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TREM2/DAP12 Signal Elicits Proinflammatory Response in Microglia and Exacerbates Neuropathic Pain

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Neuropathic pain afflicts millions of people, and the development of an effective treatment for this intractable pain is an urgent issue. Recent evidence has implicated microglia in neuropathic pain. The present study showed that the DNAX-activating protein of 12 kDa (DAP12) and its associated "triggering receptor expressed on myeloid cells 2" (TREM2) were predominantly expressed by microglia in the dorsal horn after spinal nerve injury, revealing a role for TREM2/DAP12 signaling in neuropathic pain. Nerve injury-induced proinflammatory cytokine expression in microglia and pain behaviors were significantly suppressed in *Dap12*-deficient mice. Furthermore, intrathecal administration of TREM2 agonistic antibody induced proinflammatory cytokine expression, as well as neuropathic pain, in mice without nerve injury. The agonistic antibody induced proinflammatory responses and neuropathic pain was not observed in *Dap12*-deficient mice. Together, these results suggest that TREM2/DAP12-mediated signals in microglia exacerbate nerve injury-induced neuropathic pain by inducing proinflammatory cytokine secretion from microglia. Suppression of DAP12-mediated signals could be a therapeutic target for neuropathic pain.

Key words: dap12; microglia; pain; trem2

Significance Statement

Recent studies have revealed that activated microglia in the spinal dorsal horn exacerbate neuropathic pain, which has suggested that suppression of microglial activity should be considered as a therapeutic target. However, only a few molecules have been identified as regulators of microglial activity. In this study, we focused on a receptor complex of TREM2 and DAP12, both of which are expressed by microglia and have been implicated in the pathogenesis of Alzheimer's disease, and demonstrated that TREM2/DAP12 signaling promoted proinflammatory responses in microglia and exacerbates neuropathic pain. The present results revealed the functional significance of TREM2/DAP12 signaling in microglial activation after neuronal injury, and could help in the development of treatments for neuropathic pain and neurodegenerative diseases.

Introduction

Neuropathic pain is a chronic and pathological pain that is induced by nerve injury, often due to metabolic, cancerous, infectious, chemical, or traumatic impairments (Grace et al., 2014). Allodynia, a significant sensory aberration elicited by a non-noxious stimulus, and hyperalgesia, an increased pain response to a noxious stimulus, are some of the major symptoms (Woolf and Mannion, 1999). Much work has been done to establish effective treatments for neuropathic pain, and several molecular mechanisms underlying neuropathic pain have been revealed. However, the more precise and integrative molecular and cellular

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mechanisms remain poorly understood, and to date there are no established successful or practical treatments. Recently, microglia have emerged as key players in eliciting neuropathic pain in the spinal cord (Inoue and Tsuda, 2009; Maeda et al., 2010; Tsuda et al., 2013; Grace et al., 2014; Yasui et al., 2014). Microglia, which are the immunocompetent cells in the CNS, are able to quickly respond to injury and infection, and play a pivotal role in the scavenging of pathogens and maintaining tissue homeostasis. However, when microglia are exposed to abnormal conditions, such as severe traumatic injury or inflammation in the CNS, they change their functional phenotype and secrete an excess of pro-inflammatory cytokines and reactive oxygen species. This further results in neuronal damage. Nerve injury readily induces this adverse environment and has been shown to play a role in neuropathic pain (Leung and Cahill, 2010; Yasui et al., 2014).

DNAX-activating protein of 12 kDa (DAP12) (also known as TYRO protein kinase-binding protein [TYROBP] and killer cell activating receptor-associated protein [KARAP]) is a single-pass transmembrane adaptor protein that is mainly expressed in various myeloid cells and natural killer cells (Lanier, 2009). Previous reports from our group and others have demonstrated that DAP12 is predominantly expressed in microglia in the CNS and in macrophage in the periphery (Roumier et al., 2004; Thrash et al., 2009; Kobayashi et al., 2015). DAP12 contains an immunoreceptor tyrosine-based activation motif (ITAM) in the cytoplasm and does not have ligand-binding capabilities because of its short extracellular domain, resulting in the formation of complexes with specific partner membrane receptors (Lanier, 2009). Once the ligand binds to the DAP12-associated receptors, DAP12 sends signals through the ITAM within its cytoplasmic domain and induces cellular activation (Tomasello et al., 1998; Takaki et al., 2006), such as cytokine production, proliferation, and survival of macrophages (Turnbull et al., 2005; Otero et al., 2009), osteoclast differentiation (Kaifu et al., 2003), and antigen presentation, maturation, and survival of dendritic cells (Bouchon et al., 2001). In the CNS, DAP12 and the triggering receptor expressed on myeloid cells 2 protein (TREM2), a DAP12-associated receptor expressed by myeloid cells and microglia, have been implicated in the pathogenesis of Alzheimer's disease (Guerreiro et al., 2013; Jonsson et al., 2013; Zhang et al., 2013). A *Trem2* mutation has been shown to confer a risk of late-onset Alzheimer's disease (Guerreiro et al., 2013; Jonsson et al., 2013), and *Dap12* mutations in humans results in Nasu-Hakola disease, which is characterized by progressive presenile dementia and bone cysts (Paloneva et al., 2000).

We recently reported that DAP12-mediated signals promote inflammatory polarization in microglia following motor nerve injury, and microglial DAP12-mediated signals exacerbate degeneration of injured motor neurons (Kobayashi et al., 2015). More recently, injured sensory neuron-derived colony-stimulating factor 1 (CSF1) was shown to be a critical inducer of microglia-mediated pain, with signaling via the CSF1 receptor (CSF1R) and DAP12 on microglia (Guan et al., 2016). Although this report demonstrated the significance of CSF1R/DAP12 signaling in microglial stimulation, several DAP12-associated receptors are expressed by microglia. Therefore, the present study addressed the crucial role that DAP12 plays in the microglial phenotype leading to neuropathic pain, and provided evidence that additional pathways, such as the TREM2/DAP12-mediated pathway, is also critical for determining the microglial phenotype and initiating neuropathic pain.

Table 1. Primers used for qRT-PCR

Gene	Accession number	Primer sequence (5′-3′)	
DAP12	BC056450	Forward	gattgccctggctgtgtact
		Reverse	ctggtctctgaccctgaagc
TREM2	BC052784	Forward	ggaaccgtcaccatcactct
		Reverse	atgctggctgcaagaaactt
TNF-α	NM_013693	Forward	gtggaactggcagaagaggc
		Reverse	agacagaagagcgtggtggc
IL-1β	NM_008361	Forward	ctgtgtctttcccgtggacc
		Reverse	cagctcatatgggtccgaca
IL-6	NM_031168	Forward	ttccatccagttgccttctt
		Reverse	cagaattgccattgcacaac
IRF5	AF028725	Forward	cctcagccgtacaagatctacga
		Reverse	gtagcattctctggagctcttcct
IRF8	NM_008320	Forward	gagctgcagcaattctacgc
		Reverse	aagggtctctggtgtgaggt
Ctss	BC002125	Forward	gttgggctttcagtgctgtg
		Reverse	ggataggaagcgtctgcctc
P2RX4	NM_011026	Forward	acaacgtgtctcctggctacaat
		Reverse	gtcaaacttgccagcctttcc
BDNF	BC034862	Forward	tacctggatgccgcaaacat
		Reverse	agttggcctttggataccgg

Materials and Methods

Animals. All animal experiments were conducted in accordance with the standard guidelines of the Nagoya University Graduate Schools of Medicine. The male mice used in this study were 10–15 weeks of age at the start of each experiment. Wild-type (WT) C57BL/6 mice were purchased from Charles River Laboratories Japan. *Dap12*-deficient (*Dap12*$^{-/-}$ or *Tyrobp*$^{-/-}$) mice were backcrossed into the C57BL/6 genetic background for at least 12 generations (Kaifu et al., 2003). *C-C chemokine receptor type 2* (*Ccr2*)$^{RFP/RFP}$ mice were obtained from The Jackson Laboratory. All mice were housed at a controlled temperature (23 ± 1°C) with a 12 h light-dark cycle (light on 9:00 to 21:00), and acclimated to the environment for >1 week before the experiments in accordance with the *Guide for the care and use of laboratory animals*. The study was conducted with the approval of the local animal ethics committee in accordance with the regulations for animal experiments at Nagoya University (permission 24294, 25107, 26181, 27204, and 28303), the Animal Protection and Management Law of Japan (105), and the Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983).

Surgical procedure of the neuropathic pain model. To generate neuropathic pain in mice, we used the L4 spinal nerve injury model with some modifications (Kim and Chung, 1992; Tsuda et al., 2003; Masuda et al., 2012, 2014). Under 3% isoflurane anesthesia, mice were put in a prone position. A small incision at L3-S1 was made on the left side. The paraspinal muscles were cut and partially removed from the L5 transverse process. We preserved the L5 transverse process without removing it. To expose the L4 spinal nerve, trimming around the intervertebral foramen was performed. Then, the left L4 nerve was carefully dissociated and cut. The muscle layer of the wound and skin were tightly sutured with 6-0 silk.

qRT-PCR. We harvested the L4 spinal cords from 4 mice from each group. The spinal cords were then vertically separated along the median. Hemisections of the ipsilateral and contralateral side were horizontally divided into dorsal horn (DH) and ventral horn according to the bottom line of the substantia gelatinosa under the microscope. DH tissues were subjected to total RNA extraction using the acid guanidine isothiocyanate/phenol/chloroform extraction method. Total RNA was quantified using a Nanodrop spectrophotometer (Thermo Scientific). cDNA was prepared from total RNA by reverse transcription reaction with SuperScript III (Invitrogen). qRT-PCR was performed with StepOnePlus (Applied Biosystems) using Fast SYBR Green Master Mix (Applied Biosystems). Primers are described in Table 1. The conditions for fast qRT-PCR were as follows: 1 cycle of 95°C for 20 s, 40 cycles of 95°C for 3 s, and 60°C for 30 s. At the end of the PCR, the samples were subjected to a melting analysis to confirm amplicon specificity. Results were normal-

ized to *glyceraldehyde-3-phosphate dehydrogenase* (*Gapdh*). Fold changes in gene expression were calculated using the $2^{-\Delta Ct}$ method.

