



Granulocyte-colony-stimulating factor (G-CSF) signaling in spinal microglia drives visceral sensitization following colitis

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Pain is a main symptom of inflammatory diseases and often persists beyond clinical remission. Although we have a good understanding of the mechanisms of sensitization at the periphery during inflammation, little is known about the mediators that drive central sensitization. Recent reports have identified hematopoietic colony-stimulating factors as important regulators of tumor- and nerve injury-associated pain. Using a mouse model of colitis, we identify the proinflammatory cytokine granulocyte-colony-stimulating factor (G-CSF or Csf-3) as a key mediator of visceral sensitization. We report that G-CSF is specifically up-regulated in the thoracolumbar spinal cord of colitis-affected mice. Our results show that resident spinal microglia express the G-CSF receptor and that G-CSF signaling mediates microglial activation following colitis. Furthermore, healthy mice subjected to intrathecal injection of G-CSF exhibit pronounced visceral hypersensitivity, an effect that is abolished by microglial depletion. Mechanistically, we demonstrate that G-CSF injection increases Cathepsin S activity in spinal cord tissues. When cocultured with microglia BV-2 cells exposed to G-CSF, dorsal root ganglion (DRG) nociceptors become hyperexcitable. Blocking CX3CR1 or nitric oxide production during G-CSF treatment reduces excitability and G-CSF-induced visceral pain in vivo. Finally, administration of G-CSF-neutralizing antibody can prevent the establishment of persistent visceral pain postcolitis. Overall, our work uncovers a DRG neuron-microglia interaction that responds to G-CSF by engaging Cathepsin S-CX3CR1-inducible NOS signaling. This interaction represents a central step in visceral sensitization following colonic inflammation, thereby identifying spinal G-CSF as a target for treating chronic abdominal pain.

visceral pain | microglia | colitis | G-CSF | nociceptors

Long-lasting changes in nociceptive circuits precipitate the transition from acute to persistent pain in chronic inflammatory diseases. While significant improvement has been made to reduce acute inflammation, peripheral and central sensitization often leads to debilitating persistent pain even after disease remission, thus suggesting a high level of plasticity in nociceptive pathways during inflammation or through active processes of resolution (1–6). We and others have shown that a single bout of colonic inflammation can cause subsequent visceral sensitization that persists long after the inflammation has resolved (7). This persistent postinflammatory sensitization mirrors what is seen in patients with inflammatory diseases (4, 7–10). While a number of mechanisms of peripheral sensitization have been characterized (5, 11, 12), there is a growing appreciation that, as observed at the periphery, neuro-immune interactions occurring in the spinal cord regulate pain sensitivity caused by tissue damage (8, 12–16). Microglia, the tissue-resident macrophages of the central nervous system, have been directly implicated in the initiation of mechanical hypersensitivity following peripheral nerve injury (17–19). In this pathological condition, activated microglia in the spinal cord release proinflammatory cytokines such as IL-1 β and TNF- α , which enhance pain sensation by increasing the

excitability of dorsal root ganglion (DRG) nociceptors to facilitate synaptic transmission in the spinal dorsal horn (20, 21). With regard to visceral sensitivity, microglia have recently been found to mediate central sensitization in a rat model of narcotic bowel-like syndrome (22). However, the importance of microglial activation and the spinal neuro-immune mechanisms eliciting inflammation-induced visceral hypersensitivity remain elusive. Using the dextran sulfate sodium (DSS)-induced colitis model, we have identified granulocyte-colony-stimulating factor (G-CSF or Csf-3), a blood-brain barrier-permeant cytokine, as a regulator of central sensitization. We found that G-CSF is increased in the spinal cord during colonic inflammation, which coincides with microglial activation. Our data describe the mechanism whereby G-CSF signaling, through its G-CSF Receptor (G-CSFR), also known as CD114, promotes visceral hypersensitivity. Overall, our work shows that central neuro-immune interactions involving G-CSF can precipitate the establishment of persistent pain following peripheral inflammation. Thus, G-CSF signaling may represent a therapeutic target for the treatment of pain associated with chronic inflammatory diseases.

