

The role of CX3CR1 microglia in the pathogenesis of discogenic pain

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INTRODUCTION: Disc herniation, a common spinal issue, leads to severe back and radicular pain that greatly affects patients' life qualities. CX3CR1 is a chemokine receptor expressed on microglia that binds fractalkine (CX3CL1) and regulates microglial recruitment to sites of neuroinflammation. Within this niche, the interaction between microglia and neurons regulates microglial activity, aids in monitoring neuron health, influences synaptic pruning, and impacts neuroinflammation, making it a vital aspect of spinal cord function and response to injuries or diseases. Studies on mice with spinal cord injuries and strokes have shown that CX3CR1 depletion improved neural outcomes due to a reparative behavior in the CX3CR1-expressing microglia and macrophages. Although its potential contribution is conceivable, the precise role of CX3CR1 in disc herniation remains uncertain. In our current research, we aim to explore the function of CX3CR1 regulating communication between neurons and microglia in the spinal cord, within the context of lumbar intervertebral disc herniation.

METHODS: Approved by the Institutional Animal Care and Use Committee, the study employed CX3CR1 knockin (CX3CR1^{GFP/GFP} homozygotes) and CX3CR1 (CX3CR1^{GFP/+} heterozygous) mice acquired from Jackson Laboratory. To detect the spinal cord changes in response to disc herniation, these mice underwent L4/5 and L5/6 lateral disc puncture, enabling herniated nucleus towards the adjacent spinal nerves to simulate radiculopathy condition. Lumbar segmental spinal cords were harvested at week 1 and 2, then fixed and embedded in O.C.T solution. Using ImageJ, GFP-positive microglia were counted, microglial cell morphology, including process endpoints and lengths, was assessed. GFP positive cell morphology were examined in both ipsilateral and contralateral dorsal and ventral horn sections with 3 sections per animal and 3-6 animals per group. To assess microglial phagocytic activity, CD68 immunofluorescence staining was conducted on selected lumbar spinal cord samples. Data were presented as mean ± SEM and analyzed using one-way ANOVA or t-tests, with statistical significance set at $p < 0.05$.

RESULTS SECTION: In the present study, the impact of disc herniation-induced microglial responses in the lumbar spinal cord was investigated (Figure 1a). GFP positive microglia can be detected in the dorsal and ventral horns of spinal cord in CX3CR1^{GFP/+} mice on postoperative (POD) 1wk (Figure 1b), while alterations in microglial ramification indicates potential changes in their activation states (Figure 1c). As shown in Figure 2, on both POD 1 week and 2 weeks, microglial proliferation was observed on the ipsilateral side compared to the contralateral side, and quantitative analysis confirmed elevated numbers of CX3CR1 positive microglia in the ipsilateral dorsal horn for both CX3CR1^{GFP/+} and CX3CR1^{GFP/GFP} strains, along with a reduction in endpoints per cell and process length in CX3CR1^{GFP/+} mice at POD 1 week and 2 weeks. CX3CR1^{GFP/GFP} mice exhibited decreased endpoints per cell on POD 2 weeks in the ipsilateral dorsal horn, with no significant changes in process length. Immunofluorescence images displayed enhanced lysosomal CD68 signals within microglial soma and processes on POD 1 week, indicating an augmented phagocytic phenotype (Figure 3). Further analysis on microglia phenotypic change and communication with neurons are under investigation.

DISCUSSION: The study of CX3CR1 in the context of disc herniation and pain holds significance due to its potential to uncover novel insights into the underlying mechanisms of these conditions. Exploring its role in the context of disc herniation and pain could elucidate its involvement in the modulation of nerve sensitization and pain transmission within the spinal microenvironment. In the context of pain models, such as neuropathic pain, microglia activation and CD68 expression can play a role in both amplifying and modulating pain signals. The exact mechanisms are complex and not fully understood, but it's believed that activated microglia can release pro-inflammatory molecules and other signaling factors that sensitize pain-sensing neurons, leading to increased pain perception. Higher CD68⁺ microglia activity in the spinal cord generally signifies an increased immune response, particularly a phagocytic response, which can have implications for tissue repair and pain modulation.

SIGNIFICANCE/CLINICAL RELEVANCE: This research bears clinical importance as it has the potential to shed light on the mechanisms underlying neuroinflammation, nerve sensitivity, and pain signaling within the spinal microenvironment.