In situ *hybridization.* cDNA fragments (*Dap12*: 72-352 of accession number BC056450; *Trem2*: 93-1038 of accession number BC052784) were inserted into a pGEM-T easy vector (Promega). cRNA probes were synthesized *in vitro* using T7 RNA polymerase (Promega) and digoxigenin (DIG) RNA Labeling Mix (Roche Applied Science). *In situ* hybridization was performed as previously described (Braissant and Wahli, 1998; Nakadate et al., 2009). In brief, 10-μm-thick fresh frozen sections of the spinal cord were prepared using a cryostat CM1850 (Leica Biosystems), followed by fixation in 4% PFA, and were washed twice in PBS-containing 0.1% active diethylpyrocarbonate. After equilibration in hybridization buffer (50% formamide, 5× SSC, 40 μg/ml salmon sperm DNA), sections were hybridized with DIG-labeled riboprobe in hybridization buffer for 18 h at 58°C. After washing, DIG was visualized following incubation with alkaline phosphatase-conjugated anti-DIG Fab fragment (1:2000; Roche Applied Science) for 2 h and NBT/BCIP (1:50; Roche Applied Science) for 15 h at room temperature. For double-labeling with immunohistochemistry, the sections were first processed for *in situ* hybridization and then immunohistochemistry was performed. The images were taken with a BZ-9000 microscope (Keyence).

Immunohistochemistry. Immunohistochemistry was performed as previously described (Konishi et al., 2006; Kobayashi et al., 2015). Mice were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with normal saline followed by Zamboni's fixative (0.1 M PB-containing 2% PFA and 0.2% picric acid). The L4 spinal cords were removed from perfused mice and were post-fixed in the same fixative for 15–18 h at 4°C. The tissues were then immersed in 20% sucrose in 0.1 M PB at 4°C overnight. Then, the 14-μm-thick sections of the L4 spinal cord were cut and reacted with primary antibodies for 4 h at room temperature or overnight at 4°C. Primary antibodies were as follows: anti-interferon regulatory factor 8 (IRF8) (#sc-6058, Santa Cruz Biotechnology; 1:250), anti-ionized calcium-binding adaptor molecule 1 (Iba1) (#ab5076, Abcam; 1:500), anti-DAP12 (#AB4070, Millipore; 1:250), anti-phosphorylated DAP12 (pDAP12) (a generous gift from Dr. Humphrey at University of Oklahoma Health Sciences Center) (1:250) (Peng et al., 2010), and anti-protein kinase C gamma (PKCγ) (#sc-211, Santa Cruz Biotechnology; 1:500). Signals were visualized with Alexa-488 or -594-conjugated secondary antibodies (Invitrogen). Microglial nuclei were stained with DAPI (500 ng/ml; Dojindo Laboratories) for quantifying microglial numbers. Images were taken with a confocal microscope (FV10i, Olympus) or fluorescent microscope BZ-9000.

Histological analysis. To quantify Iba1-positive cell number and area, triple immunofluorescence was performed using anti-Iba1 antibody, anti-PKCγ antibody (to identify the inner lamina II), and DAPI. The same laser power and sensitivities of confocal microscope (FV10i) were used to analyze the randomly chosen 14-μm-thick sections, and the areas of lamina I and II were defined and calculated. Iba1-positive cells that contained distinct DAPI signals were counted and normalized to the area. Iba1-positive areas were also measured after background correction by FV10-ASW software (Olympus) and normalized to the area. A total of 12 sections taken from 3 animals were examined for each time point. In these experiments, the interval of each section was at least 112 μm, and there was no possibility of repetitive counting of the same cells.

Behavioral testing. The mechanical withdrawal threshold was measured using 0.02–0.2 g von Frey filaments (Touch Test Sensory Evaluator) to evaluate mechanical sensitivity. Mice were individually placed on the wire mesh floor, covered by an opaque chamber, and habituated for at least 30 min before testing to acclimate to their new environment. Subsequently, von Frey filaments were applied to the plantar surfaces of their hindpaws. The 50% paw withdrawal threshold (PWT) was determined using the up-down method (Chaplan et al., 1994).

Flow cytometry. Microglia were isolated from DH according to a previous report with some modifications (Singh et al., 2014). Briefly, one WT and one *Dap12*-deficient mouse 3 d after L4 spinal nerve transection were perfused with PBS, and a 5 mm segment around L4 DH was dissected. The DH tissue was minced by blades, treated with 20 U/ml papain (Roche Applied Science) and 10 μg/ml DNase I (Roche Applied Science), and then dispersed by pipetting. Cells were filtrated with 70 μm cell strainer (Corning), resuspended in 37% Percoll (GE Healthcare) in PBS and centrifuged at 500 × *g* for 20 min at 25°C to enrich microglia. After washing the pellet with PBS-containing 0.5% BSA, Fc receptors were blocked with anti-CD16/CD32 antibodies (eBioscience) and then cells were stained with anti-CD11b-APC (Biolegend) and anti-CD45-Pacific Blue (Biolegend). Finally total cells obtained from one animal were resuspended in 500 μl of PBS containing 0.5% BSA with 1000 latex fluorescent beads (Flow-Count, Beckman Coulter Life Sciences) to quantify cell numbers. Flow cytometry was performed by Canto II (BD Biosciences), and data were analyzed by FlowJo software (Tree Star). A total of 5000 events were recorded, and the number of $CD11b^{+}/CD45^{low}$ microglia was normalized to that of beads. The ratio ($Dap12^{-/-}$/WT) of five independent experiments was statistically analyzed.

Conventional RT-PCR. In addition to DH cDNA, cDNA was prepared from positive control tissues, such as spleen. Primers are described in Table 2. The conditions for PCRs were as follows: 1 cycle of 94°C for 30 s, 30–35 cycles of 55°C-60°C for 30 s, and 72°C for 30 s. The amplified products were loaded on an agarose gel and stained with ethidium bromide.

Intrathecal administration of antibodies. Mice were intraperitoneally injected with 50 μg Gentacin (gentamicin sulfate, Merck Sharp & Dohme) before surgery. Under 3% isoflurane anesthesia, the mice were placed in a prone position, and an incision was made on the back. The paraspinal muscles and ligaments were partially cut, and the L5 spinous process was removed. A 32-guage catheter (ReCathCo) was intrathecally inserted to a length of 14 mm through a dural incision made by a 22 guage needle. Mice, whose hindlimb muscle contracted during catheter insertion, were eliminated to exclude the possibility that catheter insertion could affect hindlimb behavior or DH gene expression. The distal side of the catheter remained outside of the skin, and muscles and skin were tightly sutured with 6-0 silk. A total of 5 μg Gentacin was injected via catheter at the end of surgery. The next day, mice with abnormal behaviors, such as paralysis, were eliminated. Mice with paralyzed limbs after intrathecal injection of 2 μl 2% xylocaine (Astra Zeneca) were selected and used for the study. After an interval of 1 d, a total of 1 μg anti-TREM2 antibody (#MCA4772, AbD Serotec) or isotype control antibody (Rat IgG1, #12-4031, eBioscience) was intrathecally injected once a day for 3 d. A total of 5 μl saline was then injected to flush after each antibody injection. Behavioral testing ($n = 7$ or 8) and DH sampling ($n = 4$) were performed at 2 h after final administration.

