solution (PBS, pH = 7.35). The L4-L5 spinal cord was removed, post-fixed for 24 h at 4°C in the same fixative, cryoprotected in 15% sucrose in 0.1 M PBS for 24 h at 4°C, then placed in 30% sucrose in 0.1 M PBS solution at 4°C. Serial transverse sections 20 μm thick were cut on a freezing microtome at −20°C, collected in 0.1 M PBS, and processed while free floating. The sections were washed three times in 0.1 M PBS and then blocked with 10% normal goat serum plus 0.3% Triton X-100 in 0.1 M PBS (pH = 7.35) for 1 h at room temperature. Spinal sections were incubated for 1 h at room temperature followed by overnight at 4°C with rabbit anti-GPR109A (1:50, BOSTER, Pleasanton, CA), goat anti-Iba1 (a marker for microglia, 1:100, Novus, Centennial. CO), mouse anti-GFAP (a marker for astrocytes, 1:500, Cell Signaling), mouse anti-NeuN (a marker for neurons, 1:500, Cell Signaling) antibodies. The sections were then washed three times in 0.1 M PBS and incubated for 2 h at room temperature with the corresponding Texas Red antibody (1:500 Vector Laboratories) and Alexa Fluor 488 antibody (1:500 Life Technologies). After rinsing three times with 0.1 M PBS, the sections were mounted onto gelatin-coated slides, air-dried, and cover-slipped with Vectashield mounting medium (Vector Laboratories). The immunostaining for each antibody in the spinal section was recorded on a Ziess Apotome microscope. Images were processed using AxioVison Rel 4.8.

2.8 | Materials

Bicuculline, strychnine, MK1903, and tetrodotoxin were obtained from Tocris (Bristol, UK). IL-18BP was purchased from Mybiosource (San Diego, CA). SB203580 was purchased from Invivogen (San Diego, CA). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO).

3 | DATA ANALYSIS

All data are presented as the mean ± SEM. One-way and two-way ANOVAs with repeated measures were used to respectively detect differences in behavioral data over different time points in the same group, and to detect differences in behavioral data between groups over different time points. Once differences were found, the Bonferroni post hoc test was used to determine sources of differences. Student's *t*-test (paired *t*-test for data obtained within the same group; non-paired *t*-test for data obtained from different groups) was used to detect difference between groups. A p value less than .05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc.)

4 | RESULTS

4.1 | MRL/lpr mice develop thermal hyperalgesia

To determine the development of chronic pain in female MRL/lpr mice, we measured the sensitivity of both hind paws to radiant thermal stimulation in female MRL/lpr and female MRL control mice once per week starting from ages 8 to 16 weeks. We found that the latency of withdrawal response to radiant thermal stimulation in MRL/lpr mice and MRL control mice were similar and stable between 8 and 10 weeks old. Starting from the age of 11 weeks, the latency of withdrawal response in MRL/lpr became significantly (n = 8, $p <$

.05) shorter, which reached plateau between weeks 14 and 16. Consequently, the withdrawal response latency was significantly reduced from 11.25 ± 0.59 s (n = 8) at 8 weeks old mice to 9.96 ± 0.41 s (n = 8, p < .05) at 11 weeks old mice, and 7.99 ± 0.56 s (n = 8, p < .01) at 16 weeks old mice. In contrast, the latency of withdrawal response to the same thermal stimulation in female (n = 7) MRL control mice (n = 7) remained constant during the same observation period. These results indicate that MRL/lpr mice develop thermal hyperalgesia at the age of 11 weeks, and such hyperalgesia is persistent throughout ages 11–16 weeks.

4.2 | Spinal microglia and astrocytes are activated in MRL/lpr mice with thermal hyperalgesia

To determine the status of microglia and astrocytes in female MRL/lpr mice with thermal hyperalgesia, we stained microglia with Iba1 (microglial marker) antibody and astrocytes with GFAP (astrocytic marker) antibody in the spinal dorsal horn of mice at the age of 16 weeks. We found that MRL/lpr mice had heightened Iba1 and GFAP immunoreactivity in the spinal dorsal horn, likely indicative of an increased density of microglia and astrocytes respectively. Microglia and astrocytes in the spinal dorsal horn of MRL/lpr mice appeared hypertrophied with enlarged soma and thick processes, suggesting microglial and astrocytic activation (Figure 1a,b) (Milligan & Watkins, 2009; Yang et al., 2015). Our western blot experiments further confirmed protein expression of Iba1 and GFAP in female MRL/lpr mice was significantly (n = 4; p < .001) increased in comparison with MRL control mice (n = 4) (Figure 1c). p38 mitogen-activated protein kinase (p38 MAPK) is a critical kinase implicated in activation of microglia and production of pro-nociceptive mediators in the spinal cord in different pathological pain models (Chen et al., 2018). To determine whether p38 MAPK is activated, we measured phosphorylated levels of p38 MAPK (an active form of p38 MAPK) and found that global levels of phosphorylated p38 MAPK in MRL/lpr mice with thermal hyperalgesia were significantly (n = 4; p < .001) higher than MRL control mice (n = 4) (Figure 1c). Together, these data indicate that thermal hyperalgesia in MRL/lpr mice is associated with activation of microglia and astrocytes in the spinal dorsal horn, and targeting the dysfunctional glial cells may provide treatment of thermal hyperalgesia in female MRL/lpr mice.

4.3 | GPR109A protein expression in MRL/lpr mice with thermal hyperalgesia is increased and present only in microglia

GPR109A expression in the spinal dorsal horn remains elusive. To explore whether we can target GPR109A receptors at the spinal cord for the treatment of thermal hyperalgesia in MRL/lpr mice, we first determined protein expression of GPR109A in the spinal dorsal horn of mice at the age of 16 weeks using western blotting. We found that GPR109A protein was expressed in the spinal dorsal horn in both MRL/lpr (n = 4) and MRL control mice (n = 4), and its expression in MRL/lpr mice with thermal hyperalgesia was significantly (n = 4; p < .05) higher than that in MRL control mice (n = 4) (Figure 2a). Furthermore, to identify which cellular type expresses GPR109A, spinal slices obtained from MRL/lpr mice with thermal hyperalgesia and MRL control mice were used for immunohistological experiments. We found that the staining of GPR109A (stained in red) was co-labeled only with the microglial marker (Iba1, stained in green), but not with the astrocyte marker (GFAP, stained in green), or the neuronal marker (NeuN, stained in green) in the spinal dorsal horn

in MRL/lpr (Figure 2b) mice and MRL control mice (Supplement Figure 1) mice, indicating that GPR109A is only expressed in microglia in the spinal dorsal horn.

