Review

# Role of CXCL10 in Spinal Cord Injury

Xinyu Qiao[1#], Wei Zhang[1,2#], Weijiang Zhao[1,3✉]

1. Wuxi School of Medicine, Jiangnan University, Wuxi 214122, Jiangsu, China
2. Department of Pathogen Biology, Guizhou Nursing Vocational College, Guiyang, China
3. Cell Biology Department, Wuxi School of Medicine, Jiangnan University, Wuxi 214122, Jiangsu, China

#These authors contributed equally to this article

✉ Corresponding author: **Weijiang Zhao**, Cell Biology Department, Wuxi School of Medicine, Jiangnan University, 1800 Lihu Road, Wuxi 214122, Jiangsu, China. E-mail: weijiangzhao@jiangnan.edu.cn. Tel.: + 86 13794125386.

## Abstract

Spinal cord injury (SCI) results in acute inflammatory responses and secondary damages, including neuronal and glial cell death, axonal damage and demyelination, and blood-brain barrier (BBB) damage, eventually leading to neuronal dysfunction and other complications. C-X-C motif Chemokine Ligand 10 (CXCL10) is expressed after the injury, playing multiple roles in the development and progression of SCI. Moreover, the CXCL10 antagonist can restrict inflammatory immune responses and promote neuronal regeneration and functional recovery. In this review, we summarize the structure and biological functions of CXCL10, and the roles of the CXCL10 / CXCR3 axis in acute inflammatory responses, secondary damages, and complications during SCI, thus providing a potential theoretical basis by highlighting CXCL10 as a new potential drug target for the treatment of SCI.

Key words: CXCL10, Spinal cord injury (SCI), CXCR3, Inflammatory, Secondary damage, Complications

## Introduction

Spinal cord injury (SCI) refers to an injury in the spinal cord, usually caused by external physical impact [1]. Traffic accidents and falls constitute the major causes of SCI [2, 3]. SCI causes a series of complex pathological changes. It directly causes neuronal damage, cell death, ischemic injury, and inflammation. Also, it leads to complex secondary damages, such as the formation of glial scar composed of reactive astrocytes at locations both proximal and distal to the injury site, and degenerative lesions in the tissue and structure of the spinal cord namely the formation of the cystic cavity [4-9]. Glial scars and cystic cavities cause poor regeneration of myelin and axons, finally leading to permanent neurological deficits characterized by long-term sequelae and complications, including loss of motor functions, vegetative nervous system dysfunctions, neuropathic pain syndromes, and even cognitive impairment [4, 10-12].

The effects of SCI on patients result in a loss of self-care and an increased risk of death [13]. The acute mortality rate during hospitalizations ranges from 4% to 17%. After leaving the hospital, the annual mortality rate remains high: 3.8% in the first year and 1.6% in the second year after the injury, and it remains at 1.2% thereafter [14]. Given the serious long-term effects of SCI, it is critical to develop effective treatments for those cases that cannot be prevented.

The secondary damage is defined as the pathological cascade caused by the primary injury, which has been the focus of research in the past several decades. Post-traumatic inflammation is considered to be an important influencing factor of secondary damage. Inflammatory factors and inflammatory factors-mediated inflammatory responses can significantly promote or inhibit the repair process of SCI during acute, subacute, and chronic phases [15-18]. The binding of chemokines to their receptors is an essential component of many secondary inflammatory mechanisms during these phases [19]. In particular, CXCL10, known as IP-10 (Interferon-gamma inducible Protein 10 kDa), belongs to the ELR⁻ CXC subfamily of chemokines. CXCL10 functions by binding to C-X-C chemokine receptor

type 3 (CXCR3), and CXCL10 / CXCR3 axis plays a pro-inflammatory role in immunoreaction [20, 21].

CXCL10 exerts a wide range of functions in several main pathophysiological changes in SCI, such as inflammatory cell recruitment, angiogenesis inhibition, apoptosis promotion, neuronal loss, axonal injury, neuropathic pain, and impaired motor function recovery [22-25]. We therefore speculate that targeting CXCL10 and its receptor is of great potential therapeutic value in the management of SCI.

We here briefly review the structure and biological functions of CXCL10 and elucidate the critical role of CXCL10 in the pathogenesis of inflammatory responses and secondary damages to SCI, which may provide a theoretical basis for CXCL10 as a potential biomarker and therapeutic target of the central nervous system (CNS) inflammatory responses.

## CXCL10 and its Receptor CXCR3

### CXCL10

Chemokines, with a molecular weight ranging from 8 kDa to 12 kDa, are involved in a wide range of physiological and pathological processes in the CNS. Based on the presence of cysteine moieties at the N-terminal sequences of a protein, chemokines can be divided into four subgroups (C-, CC-, CXC-, and CX3C-, defined based on four conserved cysteine residues that form disulfide bonds), which act on G protein-coupled chemokine receptors [26, 27]. In the adult CNS, chemokines and their receptors are involved in developmental, physiological, and pathological processes, such as inducing cell migrations, promoting cellular interactions, activating intracellular signaling pathways, and maintaining CNS homeostasis [28].