Results

G-CSF and Its Receptor Are Up-Regulated in Spinal Cord Tissue During DSS-Induced Colitis. To examine inflammatory markers that could modulate central sensitization during colitis, we measured inflammatory cytokines in spinal cord tissues from mice treated

Significance

Visceral pain is a debilitating type of pain that afflicts adults and children. Major challenges exist in developing analgesics due to the limited understanding of the mechanisms that mediate visceral nociception. Using a model of colitis, we have identified the granulocyte-colony-stimulating factor (G-CSF) as an essential mediator of central sensitization that leads to visceral hypersensitivity, even after the resolution of inflammation. We demonstrate that G-CSF acting on spinal microglia activates a signaling platform that causes hyperexcitability of sensory neurons. We found that ablating microglia or blocking the G-CSF receptor prevents visceral sensitization. Our work establishes a microglial signaling mechanism in the transition to chronic visceral pain and likely other forms of persistent pain associated with chronic inflammatory diseases.

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with DSS for 7 d. As a surrogate marker of inflammation, both G-CSF and IL6 levels were high in the serum of colitis mice (Fig. S1A). Strikingly, we also found elevated G-CSF in gut-projecting thoracolumbar (T12–L1) spinal cord from DSS mice (Fig. 1A), whereas 29 other cytokines, including IL1 β , IL6, and TNF α , remained unchanged (Fig. S1B and Table S1). Using flow cytometry, we isolated mononuclear cells from the spinal cord and found that microglia (CD45^{low} CD11b⁺ cells) produced G-CSF (Fig. S1C) and expressed the G-CSFR (Fig. 1B). Accordingly, we observed an increase in Iba-1 immunopositive microglia in spinal cord sections of DSS mice (Fig. 1C), associated with an up-regulation in spinal G-CSFR at both mRNA (Fig. S1D) and protein levels (Fig. 1D).

G-CSF Signaling in Spinal Microglia Promotes Visceral Hypersensitivity.

To investigate the effect of G-CSF on visceral sensitization, we assessed visceromotor responses (VMR) to colorectal distension 12 h after intrathecal administration of G-CSF (Fig. 2A). Compared with sham (PBS)-treated littermates, G-CSF-treated mice showed visceral hypersensitivity at mild and noxious distension pressures (Fig. 2B). This effect was mediated by the G-CSF receptor, as simultaneous administration of G-CSF with a G-CSF receptor-neutralizing antibody (G-CSF-Rab) completely prevented the development of visceral sensitivity. To determine the role of microglia in G-CSF-induced visceral hypersensitivity, we used chow containing PLX 5622, a CSF-1 receptor inhibitor known to deplete microglia *in vivo* without altering visceral sensitivity in basal conditions (Fig. S2) (23). Ablation of microglia with PLX prevented G-CSF-induced visceral hypersensitivity (Fig. 2C), compared with the twofold increase observed in mice

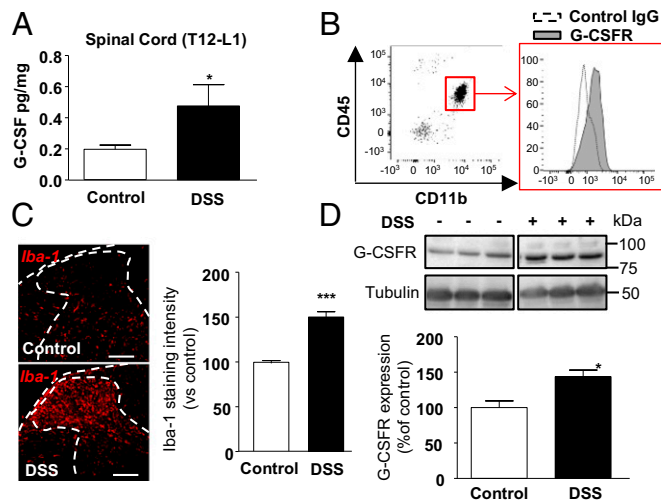


Fig. 1. G-CSF and its receptor, expressed on microglia, are increased in the spinal cord during acute DSS-induced colitis. Acute colitis was induced in mice with 2.5% DSS for 7 d. After blood removal, G-CSF levels were determined in the thoracolumbar (T12–L1) spinal cord by luminex technology in control (white bar, $n = 10$) or colitis (black bar, $n = 7$) mice (A). Mononuclear cells of the spinal cord were isolated, and CD45^{low} CD11b⁺ cells (microglia) were analyzed using flow cytometry for their expression of G-CSFR represented by increased binding of specific G-CSFR antibody (gray histogram, representative of three independent experiments) compared with control IgG (dotted histogram) (B). (C, Left) Iba-1 expression was quantified by immunostaining in spinal cord sections of control and DSS colitis mice. (C, Right) Intensity of the immunostaining was measured using Image J on a total of 35 sections from three independent experiments. (D) G-CSFR expression level was determined by Western blot in spinal cord of control (white bar, $n = 6$) or colitis (black bar, $n = 6$) mice. Statistical analyses were performed using Mann–Whitney U test; * $P < 0.05$; *** $P < 0.001$. (Scale bar: 100 μ m.)