4.4 | Intrathecal injection of the GPR109A agonist abrogates thermal hyperalgesia in MRL/lpr mice

To explore whether activation of GPR109A regulates thermal hyperalgesia in female MRL/lpr mice, we determined whether intrathecal administration of a GPR109A specific agonist, MK1903, alters the paw withdrawal latency to thermal stimuli in MRL/lpr mice. MRL/lpr mice at the age of 16 weeks with thermal hyperalgesia were grouped into the MK1903-treated group and the vehicle (saline)-treated group. Similarly, two groups of MRL control mice at the age of 16 weeks were respectively assigned into the MK1903 treated and vehicle-treated groups. After measuring the baseline withdrawal latency to thermal stimuli, MK1903 (concentration: 10 μM, volume: 5 μl) was administered through a preimplanted intrathecal catheter with an opening at the lumbar enlargement. As shown in Figure 2c, intrathecal administration of MK1903 significantly ($n = 11$, $p < .001$) raised the latency of withdrawal responses from 7.13 ± 0.52 s before injection to 10.99 ± 0.58 s at 30 min after the injection. Such effects reached plateau between 60 and 90 min and lasted more than 150 min after the injection (Figure 2c). We did not observe a significant alteration in the latency of withdrawal response in MRL/lpr mice with thermal hyperalgesia treated with vehicle ($n = 8$) in the same observation period. When compared to MRL/lpr mice with thermal hyperalgesia treated with vehicle ($n = 8$), the latencies of withdrawal responses in MRL/lpr mice with thermal hyperalgesia treated with MK1903 ($n = 11$) were also significantly ($p < .01$) increased between the 30 and 150 min time points. In contrast, intrathecal injection of the same amount of MK1903 ($n = 11$) or vehicle ($n = 6$) did not significantly alter the latency of withdrawal responses in MRL control mice (Figure 2c). We also found that intrathecal administration of MK1903 significantly reduced thermal hyperalgesia in male lupus mice at the age of 16 weeks (Supplement Figure 2). These data suggested that activation of spinal GPR109A alleviates thermal hyperalgesia in MRL/lpr mice without impairing normal nociception.

4.5 | Activation of GPR109A in the spinal dorsal horn reduces the enhanced glutamate release from presynaptic terminals in MRL/lpr mice with thermal hyperalgesia

Glutamatergic synaptic activity is a key factor for determining neuronal activation at the spinal dorsal horn (Mantyh et al., 1997; Todd, 2010). Neurons in the spinal superficial dorsal horn (laminae I and II outer layer, II_o) receive excitatory inputs from nociceptive primary fibers, with most of them being excitatory neurons (Mantyh et al., 1997; Todd, 2010). To determine synaptic mechanisms underlying the analgesic effects induced by the activation of GPR109A, mEPSCs in neurons in this area were recorded and analyzed using whole-cell voltage clamp recording techniques. Mice at the age of 16 weeks were used. We first compared glutamatergic synaptic activities in MRL/lpr mice with thermal hyperalgesia with those in MRL control mice since little is known about the spinal synaptic plasticity in animals with thermal hyperalgesia induced by SLE. We found that MRL/lpr mice with thermal hyperalgesia had significantly ($p < .001$) higher mEPSC frequencies (4.13 ± 0.45 Hz, $n = 39$) than those (2.20 ± 0.28 Hz, $n = 43$) in MRL control mice (Figure 3a,b), indicating that glutamate release from the presynaptic terminals is increased in MRL/lpr

mice with thermal hyperalgesia. We also found that the amplitudes of mEPSCs in both MRL/lpr and MRL control groups were similar (Figure 3a,c), suggesting that the activity of AMPA glutamate receptors at the postsynaptic membrane in the superficial spinal dorsal horn neurons are not significantly altered in MRL/lpr mice with thermal hyperalgesia. After recording the baseline activities of mEPSCs, we activated GPR109A in the spinal slice via perfusing MK1903 (concentration in the bath: 10 nM) into the recording chamber. We found that in spinal slices obtained from MRL/lpr mice with thermal hyperalgesia, MK1903 significantly ($p < .01$) attenuated the frequency of mEPSCs from 4.04 ± 0.85 Hz to 2.38 ± 0.52 Hz ($n = 12$) without significantly altering mEPSC amplitudes in the neurons (Figure 3d–f). When we perfused MK1903 (concentration in the bath: 10 nM) onto spinal slices obtained from MRL control mice, both the amplitudes and frequencies of mEPSCs remained unchanged ($n = 10$) (Figure 3d–f). Together, these data indicate that activation of GPR109A in the spinal cord attenuates thermal hyperalgesia and spinal neuronal activation through reducing glutamate release from the presynaptic terminals.

4.6 | Activation of GPR109A suppresses thermal hyperalgesia in MRL/lpr mice via attenuating p38 MAPK activity in MRL/lpr mice

As GPR109A is expressed in microglia but not in neurons or astrocytes, it is conceivable that the effects induced by MK1903 in MRL/lpr mice must be mediated by the action of MK1903 on microglia. Given that p38 MAPK is a master kinase in regulating the pro-inflammatory signaling pathway (Chen et al., 2018) and its activity (phosphorylation levels) is elevated in MRL/lpr mice with thermal hyperalgesia, we next determined the effects of MK1903 on p38 MAP kinase activity in the spinal cord. Spinal slices of mice at the age of 16 weeks were prepared in the same fashion as those for patch clamp experiments. Four groups of spinal slices were prepared. Spinal slices obtained from MRL/lpr mice with thermal hyperalgesia were assigned into two groups: MRL/lpr slices treated with MK1903 and MRL/lpr slices treated with vehicle. Similarly, spinal slices obtained from MRL control mice were grouped into MRL control slices treated with MK1903 and MRL control slices treated with vehicle. Spinal slices were incubated with aCSF solution plus MK1903 (concentration: 10 nM) for MK1903-treated groups, or with vehicle (aCSF) for vehicle-treated groups, for 1.5 h. The 1.5 h duration was chosen because we observed the analgesic effects induced by i.t. injection of MK1903 reached plateau between 60 and 90 min after the injection (see Figure 2c). We found that MK1903 treatment significantly ($p < .05$) attenuated the enhanced level of phosphorylated p38 MAPK in MRL/lpr mice ($n = 4$) with thermal hyperalgesia (Figure 4a). Meanwhile, MK1903 did not alter the levels of phosphorylated p38 MAPK in spinal slices obtained from MRL control mice ($n = 4$) in comparison with those treated with vehicle ($n = 4$) (Figure 4a). To further determine the role of p38 MAPK in thermal hyperalgesia in MRL/lpr mice, we inhibited the p38 MAPK activity at the spinal cord by intrathecal administration of a p38 MAPK inhibitor (SB203580, 30 μg/mouse) (Sorge et al., 2015) in MRL/lpr mice with thermal hyperalgesia. As shown in Figure 4b, we observed a significant ($n = 5$, $p < .05$) increase in the withdrawal latency in MRL/lpr mice at 60 min after the administration of SB203580. Such effects were lasted for another 90 min.

In MRL/lpr mice with thermal hyperalgesia treated with vehicle ($n = 8$), we did not observe significant changes in the latencies of withdrawal responses in the same observation period. In comparison with MRL/lpr mice with thermal hyperalgesia treated with vehicle ($n = 5$), the latencies of withdrawal responses in MRL/lpr mice with thermal hyperalgesia treated with SB203580 ($n = 5$) were also significantly ($p < .05$) increased between the 60 and 150 min time points. In contrast, the latencies of withdrawal responses were not significantly altered in control mice ($n = 4$) treated with the same amount of SB203580 or control mice ($n = 4$) treated with vehicle (Figure 2c). These results indicate that activation of GPR109A alleviates thermal hyperalgesia in MRL/lpr mice via suppressing spinal p38 MAPK activity.