Table 2. Primers used for conventional PCR

Gene	Accession number	Primer sequence (5′-3′)	
DAP12	BC056450	Forward	ctggtgccttctgttccttc
		Reverse	ctggtctctgaccctgaagc
TREM2	BC052784	Forward	ctgcacttcaagggaaaagc
		Reverse	agagtgatggtgacggttcc
TREM1	BC111875	Forward	acctcccactcaccttcctt
		Reverse	atacgtggttcagccactcc
TREM3	BC127036	Forward	ccaagctggagatgaggaag
		Reverse	cccagctttgcagagaaaga
SIRP-β	AB109404	Forward	tcacgctgaagtggttcaag
		Reverse	tctgcttgtccatcatgctc
MDL-1	BC104364	Forward	gactgaaaccagcccaggaa
		Reverse	cttttccccctccttcccac
CD300C	NM_199225	Forward	actgtgggggagtcactcag
		Reverse	gaccacgaacacctcaacct
CD300D	NM_134158	Forward	ttcgagccttgagagtggtag
		Reverse	gctcaggaacaggaacaacttc
CD300E	BC034097	Forward	catgtccctcacacacttgg
		Reverse	ctgaaacgggcatagttggt
CD200R3	BC106838	Forward	ctggccttcctgcagactac
		Reverse	ccaggagctttgcctaattg
CD200R4	BC131972	Forward	tccagcatgaggggacttac
		Reverse	ggacaggtaagctggattgc
CSF1R	NM_001037859	Forward	ctgcgatgtgtgagcaa
		Reverse	cttgcggataatgaaccct

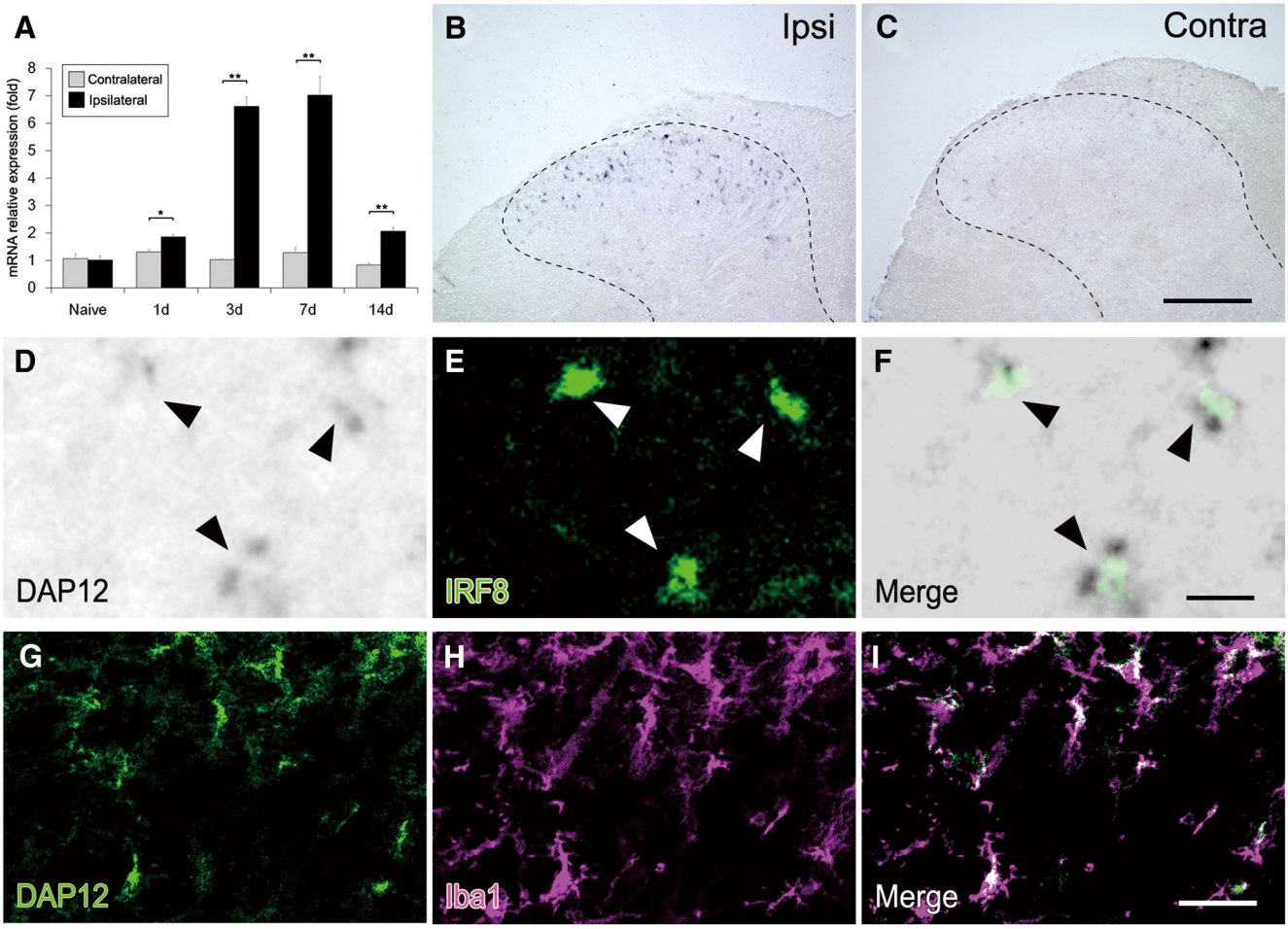

Figure 1. DAP12 expression is induced in microglia after L4 nerve injury. ***A***, qRT-PCR analysis of *Dap12* mRNA extracted from ipsilateral and contralateral L4 DH of WT mice before (Naive) and after L4 nerve injury. Results are normalized to the housekeeping gene *Gapdh* ($n = 4$ for each time point). Values are mean ± SEM. *$p < 0.05$ (two-way ANOVA with *post hoc* Turkey's test). **$p < 0.001$ (two-way ANOVA with *post hoc* Turkey's test). ***B***, ***C***, *In situ* hybridization shows increased *Dap12* mRNA expression in the spinal DH 7 d after L4 nerve injury (low-magnification images). ***B***, Ipsi, Ipsilateral side. ***C***, Contra, Contralateral side. Scale bar, 200 μm. ***D–F***, Localization of *Dap12* mRNA in microglia in ipsilateral DH (high-magnification images). Hybridized cells (***D***) have IRF8 immunoreactivity (***E***) in their nuclei (arrowheads). Scale bar, 10 μm. ***G–I***, Colocalization of DAP12 (***G***, green) and Iba1 (***H***, magenta) protein in L4 DH 7 d after L4 nerve injury. Scale bar, 20 μm.

Statistical analysis. Values are mean ± SEM. Flow cytometry data were analyzed by paired Student's *t* test. Other data were analyzed by one- or two-way ANOVA with *post hoc* Turkey's test. $p < 0.05$ was considered statistically significant.

Results

Induction of DAP12 microglial expression in the dorsal horn (DH) following nerve injury

To analyze the involvement of DAP12-mediated signals in neuropathic pain, we used the modified Chung model (Kim and Chung, 1992), which was previously demonstrated to cause reproducible mechanical allodynia and has been used in mice (Tsuda et al., 2003; Masuda et al., 2012; Masuda et al., 2014).

We transected the mouse L4 spinal nerve and performed qRT-PCR analysis using spinal DH tissues at the L4 level after nerve injury to measure *Dap12* mRNA expression changes. *Dap12* mRNA significantly increased in the ipsilateral DH from 1 d after nerve injury ($p = 0.041$, two-way ANOVA with *post hoc* Turkey's test), with a peak at 7 d ($p = 1.9 \times 10^{-15}$, two-way ANOVA with *post hoc* Turkey's test) (Fig. 1*A*).

To identify cell types that express *Dap12* mRNA in the DH after nerve injury, double-labeling of *in situ* hybridization and immunohistochemistry was conducted. Previous results have shown that *Dap12* mRNA is expressed by microglia (Kobayashi et al., 2015). Therefore, anti-IRF8 antibody, which specifically labels microglial nuclei in the CNS (Masuda et al., 2012), was used. The hybridized sections were stained with anti-IRF8 antibody following visualization of *Dap12* mRNA by *in situ* hybridization. Consistent with qRT-PCR results, there was a significant increase in *Dap12* mRNA-positive cells in the ipsilateral DH (Fig. 1*B*,*C*). Most mRNA staining colocalized with IRF8-positive immunostaining, suggesting that *Dap12*-expressing cells were microglia (Fig. 1*D–F*). To further confirm the increased DAP12 protein in the ipsilateral side, we performed double immunostaining using antibodies against DAP12 and Iba1, a microglial marker. Results showed that most DAP12-positive cells also expressed Iba1 (Fig. 1*G–I*), suggesting that DAP12 is predominantly expressed by microglia and induced in the DH after peripheral nerve injury.

DAP12 phosphorylation in microglia is induced in a neuropathic pain model

DAP12 is a transmembrane adaptor protein that does not have ligand-binding capabilities; signaling via DAP12 is transduced downstream by tyrosine phosphorylation of ITAM in the DAP12 cytoplasmic domain after ligation with DAP12-associated receptors ligand (Peng et al., 2010). To determine whether DAP12-mediated signaling in microglia is activated in response to nerve

injury, we used an antibody specific for pDAP12, the tyrosine-phosphorylated form of DAP12 (Peng et al., 2010). WT mice at 7 d after nerve injury, robust activation of DAP12 phosphorylation was detected in Iba1-positive microglia in the ipsilateral DH, whereas pDAP12 expression in the contralateral DH was very low (Fig. 2*A–F*). In the *Dap12*-deficient ($Dap12^{-/-}$) mice (Kaifu et al., 2003), there were no pDAP12-positive cells, although an intense Iba1 signal was observed (Fig. 2*G–L*). This also suggests a specificity of the antibody we used in this experiment. Together, these results suggest that microglial DAP12 could be activated by DAP12-associated receptor activation in response to nerve injury.