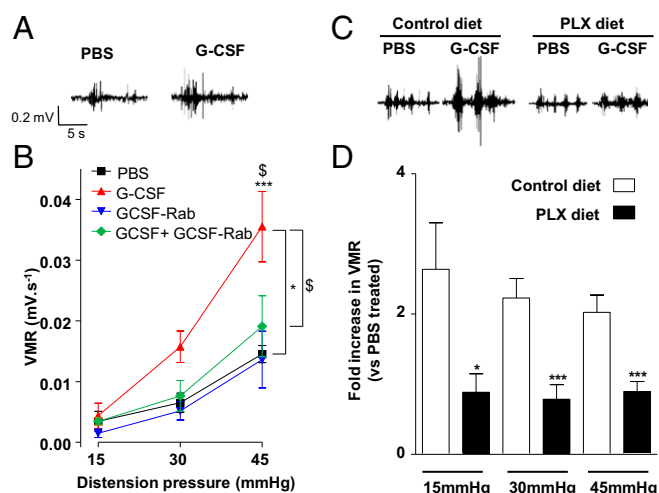


Fig. 2. G-CSF signaling in microglia induces visceral hypersensitivity. (A) Representative electromyogram recording elicited by 45 mmHg pressure at 12 h of intrathecal PBS or G-CSF (20 ng) injection. (B) VMR to colorectal distension after treatment with PBS ($n = 4$), G-CSF (20 ng; $n = 8$), anti-G-CSFR antibody (G-CSF-Rab 1 μ g; $n = 5$; *** $P < 0.001$), or a combination of both ($n = 5$). (C) Representative electromyogram recording elicited by 45 mmHg pressure at 12 h of intrathecal PBS or G-CSF (20 ng) injection in mice that received control or the PLX 5622 diet for 2 wk. (D) VMR to colorectal distension after treatment with PBS (control diet: $n = 15$, PLX diet: $n = 10$) or G-CSF (control diet: $n = 16$, PLX diet: $n = 11$). Results are expressed as fold increase in VMR (\pm SEM) for control diet (white bar) or PLX diet (black bar) animals. Statistical analysis was performed using either repeated-measures two-way ANOVA and Bonferroni post-hoc test (B; * $P < 0.05$ G-CSF; *** $P < 0.001$ vs. PBS; \$ $P < 0.05$ G-CSF vs. G-CSF+G-CSF Receptor antibody) or the Mann–Whitney U test (D; * $P < 0.05$; *** $P < 0.001$).

receiving normal chow (Fig. 2D and Fig. S2C). Our data thus indicate that activation of the G-CSF/G-CSFR–signaling axis in the spinal cord triggers microglia-dependent visceral hypersensitivity.

G-CSF Conditions Microglia to Sensitize DRG Neurons Through

Cathepsin S-CX3CR1-Inducible NOS Signaling. Intrathecal injection of colony-stimulating factor (Csf-1 also known as M-CSF) was recently reported to increase the expression of the cysteine proteinase Cathepsin S (Cat S) and CX3CR1 in the spinal cord (24). Based on the role of the Cat S/CX3CL1/CX3CR1–signaling axis in neuropathic pain (25), we examined the contribution of this pathway in driving G-CSF-induced visceral hypersensitivity. As shown in Fig. 3A, intrathecal injection of G-CSF increases Cat S activity in the spinal cord. To determine how G-CSF could engage microglia–DRG neuron interaction through Cat S activity, we developed a modified transwell coculture protocol (Materials and Methods). In this assay, BV-2 microglia cell monolayers seeded in the upper chamber were stimulated with G-CSF for 16 h (Micro^{G-CSF}), washed, and then placed above acutely dissociated DRG neurons seeded into the lower chamber (Fig. 3B). DRG neurons were exposed to factors secreted from G-CSF-primed microglia cells for a further 16 h, after which both spontaneous and evoked neuronal activity was recorded by patch clamp electrophysiology (26, 27). As shown in Fig. 3, the percentage of capsaicin-responsive (TRPV1⁺) DRG neurons exhibiting spontaneous activity increased when cultured with Micro^{G-CSF} compared with those cultured with “naïve” microglia. Neurons exposed to Micro^{G-CSF} displayed a more depolarized resting membrane potential and a lower action potential threshold, indicating overall that Micro^{G-CSF}-secreted factor(s) modulate neuronal excitability in our coculture system (Fig. 3C). Moreover, the frequency of action potential (AP) evoked by an ascending ramp of injected current was greater in Micro^{G-CSF}-cocultured