CXCL10 was initially identified as a chemokine induced by IFN-γ [29]. It belongs to the CXC subfamily of chemokines, which contains four conserved cysteine residue motifs linked by intervening amino acids between the first two conserved cysteines [27]. According to the presence or absence of the acid-leucine-arginine (Glu-Leu-Arg, ELR) motif in the N-terminal region, CXC chemokines can be divided into two subgroups: ELR+ CXC chemokines can promote angiogenesis, whereas ELR- chemokines, such as CXCL10, can inhibit angiogenesis [20, 30].

CXCL10 can be secreted by various types of cells, including immune cells such as T lymphocytes, neutrophils, eosinophils and monocytes. It can also be secreted by stromal cells, including thyroid cells, splenocytes, endothelial cells, fibroblasts, and keratinocytes [31-34]. TNFα, IFNα, β and γ are all inducers of CXCL10 [35-37].
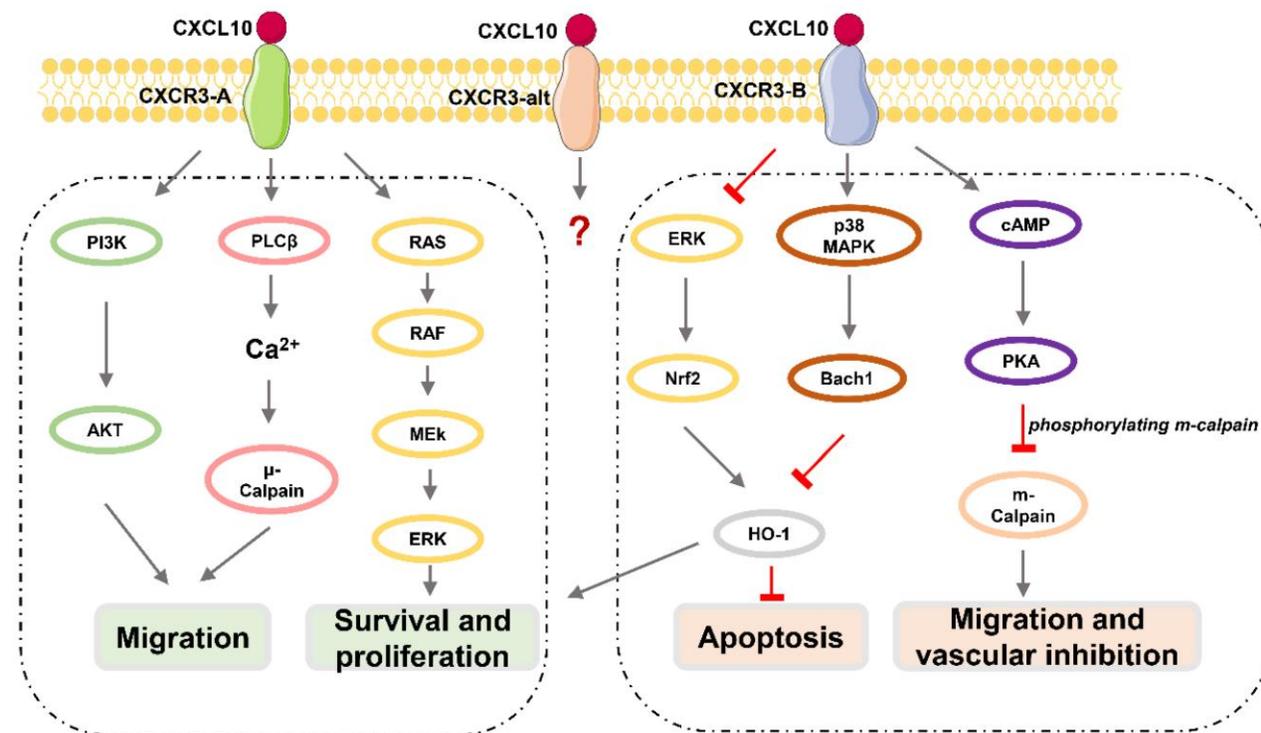


**Figure 1. Downstream effects of CXCL10 / CXCR3 signaling pathway.** CXCR3-A promotes proliferation, cell survival, chemotaxis, and invasion, while CXCR3-B mediates growth inhibition, apoptosis, and vascular inhibition.

## CXCR3

CXCR3, also known as GPR9 or CD183, is a G protein-coupled seven-transmembrane receptor that can also be expressed in immune cells such as CD4^{+} T lymphocytes, CD8^{+} T lymphocytes, and NK cells, and in stromal cells such as endothelial cells, glomerular mesangial cells, trophoblasts, and keratinocytes [38, 39]. CXCR3 can be divided into three subtypes (Figure 1): CXCR3-A, CXCR3-B, and CXCR3-alt [40-43], among which CXCR3-A and CXCR3-B are expressed in neurons [41, 44]. CXCR3-A signaling promotes cell migration and invasion via the PLCβ3 / μ-calpain signaling pathway in prostate cancer [45]. CXCL10 also binds to the CXCR3-A subtype, fully induces Gαi activation and extracellular signal-regulated kinase (ERK) 1 / 2 phosphorylation, and partially induces the recruitment of β-arrestin protein [40]. CXCR3-A mediates chemotactic responses via MAPK and PI3K / AKT signaling pathways in human airway epithelial cells [46]. Activation of CXCR3-B can inhibit angiogenesis or proliferation and promote apoptosis [47]. CXCL10 acts as an angiostatic agent, via CXCR3-B signal-mediated PKA phosphorylation of m-calpain to prevent endothelial cell motility [48-51] or via activation of p38/MAPK activation in human microvascular endothelial cells [52]. CXCR3-B mediates growth inhibition or apoptosis via p38/MAPK activation following the downregulation of heme oxygenase 1 (HO1) and the translocation of Bach1 and Nrf2 in human renal cancer cells and breast cancer cells [53]. Although CXCL10 can somewhat induce the activation of CXCR3-alt, its role in this process is unclear [54].