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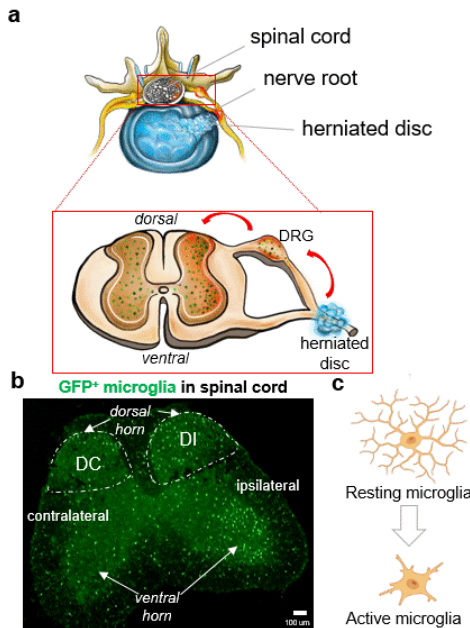


Figure 1. (a) Illustration of disc herniation induced signal transduction from "pinched" nerve root to spinal cord. (b) Fluorescence image of lumbar spinal cord in CX3CR1^{GFP/+} on POD 1wk shows GFP signals in dorsal and ventral horns. DC=dorsal contralateral; DI= dorsal ipsilateral. (c) Illustration on changes in microglial ramification indicate shifts in their activation states.

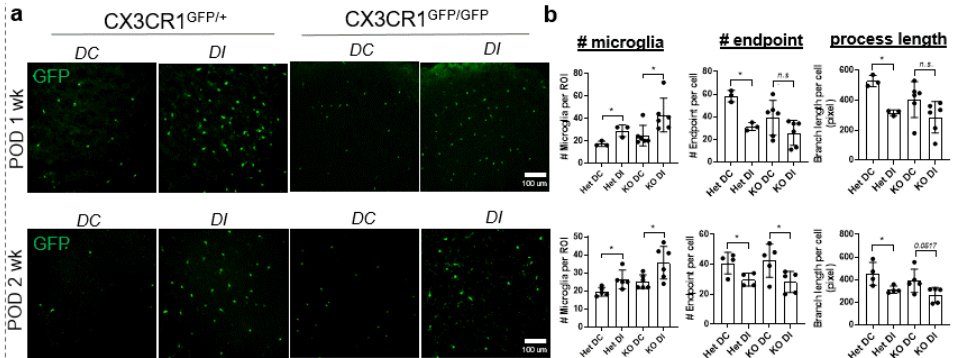


Figure 2. Proliferation and morphological change of microglia in dorsal horn of lumbar spinal cord following 1 week and 2 weeks after disc herniation. (a) Fluorescence images of dorsal horns in lumbar spinal cord showed microglia proliferation on ipsilateral side compared to contralateral side in both strains and on both POD 1wk and 2 wk. Scale bar= 100 μm (b) Quantitative assessment reveals elevated microglial counts in the ipsilateral dorsal horn (DI) in both strains at both 1 week and 2 weeks post disc herniation, compared to the contralateral side (DC). Morphological analysis indicates a noteworthy reduction in the number of endpoints per cell and process length in ipsilateral dorsal horn in CX3CR1^{GFP/+} at POD 1wk and 2wk. CX3CR1^{GFP/GFP} animals showed a decrease in endpoints per cell in ipsilateral dorsal horn on POD 2wk, and little change in process length. Het= CX3CR1^{GFP/+}; KO= CX3CR1^{GFP/GFP}

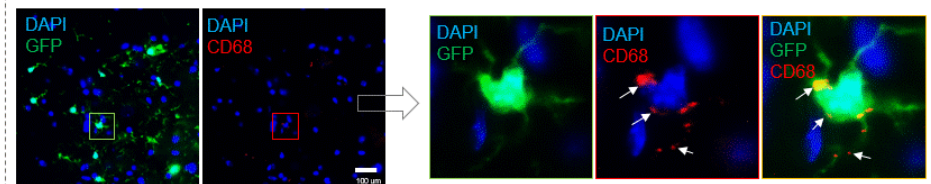


Figure 3. Immunofluorescence images in ipsilateral dorsal horn of CX3CR1^{GFP/+} on POD 1wk showed increased lysosomal CD68 (red) signals (arrows) in microglia soma and process (green), suggesting its phagocytic phenotype.