DAP12 signals augment nerve injury-induced neuropathic pain

Microglia play crucial roles in the development and prolongation of neuropathic pain (Inoue and Tsuda, 2009; Tsuda et al., 2013). We evaluated the functional relevance of microglial DAP12 expression on mechanical allodynia, a pathognomonic symptom of neuropathic pain characterized by aberrant pain hypersensitivity initiated by innocuous stimuli (Tsuda et al., 2005). Using WT and $Dap12^{-/-}$ mice, mechanical allodynia was evaluated by measuring PWT to von Frey filament stimulation of the hindpaw after unilateral nerve injury. In the ipsilateral side of WT, there was a decrease in PWT from 3 to 42 d after nerve injury, whereas $Dap12^{-/-}$ mice exhibited significantly higher PWTs compared with WT mice at all time points examined (preoperation: $p = 0.93$; 3 d: $p = 0.0017$; 7 d: $p = 0.0021$; 14 d: $p = 0.011$; 28 d: $p = 0.00019$; 35 d: $p = 0.0049$; 42 d: $p = 0.00015$, two-way ANOVA with *post hoc* Turkey's test), although *Dap12* deficiency did not suppress the pain behavior completely (Fig. 3*A*). At 42 d, PWT was ameliorated in $Dap12^{-/-}$ mice and similar to the preoperative state, whereas WT mice remained hypersensitive. There was no significant difference in pain behavior between WT and $Dap12^{-/-}$ contralateral sides throughout the experimental period (preoperation: $p = 0.95$; 3 d: $p = 0.23$; 7 d: $p = 0.93$; 14 d: $p = 0.36$; 28 d: $p = 0.87$; 35 d: $p = 0.82$; 42 d: $p = 0.79$, two-way ANOVA with *post hoc* Turkey's test) (Fig. 3*B*). Together, $Dap12^{-/-}$ mice exhibited suppressed mechanical allodynia and early recovery after nerve injury. These findings suggested that DAP12-mediated signals in microglia augment neuropathic pain.

Dap12 deficiency reduces microglial numbers in the DH after nerve injury

To elucidate the causative difference in pain behavior between WT and $Dap12^{-/-}$ mice in the neuropathic pain model, microglial numbers and morphology were examined using Iba1 staining.

Before quantifying microglia, we examined the possibility of circulating monocyte infiltration in $Ccr2^{RFP/+}$ mice, which could affect pain behavior after nerve injury (Fig. 4). CCR2 promoter-derived RFP expression allows for unequivocal distinction between infiltrating monocytes/macrophages and resident microglia (Saederup et al., 2010). In the ipsilateral dorsal root, the number of RFP-positive infiltrated monocytes/macrophages increased (Fig. 4, arrowhead), although there were almost no RFP-positive cells in the contralateral dorsal root as previously reported (Siebert et al., 2000). Simultaneously, there was an increase in Iba1-positive cells in the ipsilateral DH, but few RFP-positive cells in the ipsilateral DH (Fig. 4). These results suggested that in the present neuropathic pain model, Iba1-positive cells in the DH were derived from proliferated and/or migrated resident microglia and not from infiltrated circulating monocytes.

Iba1-positive microglia and the area of Iba1 expression were quantified in lamina I and II regions, which are critical layers of etiology in the neuropathic pain model (Fig. 5*A–H*). Antibodies against PKCγ were used to visualize the layers; PKCγ staining reveals a clear boundary of the inner lamina II in the DH (Fig. 5*B*,*F*) (Malmberg et al., 1997). There was no significant basal difference in Iba1-positive area ($p = 0.81$) and microglial numbers ($p = 0.98$) between the two genotypes of naive mice. However, after nerve injury, there were significantly smaller Iba1-positive area (1 d: $p = 0.044$; 3 d: $p = 2.0 \times 10^{-7}$; 7 d: $p = 0.0052$; 14 d: $p = 0.017$, two-way ANOVA with *post hoc* Turkey's test) and less microglial cells (1 d: $p = 0.0031$; 3 d: $p = 2.2 \times 10^{-8}$; 7 d: $p = 0.23$; 14 d: $p = 0.0051$, two-way ANOVA with *post hoc* Turkey's test) in the $Dap12^{-/-}$ mice compared with WT mice throughout time points examined (Fig. 5*I*,*J*). The Iba1-positive area and the number of microglia of $Dap12^{-/-}$ mice decreased to 59.9% and 68.5%, respectively, compared with WT mice at 3 d after nerve injury (Fig. 5*I*,*J*). To confirm the reduction of microglial numbers in $Dap12^{-/-}$ mice, we performed a flow cytometric assay at 3 d after nerve injury and demonstrated that microglial numbers in ipsilateral DH of $Dap12^{-/-}$ mice decreased to 65.7% ($p = 0.030$) (Fig. 5*K*,*L*). These results suggested that DAP12-mediated signals likely participate in microglial migration, proliferation, and/or survival after nerve injury.

Dap12 deficiency suppresses proinflammatory responses in neuropathic pain model

Representative proinflammatory cytokines, such as TNF-α, interleukin-1β (IL-1β), and IL-6, are involved in inflammatory and neuropathic pain (He et al., 2010; Leung and Cahill, 2010). These cytokines are mainly released by microglia in the CNS in response to a variety of stimuli, such as nerve injury. We previously reported that microglial DAP12-mediated signaling induces a proinflammatory response after motor nerve injury *in vivo*, as well as after LPS stimulation *in vitro* (Kobayashi et al., 2015), suggesting that a similar mechanism might be involved in neuropathic pain.

Tissues from the ipsilateral DH of WT and $Dap12^{-/-}$ mice after L4 spinal nerve injury were examined by qRT-PCR with respect to neuropathic pain-related molecules. From the early phase after injury, mRNA expression for Tnfa and *Il6* was induced in the DH; however, the induction was significantly suppressed in the $Dap12^{-/-}$ mice compared with WT mice (Fig. 6*A*,*C*). Additionally, induction of *Il1b*, whose expression peaked at 7 d after injury, was also suppressed (Fig. 6*B*). Induction of the *Irf5* and *Irf8* transcriptional factors, which are expressed by microglia in the CNS and are considered important for gene expression of proinflammatory cytokines (Takaoka et al., 2005; Krausgruber et al., 2011) and neuropathic pain (Masuda et al., 2012; Masuda et al., 2014), was also significantly suppressed in the $Dap12^{-/-}$ mice (Fig. 6*D*,*E*). Induction of *cathepsin S* (*Ctss*), which has also been shown to be involved in neuropathic pain (Clark et al., 2007), was suppressed in $Dap12^{-/-}$ mice only during the later stages after injury (Fig. 6*F*). The purinergic receptor P2X 4 (P2RX4)-BDNF axis is also considered a critical regulator of nerve injury-induced neuropathic pain (Tsuda et al., 2003; Coull et al., 2005). Although only slight statistical differences at certain time points were seen, a decreased tendency in induction of *P2rx4* and *Bdnf* mRNAs was observed throughout the experimental period (Fig. 6*G*,*H*). These findings suggest that DAP12 plays a role in the development of neuropathic pain, partly by