### The Basic Functions of CXCL10 / CXCR3 axis

CXCL10 has a variety of biological functions such as chemotaxis, differentiation, activation of immune cells, regulation of apoptosis, and neovascularization inhibition through the CXCL10 / CXCR3 axis.

One of the basic functions of CXCL10 / CXCR3 axis is that activation of the CXCL10 / CXCR3 axis activates the inflammatory cells and orchestrates inflammatory cell migration. For example, it activates microglia and directs them to the site of injury [42, 55, 56]. And it forms an amplified feedback loop in activating the inflammatory cells. In CD4^{+} T lymphocytes, CXCR3 is induced by T cell receptor (TCR), enhancing the production of IFNγ and TNFα, which stimulates various cells to secrete CXCL10, thus forming an amplified feedback loop and perpetuating the immune cascade [57, 58]. Besides CD4^{+} T lymphocytes, CXCL10 also stimulates the directional migration of CD29^{+} T lymphocytes and monocytes, as well as potentiates T lymphocyte adhesion to endothelium [59]. In addition, CXCL10 directs CD8^{+} T lymphocytes and NK cells recruiting in CNS immune response [60-63]. Besides T lymphocyte recruitment, CXCL10 induces eosinophil chemotaxis, which can be blocked by the anti-CXCR3 monoclonal antibody, via CXCR3 inactivation on eosinophils [64]. Therefore, CXCL10 plays an immunoregulatory role in innate and adaptive immunity.

In addition, CXCL10 functions as a suppressor of neovascularization by inhibiting the expression of various angiogenic factors in vivo, including VEGF a and c, and MMP13, and directly inducing endothelial cell apoptosis. In vitro, CXCL10 inhibits endothelial differentiation into tubular capillary structures in a dose-dependent manner, whereas it does not affect endothelial cell growth and migration [65, 66].

## Expressions and Roles of CXCL10 in the CNS

The CNS consists mainly of neurons and glial cells, and a small amount of vascular and connective tissues. Neurons undertake the main functions of the nervous system, and the glial cells (including astrocytes, oligodendrocytes, and microglia) participate in the formation of the blood-brain barrier (BBB), controlling immune responses in a dominant manner, and participating in nerve repair and regeneration.

Under physiological conditions, CXCL10 expression in the CNS is not detectable. However, the CNS cells can synthesize chemokines and bind to them via receptors on their surface, thus producing chemotaxis and other functions [67, 68].

### Expressions and Roles of CXCL10 in Neurons

The immune response of the nervous system is predominantly undertaken by glial cells. However, in some cases, like viral infection, neurons expressing CXCL10 trigger inflammatory responses in the absence of glial cells. Viral dsRNA binds to TLR receptors on nerve cells, leading to a virus-mediated innate immune response that results in the expression of the inflammatory cytokines IL6 and TNFα, chemokines CCL5 and CXCL10, and the antiviral molecule IFNβ [69]. CXCL10 can be toxic to cells in the CNS. For example, CXCL10 can directly cause astrocyte death along with indirect neuronal death in HIV1 Nef protein-mediated neuronal cell death [70].

In human fetal neuron/astrocyte co-culture and human NT-2 neurons, CXCL10 was sufficient to induce apoptosis. In addition, a surge in CXCL10 expression under a variety of pathological conditions can lead to neuronal death and loss. CXCL10 can serve as a functional biomarker of human cerebral malaria (HCM) mortality and promote apoptosis of brain neurons and glial cells [71, 72]. In HIV1-infected

patients, CXCL10, in combination with HIV1, can synergistically enhance neuronal toxicity, which can be inhibited by blocking CXCR3 and its downstream MAPK signaling pathway [73].

The neurotoxic mechanisms involving CXCL10 are as follows: CXCL10 induces apoptosis through the mitochondria-dependent pathway by increasing the intracellular load of calcium released from the endoplasmic reticulum, leading to the mitochondrial release of cytochrome C (Cyt C) and cleavage-activation of caspase3 [74-76].

### The Expression and Roles of CXCL10 in Glial Cells

In the CNS, chemokines, expressed primarily by glial cells, recruit lymphocytes and phagocytes to inflammatory and infected areas and promote the immune response. Astrocytes and microglia exert a definite role in CXCL10 secretion. Microglia are resident macrophages in the CNS [77]. They provide immune defense and contribute to multiple functions during CNS development and maturation. Microglia secrete CXCL10 under a variety of conditions. For example, Borrelia burgdorferi, the pathogen of neuroborreliosis, can induce increased expression and secretion of CXCL10, which may be involved in a stronger activation effect on neuroinflammation induced by dead Borrelia burgdorferi [78].