4.7 | Activation of GPR109A attenuates the elevated levels of IL-18, IL-1β, cathepsin B in the spinal cord of MRL/lpr mice with thermal hyperalgesia

It was demonstrated that exogenous application of IL-18 in the spinal dorsal horn can enhance glutamate release from presynaptic terminals, and that IL-18 is synthesized only in microglia (Yang et al., 2015). To determine whether MK1903 produces analgesic effects through suppressing the production of IL-18 from microglia, four groups of spinal slices of mice at the age of 16 weeks were prepared: MRL/lpr slices treated with MK1903, MRL/lpr slices treated with vehicle, MRL control slices treated with MK1903, and MRL control slices treated with vehicle. Spinal slices were incubated with aCSF plus MK1903 (concentration: 10 nM) for MK1903-treated groups, or vehicle (aCSF) for vehicle-treated groups for 1.5 h. Pro-IL-18 (24 KDa) and mature IL-18 (18 KDa) in these four groups were measured using western blots. As shown in Figure 5a,b, we found that MRL/lpr mice with thermal hyperalgesia had significantly ($n = 4$; $p < .001$) higher expression of Pro-IL-18 and mature IL-18 in comparison with those in MRL control mice ($n = 4$). The protein expression of Pro-IL-18 and mature IL-18 in MRL/lpr mice with thermal hyperalgesia were significantly ($n = 4$; $p < .05$) reduced when the spinal tissues were treated with MK1903. Meanwhile, protein levels of pro-IL-18 and mature IL-18 in MRL control mice ($n = 4$) were not altered by MK1903 treatment (Figure 5a,b). Cathepsin B is critically engaged in the production of mature IL-18 from pro-IL-18 (Sun et al., 2012). Thus, we asked whether MK1903 alters cathepsin B activities by examining protein levels of active cathepsin B. As shown in Figure 5c, we found that protein levels of active cathepsin B in MRL/lpr mice with thermal hyperalgesia were significantly ($n = 4$; $p < .001$) higher than those in MRL control mice ($n = 4$). Furthermore, treatment of MK1903 significantly ($n = 4$; $p < .01$) attenuated the increased levels of cathepsin B in MRL/lpr mice. Given that IL-18 and IL-β both belong to the IL-1 cytokine family, and cathepsin B is also involved in converting pro-IL-1β to mature IL-1β (Sun et al., 2012), we measured the levels of pro-IL-1β (25 KDa) and mature IL-1β (17 KDa) in the spinal slices in all the above four groups at the same time. Consistent with our previous findings (Yan et al., 2017), we found that MRL/lpr mice with thermal hyperalgesia had significantly ($n = 4$; $p < .001$) elevated levels of pro-and mature IL-1β in the spinal cord (Figure 5d,e). Importantly, these abnormalities were significantly ($n = 4$; $p < .05$) attenuated by MK1903 treatment (Figure 5d,e). These data indicate that activation of microglial GPR109A attenuates the production of IL-1β and IL-18 in the spinal dorsal horn in MRL/lpr mice with thermal hyperalgesia.

4.8 | Blocking IL-18 activity attenuates glutamate release from presynaptic terminals in the spinal dorsal horn and thermal hyperalgesia in MRL/lpr mice

We reasoned if the reduction of IL-18 production mediates the effects by MK1903, inhibition of IL-18 activity should produce effects on synaptic activities and nociceptive behaviors like those induced by MK1903. IL-18 binding protein (IL-18BP) is an endogenous inhibitor of IL-18. It binds to IL-18 and prevents the binding of IL-18 to its receptor, resulting in the inhibition of IL-18 function. We first determined the effects of IL-18BP on glutamatergic synaptic activities recorded from neurons in the spinal superficial dorsal horn (laminae I and II_o) in spinal slices from MRL/lpr mice with thermal hyperalgesia and MRL control mice at the age of 16 weeks. After recording the baseline activities of mEPSCs, we perfused IL-18BP (concentration in the bath: 10 ng/ml [Wu et al., 2003]) to selectively bind IL-18. As shown in Figure 6a–c, bath application of IL-18BP significantly ($n = 11$, $p < .01$) attenuated the mEPSC frequency from 3.89 ± 0.52 Hz to 2.24 ± 0.32 Hz, but did not alter the mEPSC amplitude in slices taken from MRL/lpr mice with thermal hyperalgesia. However, when we gave the same treatment to the spinal slices obtained from MRL control mice, both the mEPSC frequency ($n = 11$) and amplitude ($n = 11$) remained unchanged (Figure 6a–c).

We then examined whether spinal topical application of IL-18BP can attenuate thermal hyperalgesia in MRL/lpr mice using behavior tests. Mice at the age of 16 weeks were used. After measuring the baseline latency of paw withdrawal response to radiant thermal stimuli, IL-18BP (500 ng in a volume of 5 μl) was intrathecally administered onto the lumbar enlargement of the spinal cord. We found that IL-18BP treatment significantly ($p < .001$) increased the latency of paw withdrawal response in MRL/lpr mice ($n = 6$) with thermal hyperalgesia from 8.33 ± 0.68 s at baseline to 11.49 ± 0.8 s at 4 h and 13.68 ± 0.76 at 6 h after the injection, but had no effects on MRL control mice ($n = 6$) (Figure 6d). When vehicle (saline, 5 μl) was administered in the same fashion, the latency of paw withdrawal response to thermal stimuli was not significantly altered in either MRL/lpr mice ($n = 6$) or MRL control mice ($n = 6$) (Figure 6d). In comparison with MRL/lpr mice ($n = 6$) with thermal hyperalgesia treated with vehicle, MRL/lpr mice ($n = 6$) with thermal hyperalgesia receiving IL-18BP also had significantly ($p < .01$) increased latencies of withdrawal responses at 4 and 6 h after the injection (Figure 6d). These results indicate that overproduction of IL-18 leads to enhanced presynaptic glutamate release and hypersensitivity to thermal stimuli in MRL/lpr mice with thermal hyperalgesia. It is noteworthy that we have previously reported that the elevated levels of IL-1β causes suppression of glial transporter function in the spinal dorsal horn and thermal hyperalgesia in MRL/lpr mice (Yan et al., 2017). Together with findings in Figures 1–6, our results suggest that activation of GPR109A receptors on microglia produces analgesic effects on MRL/lpr mice via decreasing production of IL-18 from microglia and glutamatergic synaptic activity in the spinal cord.

5 | DISCUSSION

In this study, we provide the first evidence for the analgesic effects of a GPR109A specific agonist (MK1903) and its action mechanisms in a female SLE mouse model. Specifically,

we observed that MRL/lpr mice had a higher sensitivity to thermal stimulation at ages 11–16 weeks, which was accompanied with activation of microglia and astrocytes, and enhanced activities of p38 MAPK and glutamatergic synaptic activity in the spinal dorsal horn. Spinal administration of MK1903 reversed the thermal hypersensitivity in MRL/lpr mice. We demonstrated that GPR109A was expressed in spinal microglia but not astrocytes or neurons. Its expression in the spinal dorsal horn was significantly increased in MRL/lpr mice with thermal hyperalgesia. Activation of GPR109A reversibly attenuated the enhanced glutamate release from presynaptic terminals in the spinal dorsal horn in MRL/lpr mice with thermal hyperalgesia. We provide evidence that activation of GPR109A receptors in microglia produced analgesic effects on MRL/lpr mice via suppression of p38 MAPK activity and production of IL-18 from microglia. Our study highlights that: (a) dysfunction of glutamatergic synapses, activation of microglia, and over-production of IL-18 in the spinal dorsal horn contributes importantly to the spinal central sensitization in a SLE animals with thermal hyperalgesia; (b) targeting the microglial GPR109A signaling pathway may be a potent approach for treatment of thermal hyperalgesia caused by SLE.