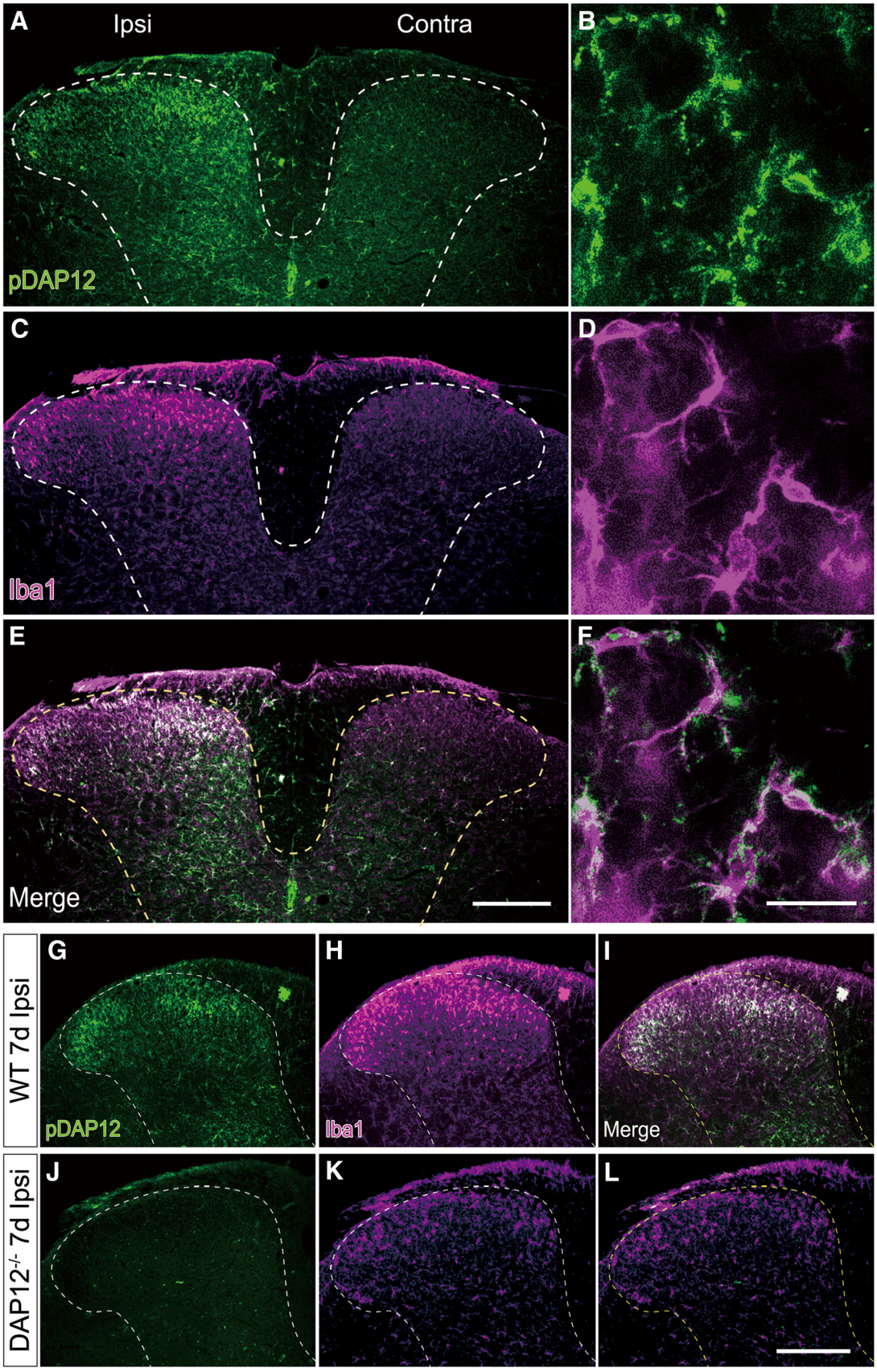

Figure 2. Microglial DAP12 is phosphorylated in neuropathic pain states. ***A–F***, Visualization of pDAP12 (***A***, ***B***, green) and Iba1 (***C***, ***D***, magenta) protein in the spinal DH 7 d after L4 nerve injury. pDAP12 signals colocalize with Iba1-positive cells. ***A***, ***C***, ***E***, Low-magnification images of ipsilateral side. ***B***, ***D***, ***F***, High-magnification images of ipsilateral side. Ipsi, Ipsilateral side; Contra, contralateral side. Scale bars: ***A***, ***C***, ***E***, 200 μm; ***B***, ***D***, ***F***, 20 μm. ***G–L***, Comparison of pDAP12 immunoreactivity in ipsilateral DH of WT (***G–I***) and *Dap12* $^{-/-}$ mouse (***J–L***) 7 d after L4 nerve injury. Only WT ipsilateral DH has robust pDAP12 immunoreactivity. Scale bar, 200 μm.

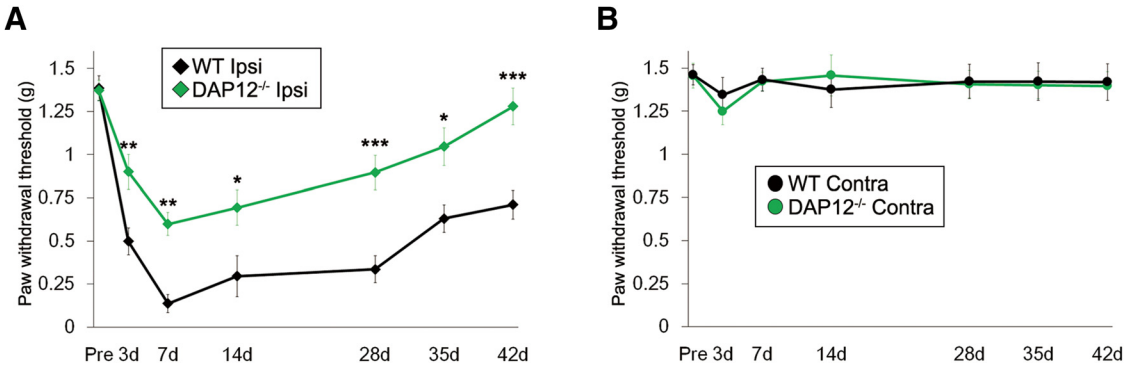

Figure 3. Nerve injury-induced mechanical allodynia is significantly reduced in *Dap12* $^{-/-}$ mice. PWT in WT and *Dap12* $^{-/-}$ mice before (pre) and after L4 nerve injury ($n = 7$–10). ***A***, PWT of ipsilateral side (Ipsi). ***B***, PWT of contralateral side (Contra). *$p < 0.05$ (two-way ANOVA with *post hoc* Turkey's test). **$p < 0.01$ (two-way ANOVA with *post hoc* Turkey's test). ***$p < 0.001$ (two-way ANOVA with *post hoc* Turkey's test). At all time points, PWT is greater in *Dap12* $^{-/-}$ mice than WT on the ipsilateral side.

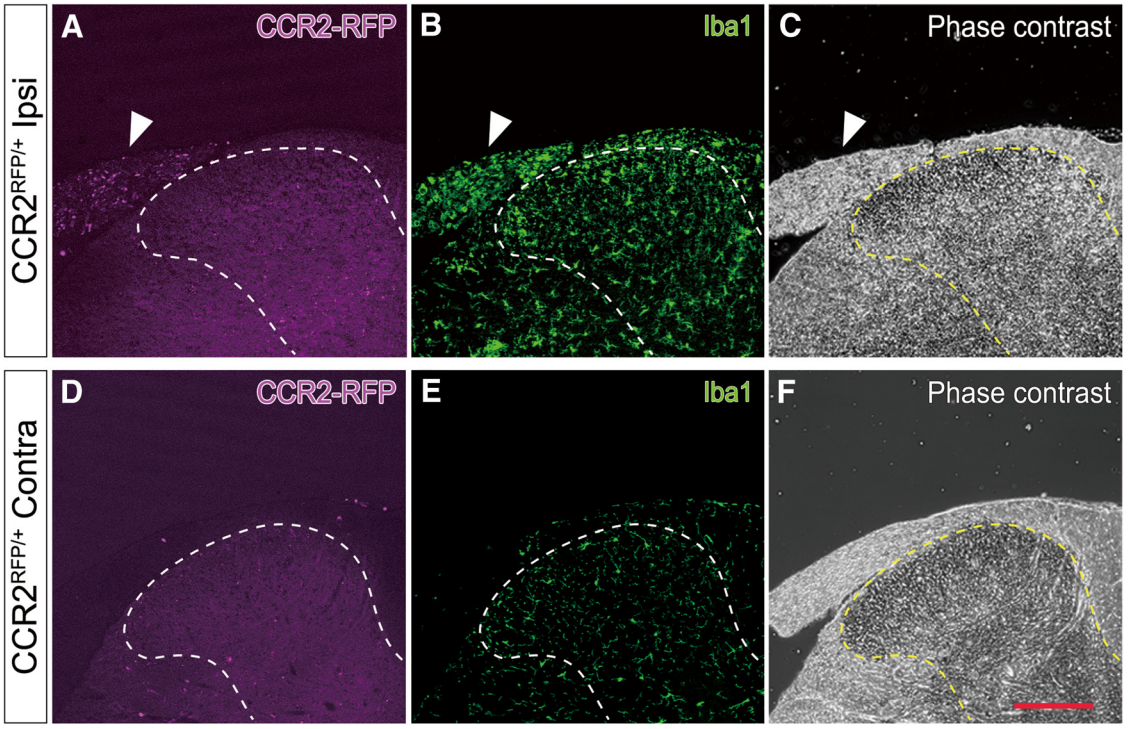

Figure 4. Peripheral monocytes do not infiltrate into spinal cord after L4 nerve injury. L4 DH with dorsal root of *Ccr2* $^{RFP/+}$ mice 7 d after L4 nerve injury. CCR2 promoter-derived RFP signals (***A***, ***D***, CCR2-RFP, magenta), Iba1 immunoreactivity (***B***, ***E***, green), and phase-contrast images (***C***, ***F***) are shown. ***A–C***, Ipsi, Ipsilateral side. ***D–F***, Contra, Contralateral side. In the injured ipsilateral L4 spinal nerve, RFP signals are observed in Iba1-positive peripheral monocytes/macrophages (arrowheads). Conversely, in the ipsilateral DH, almost all Iba1-positive microglia are negative for RFP. In the contralateral side, CCR2 signals are barely visible in the spinal nerve and dorsal horn. Scale bar, 200 μm.

promoting expression of proinflammatory cytokines and other neuropathic pain-related genes.

TREM2 role in DAP12-mediated signaling in neuropathic pain

It is assumed that DAP12 is not involved in ligand binding because of its short extracellular domain. However, DAP12 is capable of mediating signals by forming complexes with DAP12-associated receptors (Lanier, 2009). To identify the DAP12 partners in the present neuropathic pain model, we measured mRNA expression of known DAP12-associated receptors expressed by myeloid cells in the DH at 7 d after injury. Among the genes examined in Figure 7*A*, *Trem2* and *Csf1r* exhibited a significant induction (Fig. 7*A*), whereas other genes exhibited very low or no expression after nerve injury.

We focused on TREM2 as a counterpart receptor of DAP12 because CSF1R is considered to locate upstream of DAP12 (Zou et al., 2008; McVicar and Trinchieri, 2009), and no direct interaction between CSF1R and DAP12 has been reported (Hamerman et al., 2009). The *Trem2* expression profile over time in DH tissue after injury was examined by qRT-PCR analysis. Peak expression was observed at 3 to 7 d after injury, with a 7.80-fold increase compared with the nonoperated naive side at 3 d after injury (Fig. 7*B*). We then performed double-labeling with *in situ* hybridization for *Trem2* mRNA and immunohistochemistry for IRF8. Hybridization signals for *Trem2* mRNA were observed in IRF8-positive cells (Fig. 7*C–G*), indicating that *Trem2* mRNA is expressed by microglia and that TREM2 could function as a binding partner with DAP12 in microglia after nerve injury.