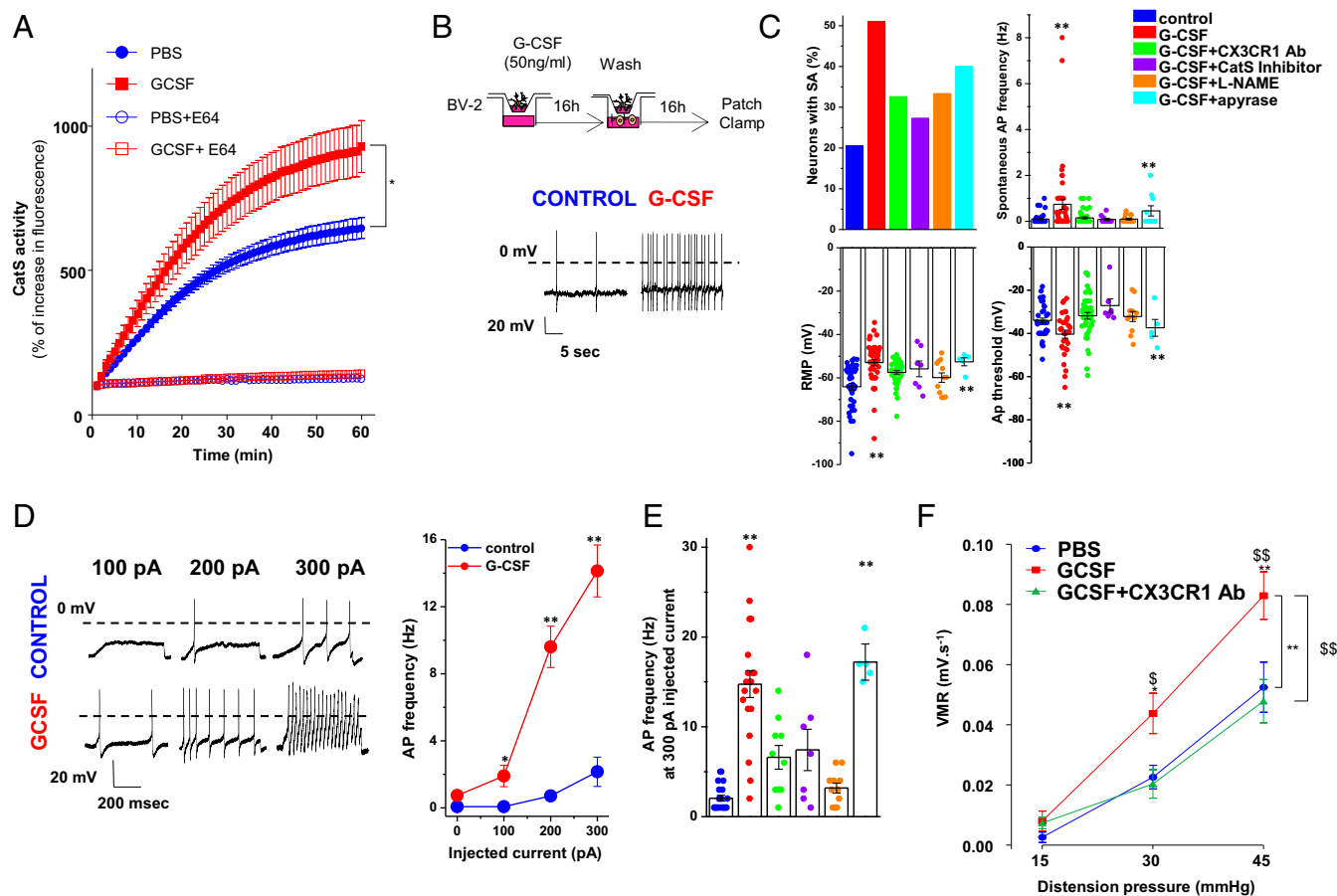


Fig. 3. G-CSF-treated microglia increases excitability of DRG neurons through Cathepsin S-CX3CR1-iNOS signaling. (A) Mice were treated intrathecally with either PBS or G-CSF (20 ng) 12 h before evaluating Cathepsin S activity in thoracolumbar spinal cord lysate ($n = 3$ experiments). (B) Cartoon illustrating the coculture system experiment. BV2 microglia cells were plated into the upper chamber of a transwell for 16 h. G-CSF was removed, and DRG neurons were plated in the lower chamber of the transwell for 16 h of coculture and then used for electrophysiological recordings. (Lower) A representative trace of spontaneous activity (SA) in a small-size TRPV1-sensitive DRG neuron cocultured with vehicle- or G-CSF-primed BV2 cells. (C) Exposure of BV2 microglia to G-CSF depolarizes the resting membrane potential (RMP) of DRG neurons and increases the percentage of neurons with SA (5/28 in control, 17/30 for G-CSF treated, 10/31 for G-CSF combined with CX3CR1 Ab-treated, 3/11 for G-CSF combined with Cat S inhibitor-treated, 5/15 for G-CSF combined with L-NAME, and 4/10 for G-CSF combined with Apyrase). (Right) G-CSF induces a decrease in the AP threshold and an increase in spontaneous AP discharge. These parameters are reversed by cotreatment with CX3CR1 Ab, Cathepsin S inhibitor, or L-NAME, but not apyrase. (D) Representative AP discharge evoked by 100-, 200-, and 300-pA current injections (1 s) in control (blue circle) and G-CSF (red circle) and for different conditions of treatment (E). (F) Measure of VMR to colorectal distension in mice injected with PBS ($n = 9$), G-CSF (20 ng; $n = 12$), or G-CSF + CX3CR1 Ab (5 μ g; $n = 9$) 12 h before recording. Results indicate mean \pm SEM (* $P < 0.05$; ** $P < 0.01$). Statistical analysis was performed using one-way (C and E) or two-way ANOVA (A, D, and F) followed by Bonferroni post hoc test (* $P < 0.05$; ** $P < 0.01$; \$ $P < 0.05$; \$\$ $P < 0.01$).