5.1 | Glutamatergic synaptic mechanisms underlying spinal central sensitization

Pathological pain is caused by abnormal activation of neurons along the pain signaling pathway. Enhanced neuronal activity in the spinal dorsal horn termed central sensitization plays a crucial role in the genesis of pathological pain (Woolf, 2011). Neuronal activity in the CNS is determined by multifaceted factors, such as the passive and active membrane properties, and the excitatory and inhibitory synaptic activities (Hardie & Pearce, 2006). Glutamate is a primary excitatory transmitter used by primary afferents and excitatory interneurons in the spinal dorsal horn to activate spinal dorsal horn neurons in the pain-signaling pathway (Yan et al., 2013; Yan & Weng, 2013). Activation of glutamatergic synapses depends on three major determinants: the amount of glutamate release from presynaptic terminals, the activity of ligand gated glutamate receptors at the postsynaptic neurons, and the efficacy of glutamate clearance by glutamate transporters as glutamate is not metabolized extracellularly (Danbolt, 2001). Increased glutamate release from primary afferents were reported in many animal models with pathological pain, such as neuropathic pain caused by nerve injury (Yan et al., 2013; Yan & Weng, 2013) or chemotherapy agents (like paclitaxel) (Yan et al., 2019), inflammatory pain induced by Complete Freund's adjuvant (Huang et al., 2019), and thermal hyperalgesia caused by bone cancer (Yang et al., 2015). NMDA and AMPA receptor functions at the postsynaptic membrane are increased in the spinal dorsal horn neuron in animals with nerve injury (Chu et al., 2012; Nie & Weng, 2010) and peripheral inflammation (Kopach et al., 2012). Currently, little is known about spinal synaptic mechanisms underlying thermal hyperalgesia caused by SLE. We recently reported that the protein expression and function of glial glutamate transporters are attenuated in the spinal dorsal horn of MRL/lpr mice with thermal hyperalgesia (Yan et al., 2017). Our present study demonstrated that there was an increased glutamate release from presynaptic terminals in the spinal dorsal horn of MRL/lpr mice with thermal hyperalgesia as evident by an increased frequency of mEPSCs in the superficial dorsal horn neurons. These findings indicate that spinal central sensitization occurs in animals with thermal hyperalgesia caused by SLE, and enhanced glutamatergic synaptic activity is a key mechanism underlying the spinal central sensitization in this animal model.

5.2 | Role of microglia and IL-18 in the genesis of pathological pain

Microglial activation in the spinal cord plays a critical role in spinal central sensitization caused by nerve injury (Guan et al., 2016), peripheral inflammation (Berta et al., 2014; Zhao et al., 2015), and bone cancer (Song et al., 2017). Inhibition of microglia with minocycline blocks the development of long-term potentiation (LTP) in the spinal cord (Chu et al., 2012). The role of microglia in the genesis of thermal hyperalgesia caused by SLE remains unclear. In this study, we found that female MRL/lpr mice with thermal hyperalgesia had microglial activation. More importantly, we found a significantly increased level of phosphorylated p38 MAPK in the spinal dorsal horn in MRL/lpr mice with thermal hyperalgesia, and that inhibition of p38 MAPK alleviated thermal hyperalgesia in MRL/lpr mice. Previous studies in animals with neuropathic or inflammatory pain have shown that increased activation of p38 MAPK in the spinal dorsal horn triggers a cascade of signaling pathways leading to increased production of many pro-inflammatory mediators, including IL-1β and IL-18 (Chen et al., 2018). We extended such findings to animals with thermal hyperalgesia caused by SLE. We found that the production of both pro-and mature IL-18 and IL-1β in the spinal dorsal horn in MRL/lpr mice with thermal hyperalgesia were significantly increased in comparison with those in MRL control mice. This was accompanied with an increased level of cathepsin B, a protease implicated in converting the pro-IL-1β and pro-IL-18 to mature IL-1β and IL-18 (Nakanishi, 2020). It was reported that spinal cord LTP induced by tetanic stimulation of the sciatic nerve is associated with overproduction of IL-18 in the spinal microglia (Chu et al., 2012). Inhibition of spinal IL-18 significantly reduces bone cancer-induced mechanical allodynia and thermal hyperalgesia in female mice (Yang et al., 2015). Bath application of exogenous IL-18 recombinant protein increases the mEPSC frequency but not amplitude in spinal lamina II_o neurons in normal rats (Yang et al., 2015). Whether IL-18 has any role in the genesis of thermal hyperalgesia induced by SLE and whether endogenous IL-18 in animals with thermal hyperalgesia regulates spinal glutamatergic synaptic activity remain unknown. Our study fills this gap by demonstrating that: (a) MRL/lpr mice with thermal hyperalgesia have elevated levels of IL-18 in the spinal cord; (b) endogenous IL-18 in these mice enhances presynaptic glutamate release at the spinal dorsal horn; (c) blocking IL-18 activity in the spinal cord attenuates thermal hyperalgesia caused by SLE in animals. Thus, normalizing the IL-18 signaling pathway at the spinal cord would be an optimal option for the management of thermal hyperalgesia caused by SLE. We reported previously that MRL/lpr mice also develop mechanical allodynia. It remains to be further studied whether IL-18 has role in the regulation of mechanical allodynia in lupus mice.

5.3 | Role of GPR109A in the CNS

GPR109A can be activated by the endogenous β-hydroxybutyric acid (BHBA) (Taggart et al., 2005), niacin (Li et al., 2010; Tunaru et al., 2003; Wise et al., 2003), or synthetic selective GPR109A agonists such as MK1903 (Boatman et al., 2012). BHBA is a major component of ketone bodies, which is produced by the liver and rises during fasting. Under physiological conditions, the concentration of BHBA is 44 ± 6 μM in the CSF and 62 ± 10 μM in the serum (Wang et al., 2017), while concentration of niacin is 0.7 μM in the CSF and 0.5 μM in the serum (Spector & Johanson, 2007). Since the EC50 for BHBA and niacin to activate GPR109A is respectively 750 μM (Gille et al., 2008) and 100 μM