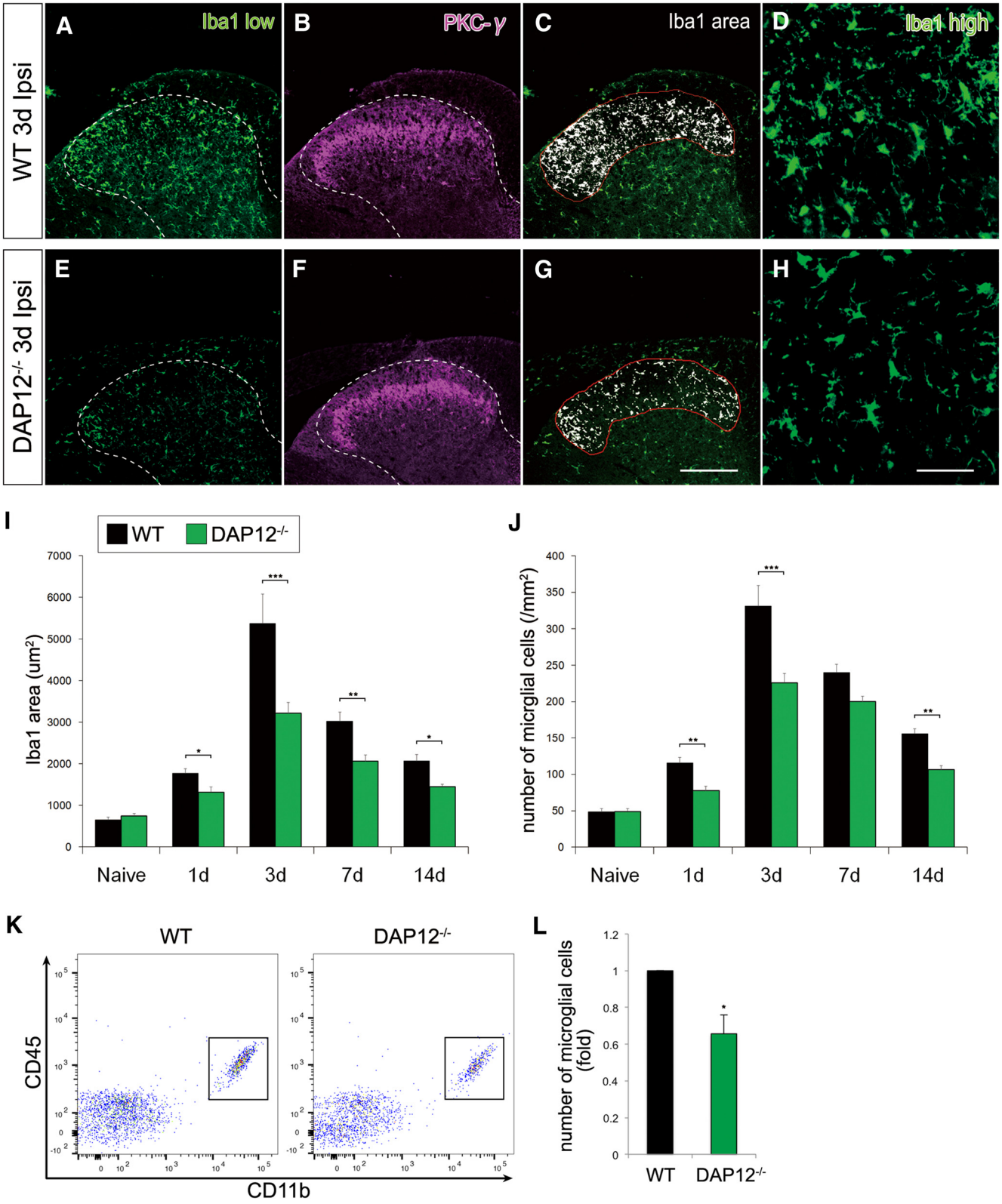

Figure 5. Microglial numbers are reduced in injured DH of *Dap12* $^{-/-}$ mice. ***A–H***, Sections of ipsilateral DH in WT (***A–D***) and *Dap12* $^{-/-}$ (***E–H***) mice 3 d after L4 nerve injury were stained by anti-Iba1 antibody (***A***, ***E***, green) and anti-PKCγ antibody (***B***, ***F***, magenta) for lamina II visualization. Iba1-positive area within lamina I and II is indicated in white (***C***, ***G***). Higher magnification of Iba1 signals in lamina I and II is also shown (***D***, ***H***). Scale bar, 200 μm (left, middle left, and middle right columns), 20 μm (right column). ***I***, ***J***, Quantification of Iba1-positive area (***I***) and microglial numbers (***J***) in lamina I and II of ipsilateral L4 DH in WT and *Dap12* $^{-/-}$ mice before (Naive) and after L4 nerve injury ($n = 3$ for each time point). Values are mean $\pm$ SEM. *$p < 0.05$ (two-way ANOVA with *post hoc* Turkey's test). **$p < 0.01$ (two-way ANOVA with *post hoc* Turkey's test). ***$p < 0.001$ (two-way ANOVA with *post hoc* Turkey's test). ***K***, Flow cytometric analysis of CD11b $^{+}$/CD45 ^{low} microglia in ipsilateral L4 DH 3 d after injury. Representative data are shown. CD11b $^{+}$/CD45 ^{high} monocytes/macrophages were hardly seen in DH tissues. ***L***, Fold changes of microglial numbers in ipsilateral L4 DH 3 d after injury determined by flow cytometry. Microglial numbers were normalized to that of fluorospheres, and then the ratio (*Dap12* $^{-/-}$/WT) was calculated ($n = 5$). Values are mean $\pm$ SEM. *$p < 0.05$ (paired Student's *t* test).

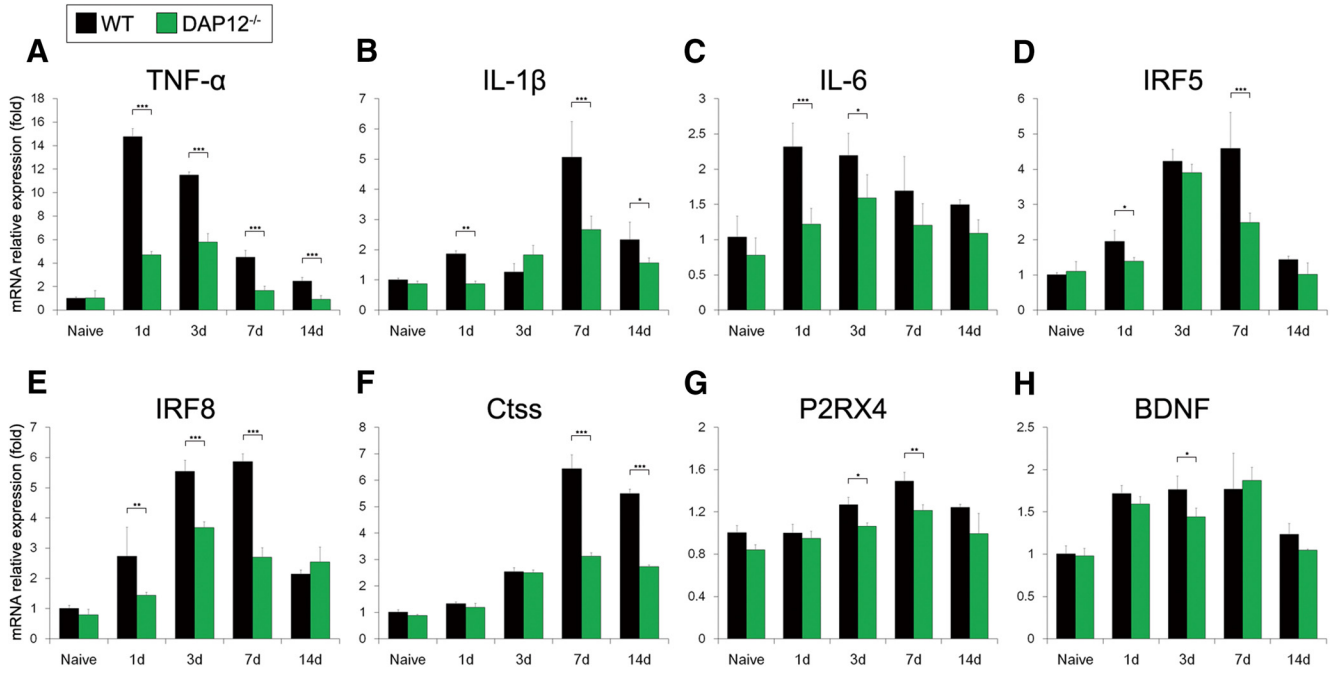

Figure 6. Neuropathic pain-related microglial molecules are suppressed in *Dap12* $^{-/-}$ mice. Ipsilateral L4 DH was obtained from WT and *Dap12* $^{-/-}$ mice before (Naive) and after L4 nerve injury ($n = 4$), and mRNA expression of neuropathic pain-related molecules as quantified by qRT-PCR. ***A***, *Tnfa*; ***B***, *Il1b*; ***C***, *Il6*; ***D***, *Irf5*; ***E***, *Irf8*; ***F***, *Ctss*; ***G***, *P2rx4*; ***H***, *Bdnf*. Results are normalized to *Gapdh* and shown as ratios to nonoperated value in WT mice. Values are mean ± SEM. *$p < 0.05$ (two-way ANOVA with *post hoc* Turkey's test). **$p < 0.01$ (two-way ANOVA with *post hoc* Turkey's test). ***$p < 0.001$ (two-way ANOVA with *post hoc* Turkey's test).