Stimulation of human microglia by LPS or IFNβ produced during spirochete infection results in enhanced transcription of CXCL10 and microglia-derived CXCL10 secretion [79-82]. IFNβ promotes CXCL10 expression in microglia through the JAK-STAT signaling pathway and LPS can bind to TLR4 receptor and induce nuclear translocations of IFN Response factor 3 (IRF 3) or NFκB to promote CXCL10 expression in microglia [83-86].

Astrocytes also play a role in secreting CXCL10 in a variety of diseases. CXCL10 attracts phagocytes and promotes inflammatory responses. And human astrocytes and microvascular endothelial cells produce specific chemokines including CXCL10 to attract phagocytic cells and promote an inflammatory response in Borrelia burgdorferi infection [87]. Astrocytes expressing CXCL10 enhance viral infection and neuronal damage through the binding of TLR3 receptors to viral dsRNA [88]. Astrocytes are the main sources of CXCL10 in Zika virus infection, Japanese encephalitis virus infection, and HIV-associated encephalitis [67, 73, 89, 90]. Astrocytes are also a major source of CXCL10 in neurodegenerative diseases. During the development of AD, the expression of CXCL10 is significantly increased in astrocytes, where it activates the ERK1 / 2 signaling pathway in mouse cortical neurons and forms a neuron-glial interaction

[43, 76, 91]. Astrocytes are also the primary source of CXCL10 in the pathogenesis of CNS immune diseases, such as multiple sclerosis (MS) [82, 92-96].

CXCL10 binds to CXCR3 expressed by microglia and astrocytes, and the effect of CXCL10 on microglia and astrocytes is mainly focused on their migration promotion effects [97, 98]. CXCL10 binds to CXCR3 to induce calcium influx and electrophysiological responses, resulting in the recruitment of distal microglia via chemotaxis [97, 99, 100]. However, the proliferation of microglia is not affected [42]. In addition, CXCL10 / CXCR3 axis is closely related to the activation of glial cells, and CXCR3 deficiency significantly weakens the activation of microglia and astrocytes [101]. Moreover, CXCL10 also induces apoptosis in a dose-dependent manner in glial cells, in which it induces the expression and activation of caspase 3 and 7, thus increasing the percentage of apoptotic human neuroglia cells from 12% to 40.6% [102].

Oligodendrocytes function as myelin-forming cells in the CNS, wrapping around multiple nerve fibers to form the myelin sheath. However, there are few studies on CXCL10 secreted by oligodendrocytes. It has been reported that oligodendrocytes can express a variety of chemokines including CXCL10 in the presence of IFNγ [103]. Therefore, most studies focus on the role of CXCL10 on oligodendrocytes in myelination. Some studies demonstrated that CXCL10 expression is highly upregulated by IL1β and IFNγ, in hypertrophic astrocytes surrounding active MS lesions, and CXCL10 finally interferes with myelin formation by acting on CXCR3 receptors on oligodendrocytes [92, 104-106].

## The Role of CXCL10 in SCI

### Basic Pathological Manifestations of SCI

According to the time course, SCI can be divided into acute (0-48 hours), subacute (48 hours-14 days), intermediate (14 days-6 months), and chronic (>6 months) phases [107]. The acute phase is characterized by bleeding, edema, inflammatory cell infiltration, the release of cytotoxic products, and cell death (Figure 2). After SCI, the reduced sympathetic tone and autonomous blood flow modulation mechanism cause a significant decrease in the spinal cord blood flow (SCBF) and the mean systemic arterial blood pressure (ABP), leading to ischemia at the injury site, which can last for days or even weeks [108, 109]. Moreover, the trauma directly leads to the displacement or rupture of the spine, resulting in compression or damage to the spinal cord and the destruction of the blood-spinal cord barrier (BSCB) environment, exposing the damaged spinal cord

tissue to peripheral cytokines and blood cells [110]. As a result, local cells die due to changes in cell permeability, initiation of pre-apoptotic signaling, and ischemia caused by vascular damage [111, 112]. Dead cells release ATP, DNA, and $K^+$, which activate the microglia to secrete pro-inflammatory cytokines including CXCL10, causing local inflammatory cell infiltration.

Microglia and endothelial cells play a role as antigen-presenting cells (APC) during the initial stage of injury. T lymphocyte infiltration preceding the bulk of monocyte influx and macrophage activation continues throughout the acute to subacute phase [113]. Inflammatory cells accumulate at the injury site, releasing cytokines such as TNF, IL1, IL18, CCL2, and MMP9, and cytotoxic molecules like glutamate, which have neuroexcitatory toxicity and can cause spinal cord white matter hypoxia and traumatic injury [114-119]. In particular, it is observed that the infiltrating macrophages develop into M1 macrophage subsets and express pro-inflammatory cytokines such as CXCL10 and IL12p70 [120].