(Offermanns, 2006), GPR109A is not activated in the body under normal conditions. Studies on the role of GPR109A are mainly based on exogenous application of BHBA or niacin into the animals or cell cultures. In the CNS, GPR109A is reportedly expressed in microglia, neurons, and astrocytes in the rostral ventrolateral medulla (Rezq & Abdel-Rahman, 2016) but only in microglia in the cortex (Rahman et al., 2014). The cellular types expressing GPR109A in the spinal cord is unknown. Our present study demonstrated that in the spinal dorsal horn, GPR109A is expressed only in microglia but not in neurons or astrocytes, consistent with previous findings by single cell RNA sequencing of microglia (Hammond et al., 2019; Zhang et al., 2014). It is conceivable that the cellular location and role of GPR109A in the CNS are region-specific. For example, topical application of niacin into the rostral ventrolateral medulla causes sympathoexcitation via glutamate release from neurons (Rezq & Abdel-Rahman, 2016). Chronic subcutaneous administration of BHBA ameliorates cognitive function in a mouse model of Alzheimer's disease by attenuating Aβ accumulation and neuroinflammation in the brain (Wu et al., 2020). Motor dysfunction in a rat Parkinson's disease model induced by intranigral injection of lipopolysaccharide is improved by BHBA treatment via the inhibition of microglial over-activation (Fu et al., 2015). More relevant to our study, intraperitoneal injection of BHBA reduces tactile allodynia in mice induced by nerve injury, and this effect is abolished in the absence of GPR109A (Boccella et al., 2019). Interestingly, an elevated level of endogenous BHBA induced by short-term starvation is sufficient to induce a GPR109A dependent analgesia in neuropathic animals (Boccella et al., 2019). Furthermore, pain-hypersensitivity induced by spinal injury is improved by systemic administration of BHBA (Qian et al., 2017). It is worth noting that while BHBA and niacin can activate GPR109A, they are not the GPR109A specific agonists because they have many other actions (Gasperi et al., 2019; Horimatsu et al., 2019). Currently, it is unknown about the role of GPR109A in the spinal dorsal horn. Our present study found that activation of spinal GPR109A with MK1903 leads to suppression of p38 MAPK activity, and reduced production of IL-18 and IL-1β in MRL/lpr mice. At the synaptic level, activation of GPR109A reduces the presynaptic glutamate release in the spinal dorsal horn in MRL/lpr mice with thermal hyperalgesia via suppressing the production of IL-18 from microglia. GPR109A is reportedly expressed in peripheral macrophages although it remains unknown whether dorsal root ganglion (DRG) macrophages express GPR109A. Our experiments cannot rule out the possibility that the analgesic effects induced by intrathecal injection of MK1903 may be in part due to its action of DRG macrophages. Nevertheless, two experiments in this study support that activation of spinal GPR109A at least in part contributes to the analgesic effects induced by intrathecal injection of MK1903: a. GPR109A is expressed in spinal microglia; b. in the spinal slice preparations without DRGs, treatment of spinal slices with MK1903 suppresses spinal glutamatergic activity and production of IL-1β and IL-18. Given that we have previously demonstrated that inhibition of IL-1β attenuates thermal hyperalgesia in MRL/lpr mice through improving glial glutamate transporter function (Yan et al., 2017), our results indicate that activation of GPR109A produces analgesic effects in MRL/lpr mice with thermal hyperalgesia through suppressing p38 MAPK activity, production of IL-18 and IL-1β, and reducing glutamatergic activity at the spinal dorsal horn.

6 | CONCLUSIONS

We reported in this study that lupus mice with thermal hyperalgesia have activation of microglia and astrocytes, and enhanced glutamate release from presynaptic terminals in the spinal dorsal horn. We demonstrated that activation of spinal microglial GPR109A receptors produces analgesic effects via suppressing IL-18 production and glutamatergic activity at the spinal dorsal horn. Given that GPR109A can be activated by the endogenous β-hydroxybutyric acid (Taggart et al., 2005), niacin (Li et al., 2010; Tunaru et al., 2003; Wise et al., 2003), or synthetic selective GPR109A agonists such as MK1903 (Boatman et al., 2012), our findings suggest the potential usage of these compounds for the treatment of chronic pain caused by lupus disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

This project was supported by the NIH RO1 grant (NS107569) to H.R. W. The authors declare no conflict of interest.

Funding information

National Institute of Neurological Disorders and Stroke, Grant/Award Number: NS107569

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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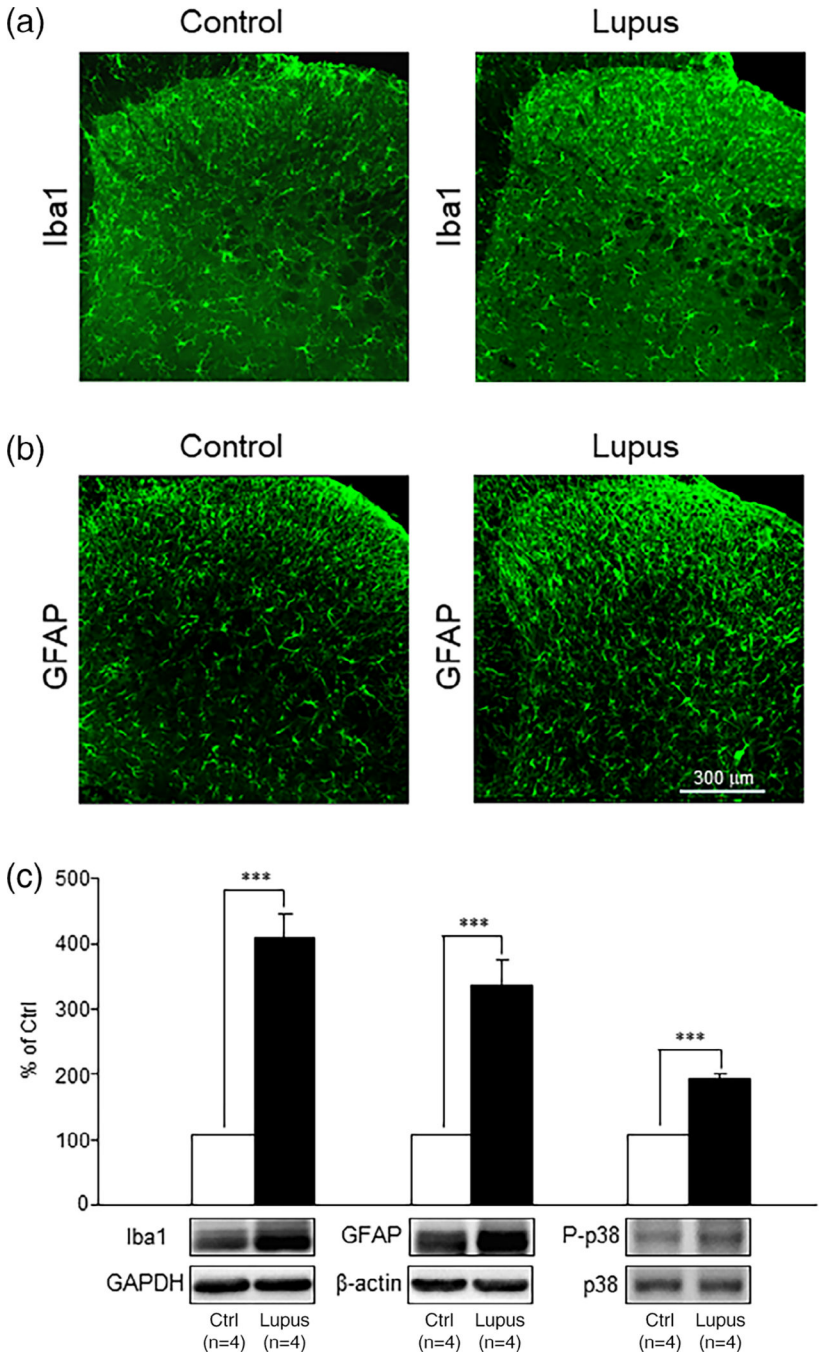

FIGURE 1.