neurons (Fig. 3 D and E). Both spontaneous and evoked electrical activities were partially blocked by treatment of cocultures with an anti-CX3CR1 antibody or a Cathepsin S inhibitor. To identify what factor(s) downstream of the microglial G-CSF receptor promote neuronal excitability, we examined the role of ATP and nitric oxide (NO), two known regulators of microglial activation and migration. While depleting microglial ATP with apyrase did not alter G-CSF-mediated hyperexcitability, blocking the NO pathway with the broad spectrum NOS inhibitor N(ω)-nitro-L arginine methyl ester (L-NAME) prevented it (Fig. 3 C and D). Of note, L-NAME alone had no effect on DRG neuron excitability (Fig. S3 A–D). These results correlated directly with an elevation of inducible NOS (iNOS) level in the spinal cord following G-CSF treatment in vivo (Fig. S3E) and therefore point to an excitatory effect of NO in response to the microglial CX3CR1-Cat S pathway. The anti-CX3CR1 antibody also blocked the ability of G-CSF to induce visceral hypersensitivity in vivo wherein the G-CSF-induced visceral hypersensitivity returned to basal levels upon G-CSF+CX3CR1 antibody administration (Fig. 3E). Our data indicate that G-CSF sensitizes DRG neurons

through microglial stimulation and activation of Cat S-CX3CR1 signaling. This activation leads to an NO-driven neuronal hyperexcitability that underlies visceral hypersensitivity.

G-CSF Receptor Blockade in the Spinal Cord Prevents Persistent Visceral Hypersensitivity Postcolitis. To test whether G-CSF could participate in the establishment of inflammation-induced chronic visceral pain, we blocked G-CSFR signaling in the spinal cord of DSS-treated animals that develop postcolitis pain (7). Briefly, mice were given 2.5% DSS for 5 d, after which they received control IgG or a G-CSFR-blocking antibody (G-CSF-RAB) twice a week for a 5-wk recovery period (Fig. 4A). We previously reported that visceral hypersensitivity persists for at least 5 wk following acute colitis, despite the complete resolution of inflammation (7). Injection of G-CSF-RAB intrathecally did not alter the acute inflammatory response measured by macroscopic scoring and myeloperoxidase (MPO) activity (Fig. S4 A and B) nor the resolution of inflammation following DSS discontinuation (Fig. S5). When we assessed VMR, postcolitis mice chronically treated with control IgG exhibited increased VMR to