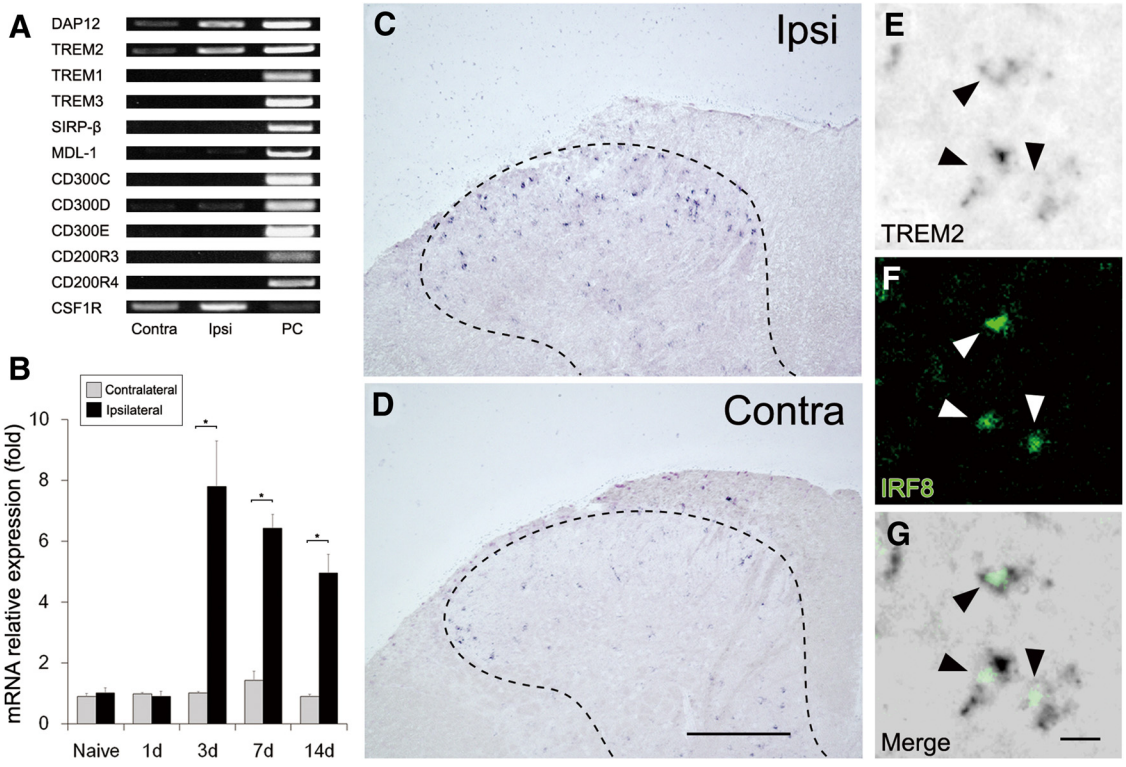

Figure 7. DAP12-associated receptor TREM2 is induced in microglia after nerve injury. ***A***, Conventional RT-PCR results of DAP12-associated receptors expressed by myeloid cells. In addition to cDNA prepared from ipsilateral (Ipsi) and contralateral (Contra) DH 7 d after L4 nerve injury, positive control (PC) tissues, such as spleen, were used. ***B***, qRT-PCR analysis of *Trem2* mRNA extracted from contralateral and ipsilateral L4 DH of WT mice before (Naive) and after L4 nerve injury. Results are normalized to *Gapdh* ($n = 4$ for each time point). Values are mean ± SEM. *$p < 0.001$ (two-way ANOVA with *post hoc* Turkey's test). ***C***, ***D***, *In situ* hybridization shows induction of *Trem2* mRNA expression in the spinal DH 7 d after L4 nerve injury (low-magnification images). ***C***, Ipsi, Ipsilateral side. ***D***, Contra, Contralateral side. Scale bar, 200 μm. ***E–G***, Localization of *Trem2* mRNA in microglia in L4 DH (high-magnification images). Hybridized cells (***E***) have IRF8 immunoreactivity (***F***) in their nuclei (arrowheads). Scale bar, 10 μm.

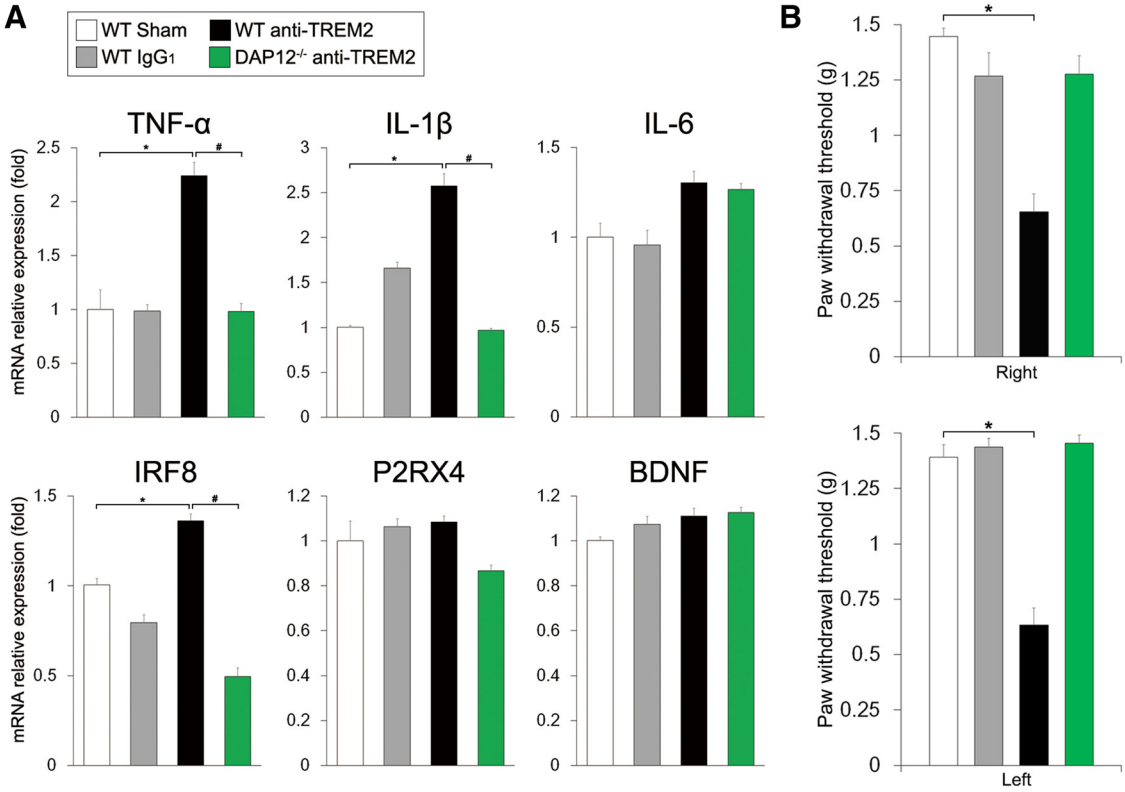

Figure 8. Intrathecal administration of TREM2 agonistic antibody induces mechanical allodynia via DAP12. ***A***, qRT-PCR analysis of neuropathic pain-related microglial molecules in L4 DH of noninjured WT and *Dap12* $^{-/-}$ mice after intrathecal administration of TREM2 agonistic antibody (anti-TREM2) or control IgG (IgG_1) for 3 consecutive days. Results are normalized to *Gapdh* ($n = 4$ for each time point). Values are mean ± SEM. $^{*}p < 0.001$ versus WT Sham (one-way ANOVA with *post hoc* Turkey's test). $^{\#}p < 0.001$ versus WT anti-TREM2 (one-way ANOVA with *post hoc* Turkey's test). ***B***, PWT for right and left paw of mice intrathecally administered TREM2 agonistic antibody for 3 consecutive days ($n = 7$ or 8). Values are mean ± SEM. $^{*}p < 0.001$ versus WT Sham (one-way ANOVA with *post hoc* Turkey's test).

To determine the functional involvement of TREM2 in neuropathic pain, we examined the effect of intrathecal administration of agonistic TREM2 antibody in noninjured WT mice (Fig. 8). This monoclonal antibody is reported to cross-link TREM2 on the cellular surface and exert DAP12 signaling (Humphrey et al., 2006). *Tnfa*, *Il1b*, and *Irf8* mRNA expression levels were upregulated in the DH after 3 consecutive days of TREM2 antibody administration compared with the sham-operated group (*Tnfa*: $p = 9.0 \times 10^{-6}$; *Il1b*: $p = 7.2 \times 10^{-13}$; *Irf8*: $p = 7.1 \times 10^{-13}$, one-way ANOVA with *post hoc* Turkey's test). However, these expression changes were not observed in the *Dap12* $^{-/-}$ mice (Fig. 8*A*). Additionally, PWTs in the TREM2 administration group were significantly less than in the control groups and *Dap12* $^{-/-}$ mice, indicating that the TREM2 agonistic antibody induced pain behavior without nerve injury (right paw: $p = 7.9 \times 10^{-7}$; left paw: $p = 1.5 \times 10^{-9}$; vs WT sham-operated group, one-way ANOVA with *post hoc* Turkey's test), and that this type of pain induction was DAP12-dependent (Fig. 8*B*).