Spinal microglia and astrocytes are activated in MRL/lpr mice with thermal hyperalgesia. Results were collected from female mice at the age of 16 weeks. Samples of spinal sections show immunostaining of microglia with Iba1 antibody (a) and astrocytes (b) with GFAP antibody in female MRL control and MRL/lpr mice. Note that in the spinal dorsal horn of MRL/lpr mice, Iba1, and GFAP immunoreactivity was heightened; microglia and astrocytes appeared hypertrophied with enlarged soma and thick processes. Bar graphs in (c) show the relative protein expression (mean + SEM) of Iba1, GFAP, and phosphorylated p38 MAPK

(P-p38) in MRL/lpr mice ($n = 4$) compared to MRL control mice ($n = 4$). Samples of each protein molecule expression in each group are shown below. ***$p < .001$

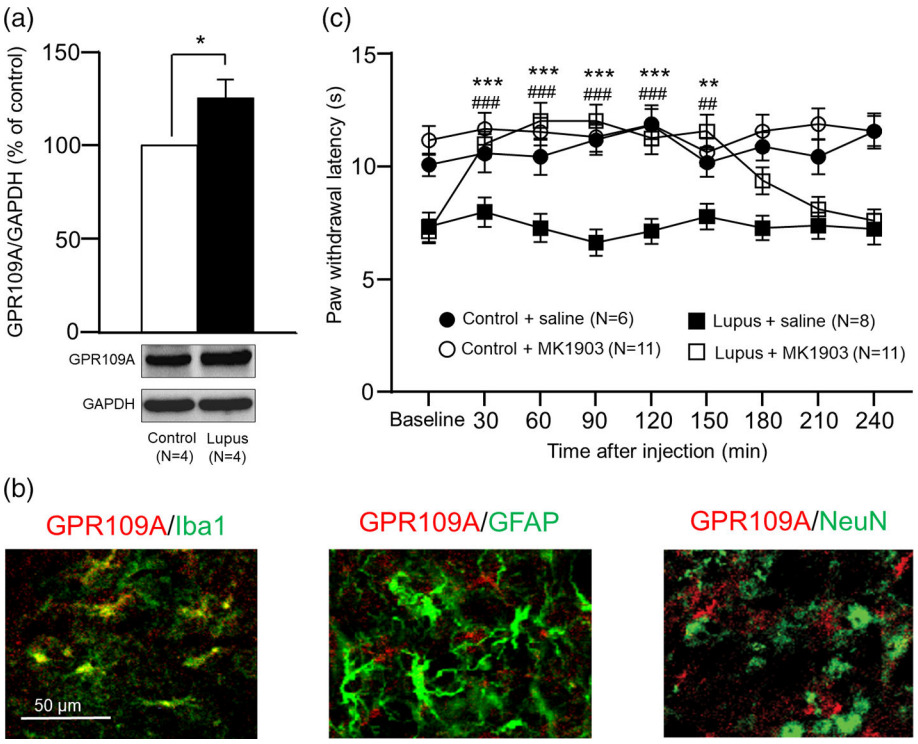

FIGURE 2.

GPR109A protein expression is increased in MRL/lpr mice with thermal hyperalgesia and present only in microglia; and intrathecal injection of a GPR109A agonist (MK1903) attenuates thermal hyperalgesia in MRL/lpr mice. Bar graphs (a) show the relative protein expression (mean + SEM) of GPR109A in MRL/lpr mice with thermal hyperalgesia compared to MRL control mice ($n = 4$). Samples of GPR109A protein molecule expression in each group are shown below. Images in (b) show double staining obtained from the spinal dorsal horn of MRL/lpr mice. Microglia, astrocytes, and neurons in spinal slices were respectively labeled with Iba1, GFAP, and NeuN antibodies (in green) while GPR109A was labeled in red. Line plots in (c) show summaries of the withdrawal latency to thermal stimuli before and after intrathecal injection of MK1903 (concentration: 10 μM, volume: 5 μl) or vehicle (5 μl saline) in MRL/lpr mice and MRL control mice. Baseline indicates the measurement immediately prior to the injection. Comparisons between baseline and at each time point for the MRL/lpr mice+MK1903 group are indicated with #. Comparisons between the MRL/lpr mice+MK1903 group and MRL/lpr mice+saline group are labeled with *. One symbol: $p < .05$; two symbols: $p < .01$; three symbols: $p < .001$