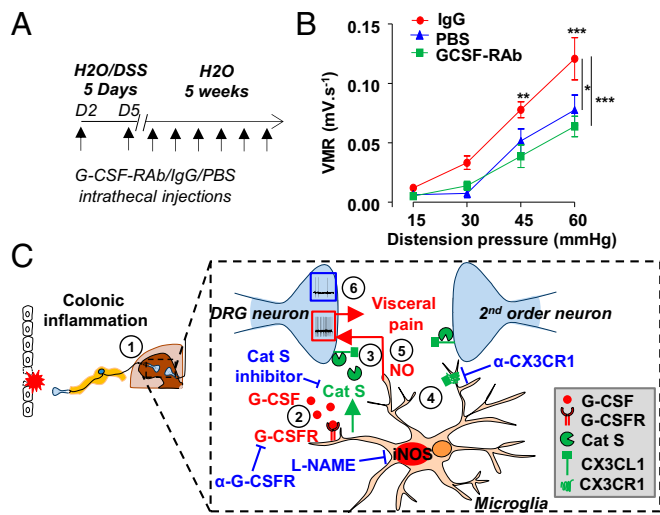


Fig. 4. Blocking G-CSF receptor signaling in the spinal cord alleviates DSS-induced postinflammatory visceral hypersensitivity. (A) Colitis was induced with 2.5% DSS for 5 d, after which mice received only water for the next 5 wk. During the recovery period, mice were treated twice a week with either G-CSFR-blocking antibodies or control IgG. A healthy control group that did not receive DSS was intrathecally injected with PBS. (B) Visceromotor responses to colorectal distension post-DSS-induced colitis in G-CSF-RAb (green squares; $n = 11$) and control IgG (red circles; $n = 6$) treated mice compared with healthy mice injected with PBS (blue triangle; $n = 4$). Data are expressed as mean of VMR \pm SEM. Statistical analysis was performed using repeated-measures two-way ANOVA and the Bonferroni post hoc test ($*P < 0.05$; $**P < 0.01$; $***P < 0.001$). (C) Schematic representation of G-CSF-mediated visceral sensitization in the spinal cord. (1) Acute DSS colitis increases G-CSF in the spinal cord. (2) G-CSF binds to its receptor on microglia and (3) induces the secretion of Cathepsin S. (4) Cathepsin S triggers the release of soluble fractalkine that activates its receptor CX3CR1 at the microglial cell surface. (5) This drives a hyperexcitability of DRG neurons through a NO-dependent process and the establishment of visceral sensitization. (6) Inhibition of either G-CSFR or CX3CR1 prevents both DRG hyperexcitability and visceral hypersensitivity.

colorectal distension compared with healthy mice treated with saline. Strikingly, blockade of G-CSFR signaling completely abrogated visceral hypersensitivity in the postcolitis mice (Fig. 4B). In contrast, G-CSF-RAb did not have antinociceptive effects in the acute phase of colitis (Fig. S4C), thus indicating that G-CSF signaling in the spinal cord is responsible for the establishment of persistent visceral hypersensitivity post resolution but not during acute inflammation.

Discussion

Here, we report that G-CSF signaling drives persistent pain that lasts post resolution of inflammation via activating a Cathepsin S-CX3CR1-NO-signaling pathway between spinal microglia and DRG neurons (Fig. 4C). We demonstrate that (i) G-CSF is increased in the spinal cord of colitis mice and that intrathecal injection of G-CSF triggers visceral hypersensitivity through activation of the G-CSFR in microglia; (ii) G-CSF administration increases Cathepsin-S activity in the spinal cord; (iii) supernatants from G-CSF-treated microglia promote increased excitability of cocultured sensory neurons; and (iv) G-CSF-mediated hyperexcitability of DRG neurons involves the Cathepsin S-CX3CR1-NO pathway from microglia, which plays a major modulatory role in pain sensitization (28). Accordingly, injection of mice with a CX3CR1 antibody suppressed G-CSF-induced visceral hypersensitivity in vivo. Finally, we show that chronic treatment with a G-CSF-RAb alleviates postinflammatory visceral pain. Our data thus single out a role for G-CSF and its receptor in the establishment of postinflammatory pain that persists after tissue healing.