Discussion

Results from the present study revealed a critical role for DAP12 and its associated receptor TREM2 in neuropathic pain. Both DAP12 and TREM2 were expressed by microglia (Figs. 1, 7), and DAP12 were phosphorylated in activated microglia in response to nerve injury (Fig. 2). These results suggested that DAP12-mediated signaling is induced in microglia after nerve injury. Consistent with these results, *Dap12* deficiency also suppressed nerve injury-induced mechanical allodynia and TREM2 activation induced allodynia (Figs. 3, 8*B*), suggesting that microglial TREM2/DAP12-mediated signaling contributes to the development and persistence of neuropathic pain.

DAP12 signaling in microglia

In the CNS, DAP12 is predominantly expressed by microglia (Fig. 1). Results from the present study showed that *Dap12* mRNA levels (Fig. 1*A–C*) and microglial numbers simultaneously increase in response to sensory nerve injury. Because the fold changes of the mRNA expression in the ipsilateral DH after injury (Fig. 1*A*) were equivalent to those of microglial numbers (Fig. 5*J–L*), the increase in *Dap12* mRNA in the ipsilateral DH may be due to increased number of microglia rather than upregulated expression. The phosphorylation of tyrosine residues within the ITAM motifs of DAP12 was significantly induced in microglia in response to sensory nerve injury (Fig. 2), suggesting that DAP12 activation elicits further intracellular signals. Several signaling pathways, such as mitogen-activated protein kinases, phospholipase Cγ, PKC, phosphoinositide 3 kinase, and nuclear factor-κB, are associated with proinflammatory responses, phagocytosis, and microglial migration and proliferation (Xing et al., 2015). In particular, induction of proinflammatory cytokines and their associated molecules in microglia could result in a phenotypic shift to a toxic phenotype, resulting in neuronal damage (Maeda et al., 2010; Kobayashi et al., 2015). We clearly demonstrated that nerve injury increases mRNA expression of proinflammatory cytokines, such as *Tnfa*, *Il1b*, and *Il6*, as well as other factors associated with neuropathic pain, such as *Irf5*, *Irf8*, and *Ctss* in the ipsilateral DH; however, expression of these genes was suppressed in *Dap12*-deficient mice (Fig. 6). The decreases in the expression of these molecules were greater than the decreases in microglial num-

bers at the expression peak (Fig. 5*J*), suggesting that expression of those proinflammatory molecules might be suppressed in activated microglia in *Dap12*-deficient mice. These results are in line with our previous study demonstrating downregulation of proinflammatory molecules in LPS-activated cultured microglia of *Dap12*-deficient mice (Kobayashi et al., 2015). *Dap12* deficiency likely suppresses excessive activation of microglia and reduced allodynia (Fig. 3).

Recently, Guan et al. (2016) demonstrated through the use of BrdU labeling that *Dap12* (*Tyrobp*) deletion has no effect on nerve injury-induced microglial proliferation. However, our findings showed decreased microglial cell numbers and the area of Iba1 expression in the DH of *Dap12*-deficient mice (Fig. 5). Our findings suggest that the increased microglial cell number and occupying area are due to microglial migration from surrounding areas or changes in the rate of microglial survival. DAP12 has been proposed to be involved in cytoskeletal remodeling and migration in other cells (Turnbull and Colonna, 2007), which supports the theory that suppression of microglial migration in DAP12-deficient mice is likely. Additionally, *Dap12*-deficient mice had fewer microglia in the spinal cord and exhibited microglial degeneration and apoptotic cell death features at 10 months of age, suggesting that microglial survival is diminished in *Dap12*-deficient mice (Otero et al., 2009). Furthermore, Wang et al. (2015) demonstrated a role for TREM2 in maintaining microglial survival during reactive microgliosis using *Trem2*-deficient Alzheimer's disease model mice. Nevertheless, further studies are needed to determine the mechanisms involved in the decreased microglial numbers observed in this study.

The DAP12 counterpart receptor

DAP12 consists of a short extracellular domain, a single transmembrane domain, and a cytoplasmic ITAM, and has been identified as the signaling chain for some ligand-binding receptors. More than 30 receptor chains have been shown to be associated with DAP12 (Lanier, 2009). Among the associated receptors examined, the mRNA expression profile for *Trem2* and *Csf1r* was most similar to *Dap12* mRNA levels in our allodynia model (Figs. 1*A*, 7*A*,*B*). While preparing this article, a report was published showing that DAP12 is responsible for microglial CSF1R-induced neuropathic pain (Guan et al., 2016). In terms of pain sensation, they demonstrated that DAP12 is located downstream of CSF1R, whereas we showed that TREM2 is a potent counterpart receptor of DAP12. Although no direct interaction between CSF1R and DAP12 has been reported (Hamerman et al., 2009), CSF1R is considered an activator of Src tyrosine kinase, which phosphorylates the ITAM motif of DAP12 (Zou et al., 2008; McVicar and Trinchieri, 2009). Therefore, DAP12 could be located at a position of signal convergence between CSF1R-mediated and TREM2-mediated signals. Their findings primarily support our data showing that the microglial DAP12 pathway plays a critical role in neuropathic pain development. It is likely that multiple signals associated with pain sensation converge at DAP12 because CSF1R and TREM2 are abundantly expressed by microglia after nerve injury (Fig. 7), and *Dap12* deficiency suppressed the receptor-mediated pain behavior of these two molecules (Fig. 3) (Guan et al., 2016). Although the significance of DAP12 in pain sensation is supported by both studies, Guan et al. (2016) demonstrated almost total suppression of pain behavior in *Dap12* knock-out mice, whereas our *Dap12* knock-out mice exhibited partial, but significant, suppression of pain behavior (Fig. 3). These differences could be due to differences between animal models used.

TREM2 function

As with *Dap12* mRNA, the rate of increase in *Trem2* mRNA in the injured ipsilateral DH (Fig. 7*B*) was nearly equal to those of microglial numbers (Fig. 5*J–L*) at the expression peak, suggesting that TREM2 may be expressed by and function in microglia in a similar manner between steady and an activation states. *Trem2* was recently reported to be a causative gene of Alzheimer's disease (Guerreiro et al., 2013; Jonsson et al., 2013), Parkinson's disease (Rayaprolu et al., 2013), and amyotrophic lateral sclerosis (Cady et al., 2014). Because TREM2 is expressed by microglia, these neurodegenerative diseases are assumed to be partly associated with microglial disorders. TREM2 has been identified as a phagocytic receptor in an experimental autoimmune encephalomyelitis model (Takahashi et al., 2005) and ischemia model (Kawabori et al., 2015), and TREM2 signals sustain microglial responses by promoting microglia survival in an Alzheimer's disease model (Wang et al., 2015). TREM2 is assumed to be an essential molecule that determines microglial phenotype and activation status. Therefore, microglial alteration by TREM2 signaling could lead to neuropathic pain. In the present study, intrathecal administration of agonistic TREM2 antibody elicited pain behavior in mice without nerve injury, and this pain behavior was totally abolished in *Dap12*-deficient mice (Fig. 8*B*), suggesting that TREM2 signaling is predominantly transferred to DAP12. Concomitantly induced expression of proinflammatory cytokine genes have been observed in the DH after nerve injury, although these inductions do not occur in *Dap12*-deficient mice (Fig. 8*A*). Together, DAP12-mediated signals could be one of key pathways in pain signaling.

Although the ligand that binds to and activates TREM2 remains elusive, a broad array of lipids produced directly or indirectly as a result of damage to primary sensory neurons has been proposed as potential TREM2 ligands (Wang et al., 2015). TREM2 could serve as a microglial sensor for lipid mediators that are released from damaged sensory nerve terminals or other cells in the DH. Recently, apolipoprotein E (ApoE) was reported to be a potent ligand for TREM2, and an Alzheimer's disease-associated R47H mutation and other artificial mutations introduced at the same location significantly reduce the affinity of TREM2 for ApoE (Atagi et al., 2015; Bailey et al., 2015). Additionally, ApoE protein was shown to be upregulated in injured DRG neurons in a spinal nerve ligation model (Melemedjian et al., 2013). These reports suggest that ApoE could be a potent activator of the TREM2/DAP12 system in the present pain model. Furthermore, recent evidence shows that exosomes play important roles in intercellular communication networks, enabling the exchange of information, as well as proteins and lipids, between producing cells and target cells (Turturici et al., 2014). Cortical neurons (Fauré et al., 2006), microglia (Potolicchio et al., 2005), and astrocytes (Frühbeis et al., 2013) release exosomes. The activation of transcription factor EB, a key transcription factor for lysosomal exocytosis (Medina et al., 2011), together with lysosome transportation into central branches of DRG neurons, has been observed in nerve-injured DRG neurons (Jung et al., 2016). Those findings suggest that exosomes and lysosomes released from primary sensory nerve terminals after nerve injury are additional source of lipids for TREM2 activation, although other ligands, such as nucleic acids, may exist for microglial TREM2 activation (Kawabori et al., 2015).

In conclusion, this study provides evidence that DAP12 could be a key microglial membrane molecule that modulates microglial function and elicits abnormal pain after sensory nerve injury. Our results demonstrated that TREM2 is a partner receptor

for microglial activation, although additional receptor-mediated pathways, such as CSF1R, may also be involved in DAP12-mediated microglial activation. DAP12 and TREM2 could serve as potential therapeutic targets for neuropathic pain, although further studies are needed to determine the mechanisms of TREM2/DAP12 intracellular signaling.

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