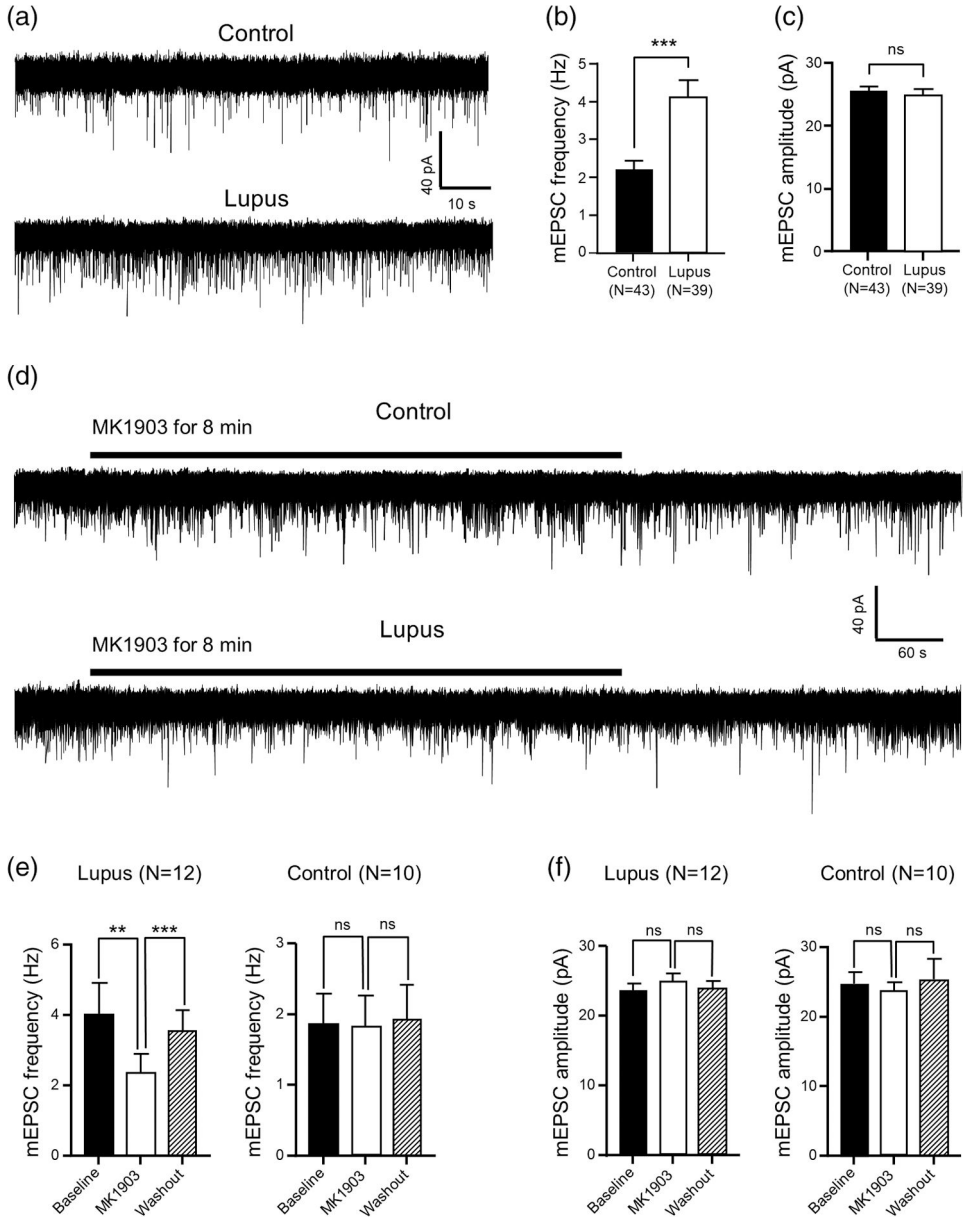

FIGURE 3.

Activation of GPR109A in the spinal dorsal horn reduces the enhanced glutamate release from presynaptic terminals in MRL/lpr mice with thermal hyperalgesia. (a) Raw data show mEPSCs recorded from a neuron from an MRL control mouse (upper trace) and a neuron of an MRL/lpr mouse with thermal hyperalgesia (lower trace). Bar graphs show the mean (+SEM) mEPSC frequency (b) and amplitude (c) from MRL/lpr mice and MRL control mice. (d) Raw data show mEPSCs recorded before, during perfusion and after washout of MK1903 (10 nM) from a neuron of an MRL control mouse (upper trace) and a neuron of an MRL/lpr mouse with thermal hyperalgesia (lower trace). The mean (+SEM) mEPSC frequency (e) and amplitude (f) before, during perfusion and after washout of MK1903 from MRL control and MRL/lpr mice are shown. “N” indicates number of neurons used per

group. One to two neurons per mouse were collected. Two symbols: $p < .01$; three symbols: $p < .001$; ns, no significance

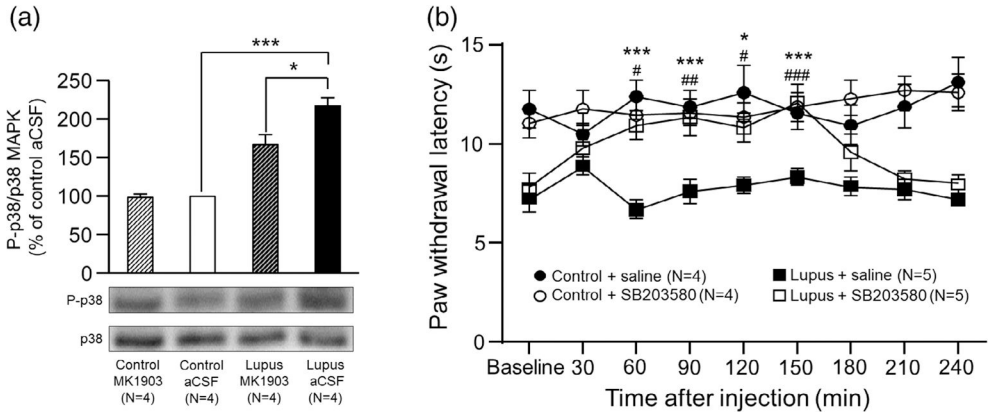

FIGURE 4.

Activation of GPR109A suppresses thermal hyperalgesia in MRL/lpr mice via attenuating p38 MAPK activity in MRL/lpr mice. (a) Four groups (four animals/group) of spinal slices were used: MRL/lpr slices treated with MK1903 (10 nM), and MRL/lpr slices treated with vehicle (aCSF), MRL control slices treated with MK1903 (10 nM), and MRL control slices treated with vehicle (aCSF). Bar graphs show the relative protein expression (mean + SEM) of phosphorylated p38 MAPK in MRL/lpr mice (N = 4) compared to MRL control mice (N = 4) in spinal slices treated with MK1903 (10 nM) or vehicle (aCSF). Samples of each protein molecule expression in each group are shown below. Line plots in (b) show the mean (±SEM) of the withdrawal latency to thermal stimuli before and after intrathecal injection of SB203580 (30 μg/mouse, in 5 μl) or vehicles (5 μl saline) in MRL/lpr mice and MRL control mice. Baseline indicates the measurement immediately prior to the injection. Comparisons between baseline and at each time point for the MRL/lpr mice+SB203580 group are indicated with #. Comparisons between the MRL/lpr mice+SB203580 group and MRL/lpr mice+saline group are labeled with *. One symbol: $p < .05$; two symbols: $p < .01$; three symbols: $p < .001$