In the later acute phase to subacute phase, progressive edema exacerbates the post-injury microenvironment. In this case, the inflammatory cells stay in the spinal cord and continuously intensify the inflammatory response from the acute to subacute phase, resulting in further edema of multiple adjacent spinal segments. In the subacute phase, edema, combined with vascular thrombosis and vasospasm, causes ischemia and exacerbates infiltration of inflammatory cells, further leading to cell death. Cystic cavities containing extracellular fluids, small bands of connective tissue, and macrophages start to form as a consequence [121, 122]. Astrocytes proliferate and deposit into the surrounding area of the lesion core. During the intermediate phase to the chronic phase, axons continue to degenerate. And mature glial scars and cystic cavities further inhibit axonal regeneration and cell migration [123].

## SCI-induced CXCL10 Changes in the Spinal Cord, the Cerebrospinal Fluid and the Blood Serum

The changes in the CXCL10 expression level show a certain correlation with the process of secondary damages. In humans or a variety of animal models like those in rats or mice, increased CXCL10 expression levels can be detected in the core area of the spinal cord, serum, and cerebrospinal fluid (CSF).

As reported by McTigue et al, the mRNA expression of CXCL10 can be detected from 6 hours to 28 days in the lesion core of rats after SCI, with the peak identified at 6 hours post-injury [124]. Although the expression level of CXCL10 decreases after 6 hours post-injury, it is still significantly high at 12 hours post-injury and is attenuated *to* the control level subsequently [124]. In particular, the mRNA expression of CXCL10 is visible in the mouse spinal cord 30 minutes post-injury [125]. All these reports indicate that CXCL10 is mainly expressed by cells in the lesion core during the acute phase of SCI including microglia, astrocytes, and other inflammatory cells such as NK cells, CD8+ T lymphocytes, and macrophages [22, 123, 126, 127]. Furthermore, inflammatory cells like microglia in the spinal cord are sufficient to trigger an inflammatory response without the presence of peripheral blood-derived cells in the mouse SCI model [125]. Microglia are the main source of CXCL10 in early acute injury, and CXCL10 is co-expressed in microglia and other inflammatory cells, including astrocytes and macrophages [56, 120, 128].
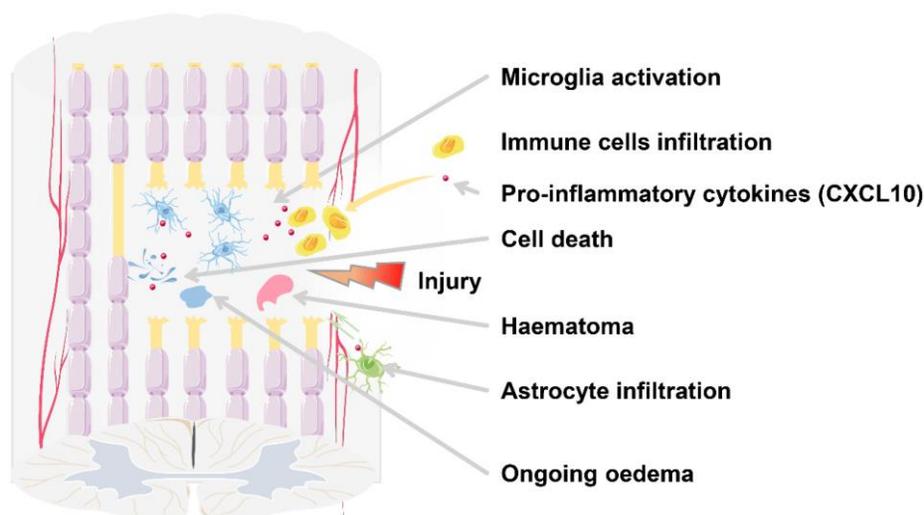


**Figure 2. SCI results in secondary injuries, as well as local and systemic complications.** In the lesion core, the injury leads to neuronal death, axonal damage and demyelination, and other complications.

There are few studies about the expression of CXCL10 in CSF. Casha et al reported that CXCL10 significantly increases at 6 h post-injury, and then reaches the peak within 2 days in the acute phase, followed by a slight decrease at day 3, and a more sustained increase between days 4 and 7, exhibiting a bimodal release mode [118].

The trend of CXCL10 concentration changes in the peripheral blood is relatively consistent with that in the CSF. Hassanshahi et al reported that the concentration of CXCL10 increases significantly in the acute SCI phase (3-6 hours post-injury), reaches the maximum value after the subacute phase (7 days), and then decreases. Notably, significantly increased CXCL10 concentration can still be detected after the intermediate phase (28 d) until 30 days post-injury when the concentration of CXCL10 is equal to that of the control [129, 130].

To summarize, the level of CXCL10 in the CSF, the peripheral blood, and the lesion core is relatively consistent over time. CXCL10 exhibits a high level in the acute phase post-injury, and the level maintains at higher concentrations in the subacute phase, and remains obvious in the intermediate period, suggesting a certain relationship between the changes of CXCL10 and the secondary damage.

## Roles of CXCL10 in SCI-induced Neuroinflammation

In the acute phase of mice injury, Rice et al reported that the activated microglia of the CNS release a large amount of CXCL10 at 3 hours post-injury, playing a key role in inflammatory cell aggregation [125]. CNS-derived CXCL10 performs their function of CD4+ Th1 cells recruitment by binding to the CXCR3-A receptor, and then CD4+ Th1 cells secrete IL2, IFN, TNF, and other cytokines to participate in the regulation of cellular immunity, enhance macrophage toxicity, regulate the differentiation of CD8+ T lymphocytes, and trigger the immune response. In the meantime, the immune cells recruited in response to CXCL10 also release CXCL10, resulting in a cascade reaction [24, 120].