Our work supports data obtained over the past few years demonstrating a key role for the CNS in regulating pain sensitization and altered behavior following peripheral inflammation (29, 30). In the gastrointestinal tract, inflammation induced by either 2,4,6-trinitrobenzene sulfonic acid (TNBS) (31) or DSS (32), correlates with microglial activation in the hippocampus, which leads to increased susceptibility to seizures in mice (31). In addition, neonatal colon irritation is known to mediate microglial activation in the hippocampus and hence precipitate visceral hypersensitivity (33). To date, activation of microglia in the brain is linked to increases in hippocampal TNF α and IL1 β , two cytokines thought to be responsible for behavioral changes (31, 33). In our new work, we did not observe any increase of IL1 β in the spinal cord of acute DSS-treated mice, and no TNF α could be detected in either group, suggesting a different pattern of cytokines at play in the spinal cord compared with the ones identified in the hippocampus after peripheral inflammation. From a panel of 30 cytokines analyzed (Table S1), we found that only G-CSF, a hematopoietic stem cell factor that has been known to drive the differentiation of neutrophils from myeloid progenitors and trigger their release from the bone marrow (34), was significantly up-regulated. Although G-CSF is primarily known to be produced by monocytes/macrophages, fibroblasts, and endothelial and mesothelial cells upon stimulation, several studies have reported that hippocampal neurons (35) and astrocytes (36, 37), as well as microglia (38–40), can synthesize G-CSF in the central nervous system. Our data also show that peripheral inflammation can induce the production of this factor centrally in the spinal cord. Given that we observed activation of the microglia in the spinal cord during colitis, as attested to by increased Iba-1 expression, and based on our findings that microglia can produce G-CSF, we propose that microglia represent the source of G-CSF during DSS-induced colitis. Nevertheless, further studies using *in situ* hybridization, for example, would delineate whether other cells in the spinal cord are enabled to produce the cytokine. G-CSF, acting via its G-CSFR, stimulates several classes of cells in the nervous system such as neurons (35, 41), astrocytes, or microglia (42). G-CSFR-mediated signaling can also drive microglial proliferation (43) and activation in a model of amyotrophic lateral sclerosis (44). Accordingly, not only did we find that spinal cord microglia express G-CSFR, but its expression was increased in the spinal cord during colitis.

It has been shown that paw injection of hematopoietic growth factors such as G-CSF or granulocyte macrophage-CSF (GM-CSF) induces both thermal and mechanical hyperalgesia in mice and that locally secreted G-CSF is responsible for bone cancer-associated pain in mice (41). Also, recent work has reported that neutralizing G-CSFR with a monoclonal antibody could prevent and reverse arthritic pain in both adaptive and innate immune arthritis models, thus bringing forward the concept that G-CSF could be a key player in generating chronic pain associated with various inflammatory conditions (45). We and others have previously shown that, in a mouse model of DSS colitis, animals exhibit substantial visceral discomfort and pain both in the acute and the postresolution phase of the inflammation (7, 46, 47). Our data indicate that G-CSF could mediate this effect. Although we acknowledge that G-CSF may modulate pain sensation locally at the site of inflammation, G-CSF acting on microglia likely regulates the plasticity of spinal nociceptive circuits and therefore drives persistent visceral pain following colonic inflammation.

G-CSF was reported to activate nociceptors directly (41), inducing transcriptional changes concordant with an increase in nerve activity (48). Other researchers have proposed an indirect effect of G-CSF through prostanoid production (49) and were unable to detect G-CSFR expression in DRGs (45). Our model provides three major findings to suggest that G-CSF-promoted

visceral hypersensitivity results from a direct action of G-CSF on microglia. We show that (i) G-CSFR is expressed by spinal cord microglia; (ii) G-CSF-induced visceral pain is abolished in mice depleted of microglia; and (iii) G-CSF-stimulated microglia can sensitize naive DRG neurons. Our results thus uncover a mechanism of G-CSF-induced visceral pain involving microglia. Along these lines, two recent papers concomitantly show that another hematopoietic growth factor, macrophage-CSF (M-CSF), secreted by injured nociceptors, leads to chronic pain caused by nerve injury. This effect of M-CSF, in keeping with our findings, is also mediated by activation of spinal cord microglial M-CSF receptors. Strikingly, spinal injection of M-CSF alone can induce mechanical allodynia, indicating that activation of microglia by the hematopoietic growth factor is sufficient to drive pain phenotypes (24, 50).