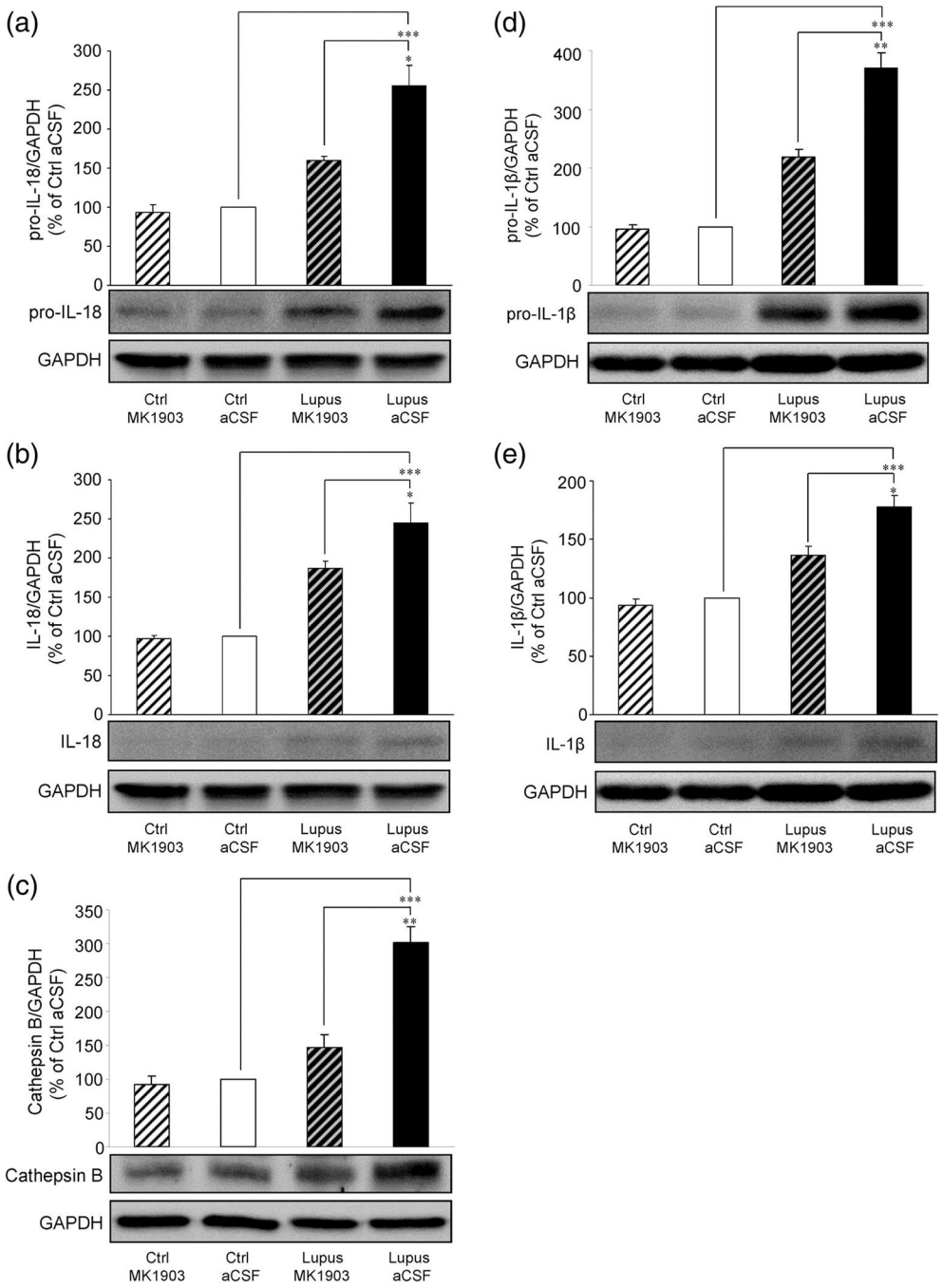

FIGURE 5.

Activation of GPR109A attenuates the elevated levels of IL-18, IL-1β, and cathepsin B in the spinal cord of MRL/lpr mice with thermal hyperalgesia. Bar graphs show the relative protein expression (mean + SEM) of pro-IL-18 (24 KDa) (a), mature IL-18 (18 KDa) (b), cathepsin B (c), pro-IL-1β (25 KDa) (d), and mature IL-1β (17 KDa) (e) in MRL/lpr mice (N = 4) and MRL control mice (N = 4) in spinal slices treated with MK1903 (10 nM) or vehicle (aCSF). Samples of each protein molecule expression in each group are shown below. One symbol: $p < .05$; two symbols: $p < .01$; three symbols: $p < .001$

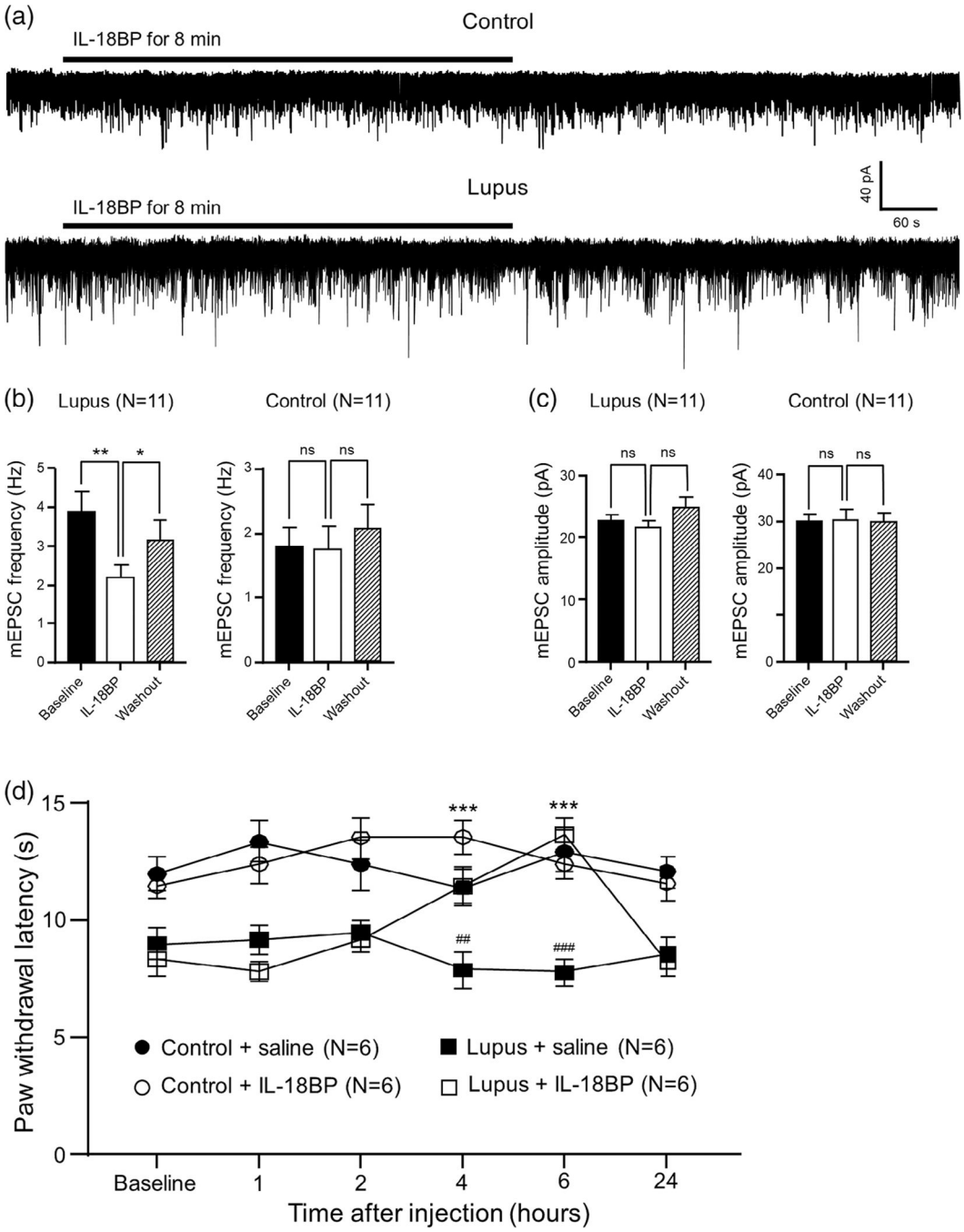

FIGURE 6.

Blocking IL-18 activity attenuates glutamate release from presynaptic terminals in the spinal dorsal horn and thermal hyperalgesia in MRL/lpr mice. (a) Raw data show mEPSCs recorded before, during perfusion and after washout of IL-18BP (10 ng/ml) from a neuron of an MRL control mouse (*upper trace*) and a neuron of an MRL/lpr mouse (*lower trace*). The mean (+SEM) mEPSC frequency (b) and amplitude (c) before (baseline), during perfusion, and after washout of IL-18BP from MRL control mice and MRL/lpr mice with thermal hyperalgesia are shown. Line plots in (d) show summaries of the withdrawal latency to

thermal stimuli before and after intrathecal injection of IL-18BP (500 ng/mouse, volume: 5 μl) or vehicles (5 μl saline) in MRL/lpr mice and MRL control mice. Baseline indicates the measurement immediately prior to the injection. Comparisons between baseline and at each time point for the MRL/lpr mice +IL-18BP group are indicated with #. Comparisons between the MRL/lpr mice+IL-18BP group and MRL/lpr mice+saline group are labeled with *. One symbol: $p < .05$; two symbols: $p < .01$; three symbols: $p < .001$; ns, no significance