There may be two reasons for the destruction of the BBB caused by CXCL10 in the acute phase. On the one hand, the BBB structure maintains the homeostasis of the CNS and restricts the recruitment of peripheral immune cells into the brain parenchyma composed of brain microvascular endothelial cells, pericytes, and astrocyte endfeet. CXCL10 inhibits the migration of endothelial cells through the CXCR3-B receptor, inhibits DNA synthesis, and promotes endothelial cell apoptosis, thus destroying the BBB [47, 102]. On the other hand, CXCL10 drives the aggravation of inflammatory responses and indirectly disrupts the BBB by oxidative stress and neurotoxic molecules [131]. This oxidative stress is generated by reactive oxygen species and nitric oxides released by inflammatory cells such as microglia and astrocytes, and neurotoxic molecules including prostaglandin, cyclooxygenase 2, MCP1 and MIP1α, as well as pro-inflammatory cytokines IL6, TNFα, and IL1β [131]. Therefore, CXCL10 involved in destruction of BBB in the acute phase, which creates a unique condition for the accumulation of peripheral immune cells, expanding the immune response from the local region to the whole CNS.

The early immune response plays a positive role in the recovery of SCI. To illustrate, immune cells can help to remove tissue debris and trigger the release of various neurotrophic factors [16, 132]. However, this autoimmune response gets out of control over time, resulting in secondary degenerative pathologies followed by continued axonal degeneration and glial scar maturation, thus causing detrimental consequences to spontaneous recovery from SCI. A high level of CXCL10 in the CSF during the subacute stages of SCI plays a key role in the secondary degenerative pathologies by restricting revascularization and axonal regeneration, resulting in the loss of the spinal cord tissue [22].

## Potential Underlying Mechanisms for the Role of CXCL10 in SCI

CXCL10 generates biological effects in SCI through interacting directly or indirectly with neurons, astrocytes, microglia, oligodendrocytes, endothelial cells and T lymphocytes (Figure 3) [18]. First of all, the effect of local ischemia on necrotic tissue cannot be ignored. The rupture of blood vessels, thromboses and vasospasm, and neurogenic hypotension are all caused by the injury and lead to the loss of blood. At this point, angiogenesis that can relieve the ischemic state is unfortunately directly or indirectly inhibited by CXCL10 [24, 133]. This means CXCL10 not only directly inhibits differentiation and promotes apoptosis of endothelial cells but also works together with inflammatory cells to involve in subsequent functions [47]. CXCL10 can inhibit the expression of various pro-angiogenesis cytokines and receptors such as angiogenesis-associated growth factor VEGF, the receptors Flt1, Flt4, and the thrombin receptor, and the endothelial cell marker CD31 / PECAM1 and the growth factor angiopoietin1. It also causes apoptosis of vascular endothelial cells by oxidative stress as well as a variety of other cytotoxic molecules which plays an indirect role in inhibiting angiogenesis [24, 131].
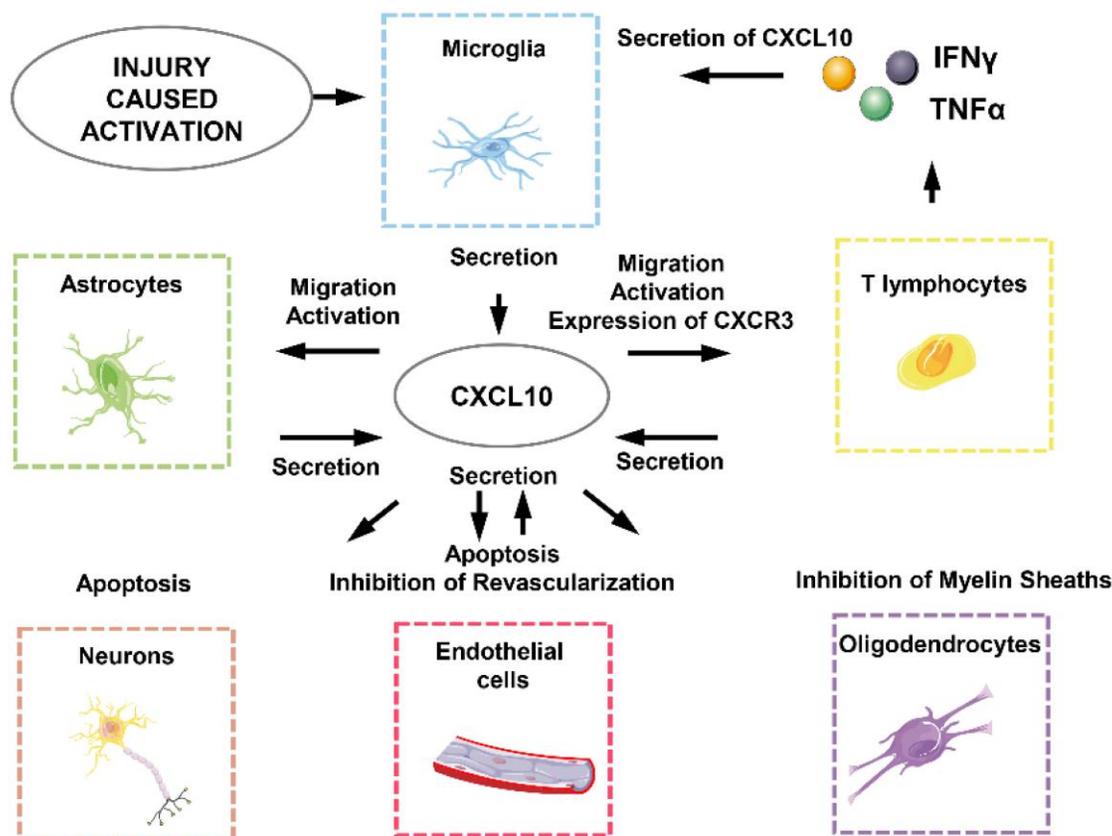
**Figure 3.** CXCL10-related cellular response in SCI-induced neuroinflammation.