Although activation of microglia and their role in chronic hyperalgesic pain states is now well established in the settings of nerve injury (25), chronic pancreatitis (51), chronic stress (52, 53), or colonic inflammation (54), the mechanisms that underlie microglia-dependent pain sensitization are still unclear. While Cathepsin S was recently identified as a biased agonist of proteinase-activated receptor-2 (PAR2) (55), our results obtained using the anti-CX3CR1 antibody point to the contribution of the CX3CR1 agonist ligand, fractalkine/CX3CL1, known to promote neuropathic pain (56) or chronic pain associated with arthritis (28, 57). This pathway involves the microglial release of Cathepsin S, which then cleaves membrane-bound fractalkine on sensory neurons (58). Cleaved fractalkine in turn activates the CX3CR1 receptor on microglia, thus leading to the release of proinflammatory mediators, including NO, that strengthen synaptic activity in the spinal dorsal horn (25). Indeed, NO is a central factor of sensitization in the spinal dorsal horn, and targeting iNOS can attenuate inflammatory pain hypersensitivity (59–62). During colitis, this pathway is likely stimulated by spinal G-CSF. Collectively, our results agree with previous studies reporting that fractalkine can induce visceral hypersensitivity when injected intrathecally (53), that Cathepsin S activity is increased in the spinal cord during acute colitis (47), and that hematopoietic growth factor can induce Cathepsin S mRNA expression when injected in the spinal cord (24). Altogether, our findings demonstrate the importance of the proteinase-regulated pathway in our newly described G-CSF-mediated effect on visceral hypersensitivity in the context of colitis. Given that we observed only a partial inhibition of DRG neuron excitability using the CX3CR1 Ab in our in vitro setting, it is possible that other factors or mechanisms contribute to neuron sensitization. Indeed, G-CSF-treated BV.2 microglia also produce brain-derived neurotrophic factor (42), a factor broadly implicated in central sensitization processes (25). Nevertheless, the use of a CX3CR1 antibody was able to reverse hypersensitivity fully when administered intrathecally to mice, thus confirming that

the Cathepsin S-CX3CR1–signaling axis plays a pivotal role in G-CSF-mediated central sensitization.

Central sensitization is often associated with chronic widespread pain states in inflammatory diseases such as inflammatory bowel disease (IBD) or arthritis. While anti-TNF α therapy, steroids, or nonsteroidal anti-inflammatory drugs can be effective at reducing acute inflammation in these conditions, many patients experience persistent pain after disease remission. Our work suggests that, in IBD, G-CSF signaling in microglia may contribute to this central sensitization process. We found that chronic spinal inhibition of G-CSF signaling by the GCSF-RAB prevented the maintenance of postinflammatory visceral pain in colitis. Interestingly, inhibition of G-CSF signaling did not affect peripheral inflammation, most likely because the antibody cannot cross the blood–brain barrier to reach the periphery (63) (Figs. S4 and S5).

Pharmacological inhibition of microglial activation in other mouse models of gastro-intestinal inflammation (TNBS) has been shown to reduce both acute (54) and chronic pain (51), suggesting that additional microglial mechanisms may be implicated during acute inflammation, whereas G-CSF acting on microglia may be required for maintaining persistent pain postresolution.

Of relevance, post-IBD irritable bowel syndrome patients with chronic pain symptoms represent a substantial clinical challenge due to the absence of treatment options (64). Our work identifying G-CSF as a central regulator of the transition to post-inflammatory chronic visceral pain thus offers a potential therapeutic target to treat chronic abdominal pain in IBD and possibly in other pain conditions that persist after inflammatory disease remission.

Materials and Methods

Detailed methods, including reagents, mice strains, in vivo study design, induction of colitis, depletion of microglia with PLX diet, intrathecal injections and visceral pain assessment, colonic inflammation assessment, mRNA extraction, real-time PCR and Western blot detection of G-CSFR, immunocytochemical detection of Iba-1 expression in the mouse spinal cord, measurement of spinal cord Cathepsin S activity, measurements of spinal cord cytokine levels, coculture of BV-2 microglia and DRG neurons and in vitro study design, electrophysiological studies, and statistical analyses appear in *SI Materials and Methods*. All animal experiments were approved by the University of Calgary Animal Care Committee and were performed in accordance with the international guidelines for the ethical use of animals in research and guidelines of the Canadian Council on Animal Care.

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