Secondly, CXCL10 inhibits axonal regeneration. Previous studies have revealed that major phenotypic changes that astrocytes undergo are prerequisites for promoting spinal cord myelination. Astrocytes release CXCL10 to interfere with oligodendrocytes, thereby inhibiting the regeneration of axons of the spinal cord neurons [105]. In addition, CXCL10 impairs axonal extension and inhibits axonal regeneration by recruiting inflammatory cells, although the molecular mechanisms are poorly understood [25, 134].

Finally, CXCL10 not only exerts a direct apoptosis-promoting effect on neurons and glial cells but also recruits CD4+ T lymphocytes secreting various cytotoxic molecules such as IFNγ and TNFα to induce apoptosis of neurons and glial cells [135-137].

## The Role of CXCL10 in the Complications of SCI

Studies have shown that there is a certain correlation between CXCL10 and neuropathic pain, joint inflammation, dysfunction of the genitourinary system, and pressure sores after SCI.

### Neuropathic Pain

More than 70% of patients suffer from pain after SCI, and about 40% of patients experience chronic nerve pain. The pain can last for years and significantly impact the physical and emotional functions and quality of life [138-140].

Neuropathic pain has not been sufficiently researched to identify the underlying mechanisms. Some studies have shown that nerve root damage may lead to sprouting of spinal cord fibers and activation of primary afferent fibers, causing allodynia and hyperalgesia [141, 142]. Several recent clinical studies have found that inflammatory cytokines and chemokines play an important role in the development and persistence of neuropathic pain. Statistical analysis of the relationship between CXCL10 levels and pain in patients with SCI shows that CXCL10 concentrations are positively associated with pain intensity [23]. Patients with constant or increased CXCL10 over time have an increased risk of pain at one-year post-injury [23]. Therefore, CXCL10 has the potential to serve as a biomarker of pain development in SCI patients. In recent years, many studies have recognized that the interaction between CXCL10 and CXCR3 can participate in neuropathic pain in both the spinal cord and brain [55, 126, 143-148]. In rats with SCI, CXCL10 and CXCR3 are significantly expressed in the spinal cord, and CXCL10 / CXCR3 interaction is directly involved in neuropathic pain by regulating the ERK signaling

pathway [55].

In addition to its role at the site of injury, CXCL10 / CXCR3 interaction is also involved in the emotional process of pain by aberrant activation of the anterior cingulate cortex (ACC), which is part of the limbic system in the frontal region of the cingulate cortex. The pain involves affective, sensory, and cognitive dimensions [143], and neuronal activity in this structure can lead to an aversive emotion to noxious stimuli [144, 145]. One study suggests that CXCR3 can be increased by enhanced interaction between C / EBPα and DNA demethylation post-injury and activated by CXCL10 to maintain neuropathic pain, via the downstream pERK pathway, causing hyperalgesia [146, 147]. And the CXCR3 inhibitor AMG487 or (±)-NBI-74330 can significantly improve the pain threshold and can reduce neuropathic pain [55, 148]. Another study showed that only CXCL10 increases excitatory synaptic transmission and the risk of pain onset, and it also exacerbates existing pain in mice with SCI. In contrast, CXCL9 and CXCL11, the same subfamily chemokines jointly binding to CXCR3, can enhance both excitatory and inhibitory synaptic transmission without exacerbating pain [126]. These combined data suggest an excellent application prospect of anti-CXCL10 means in the treatment of neuropathic pain after SCI.

### Joint Inflammation

Inflammations, heterotopic ossifications, and fibrosis have been observed in the joints following SCI [149]. In recent years, most studies on joint complications after SCI focus on heterotopic ossification with an incidence rate of 10-53%. Its clinical presentations include pain, fever, redness, swelling of surrounding tissues, and limited joint movements, followed by the formation of ectopic bones in the periarticular connective tissues [150].

The exact pathophysiology of the joint inflammation remains unclear, while a recent study confirms the role of CXCL10 in this disease [151]. CXCL10 increases the migration of F4 / 80$^+$ macrophages and CD4$^+$ T lymphocytes into the synovium in collagen antibody-induced arthritis (CAIA) mice through ERK activation mediated by CXCR3 [151]. CXCL10 / CXCR3 axis works synergistically with TLR4 and increases joint inflammations by stimulating the production of osteoclastogenic cytokines in CD4$^+$ T lymphocytes [151].

### Dysfunctions of the Genitourinary Systems

The bladder problem (44%) was one of the most common medical problems in patients with SCI [140].

SCI blocks the spino-bulbo-spinal reflex pathway, resulting in bladder areflexia with complete urinary retention [152].

CXCL10 is significantly expressed in bladder tissues at 8 weeks and 12 weeks after rat SCI and the poor prognosis with the low recovery of urination function is related to the up-regulated expression of chemokines (represented by CXCL10), other pro-inflammatory factors and anti-inflammatory cytokines [153]. CXCL10 can exert an inhibitory effect on the spontaneous recovery of the urination reflex, which might be related to the demyelination of related neuronal fibers.

### Pressure Sores

In clinical practice, pressure sores are one of the common skin diseases which usually occur after SCI. Pressure sores can cause pain and increase the burden of nursing, resulting in a dramatic deterioration of patients' life quality. Pressure sores occur commonly on the buttocks (31%), lateral thighs (26%), sacrum (18%), feet (7%), and ankles (4%) [154]. A recent study found that plasma concentrations of CXCL10 are associated with the development of pressure sores before its first occurrence. CXCL10 has the potential to be a biomarker to identify patients at risk for pressure sores progression [155].

## Concluding Remarks

CXCL10 is involved in the process of acute inflammatory responses and secondary damages, and many complications after SCI via CXCR3 receptor [18, 22, 24, 25, 47, 55, 105, 118, 120, 123-127, 129-131, 133-137, 143-148, 150, 151, 153, 155]. After SCI, CXCL10 secreted by microglia plays an important role in recruiting and activating inflammatory cells [124, 125]. CXCL10 and recruited inflammatory cells work together to aggravate the inflammatory response, inhibit angiogenesis by inhibiting the differentiation of endothelial cells, decrease myelin formation by inhibiting oligodendrocytes, and then promote apoptosis resulting in the progressive loss of tissue at the lesion core [24, 25, 47, 105, 133-137]. All these effects lead to the inhibition of spinal cord regeneration. In addition, CXCL10 causes an increased disruption of BBB due to its effects on endothelial cells, resulting in CNS homeostasis disruption, and exacerbating CNS inflammation [47, 102, 131]. Furthermore, CXCL10 has proved to be associated with four complications after SCI, including joint inflammation, neuropathic pain, dysfunction of the genitourinary systems, and pressure sores [23, 55, 126, 143-148, 151, 155].

Existing clinical treatments of SCI include a variety of pharmacological and non-pharmacological

approaches to alleviate and delay the secondary damage cascade after SCI, including sodium channel-blocking antiseizure drugs Riluzole, the NMDA receptor antagonist magnesium, the antibiotic minocycline, the glycosphingolipid GM1 as a kind of ganglioside with neuroprotective effects, the fibroblast growth factor, the hepatocyte growth factor and G-CSF with anti-inflammatory effects. Although all these drug treatments have achieved certain clinical results, they fail to completely meet the expected therapeutic efficiency for patients [156].

It is undoubtedly a novel means for the treatment of SCI by regulating the immune process via the neutralization of pro-inflammatory factors [157]. In both the rat and the mouse model mimicking SCI, CXCL10 antagonist can reduce T lymphocyte infiltration and inhibit neuronal death, thus increasing axonal and blood vessel regeneration and improving functional recovery after SCI [22, 25, 157, 158].

Despite the encouraging results from these aforementioned studies, CXCL10 antagonist alone cannot achieve the desired therapeutic effect. Although CXCL10 can affect regeneration and functional recovery after SCI as a key factor in the microenvironment of the injured spinal cord, several other growth-inhibiting molecules (chemokine and cytokines) also involve in a time-dependent manner [159, 160]. For instance, the serum level of TNF-α, MCP-1, IL-1β, IL-2, IL-6, IL-4 and IL-10 is highly time dependent during the progression of SCI and abnormal expression of these cytokines is also related to poor neurological outcomes [161]. These findings further emphasize that CXCL10, particularly along with other factors in the microenvironment of the spinal cord, is necessary for SCI deterioration. CXCL10-induced inflammation may also play a neuroprotective role in the CNS. For instance, neurons infected with the neurotropic flavivirus West Nile virus can express CXCL10 to promote adaptive immune responses to clear the virus, casting a new light on the neuroprotective function of CXCL10 [60]. And other results show that infiltrating blood-derived macrophages displayed an anti-inflammatory beneficial role, and microglia prevented lesion expansion in brain damage and astrocytes to aid CNS axon regeneration in SCI [162-164]. It also suggests that inflammation might represent a front-line defense against CNS damage. Furthermore, most results of CXCL10 antagonist currently available have been conducted using animal models with a lack of evidence in human beings, and some specific issues remain to be solved. For instance, the most appropriate therapeutic time window needs further evaluation. Different spinal segments with injury exhibit differential responses among individuals, showing different expression levels of the inflammatory cytokines. Taken together, personalized treatment strategies are still needed to optimize the treatment via targeting CXCL10 signaling pathways and the CXCL10 antagonist treatment should be combinedly utilized with other therapeutic means.

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## Competing Interests

The authors have declared that no competing interest exists.

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