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Critical role of microglia in the inflammatory response after spinal injury

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Abstract

The spinal cord, comprising nerve cells and supporting cells, is a vital component of the central nervous system (CNS) that connects the brain to the body. It is part of a bony structure, the vertebral column, which protects the delicate cord from external damage. Various factors can cause spinal cord injuries (SCIs) that disrupt its structure and function, leading to devastating consequences for affected individuals. It is important to understand SCIs, their causes, structure, consequences, and pathology to inform research and therapeutic efforts. As SCIs severely damage the CNS, which doesn't regenerate after injury, efficient pathways to repair the nervous system must be found. This is understood as a cellular response to injury, currently focusing on immune and glial inflammation. Microglia are the resident immune cells of the brain, fulfilling roles in the maintenance, repair, and homeostasis of the healthy CNS, and responding to injury and disease. While they perform beneficial, protective roles under some circumstances, they can also harm cells by the release of neurotoxic molecules. This dual action makes them a prime candidate for possible therapeutic interventions in various neurological disorders, including SCIs. The proper functioning of the spinal cord and the integrity of the spinal cord pathway mechanisms are essential for an unaffected or intact interchange between the body and the brain. However, a spinal injury or the rupture of the spinal cord-producing lesion interrupts these pathways. The kinds of lesions can happen through disease, trauma, congenital malformation, or injection of a drug. The types or classes of injury producing insults can be classified into two categories: complete and incomplete. In a complete injury, the spinal cord is completely damaged, and there is a complete loss of function below the level of injury. In an incomplete injury, the spinal cord is partially damaged, function below the level of injury is not completely lost, and the deficit varies in the extent of damage to different tracts.

Keywords: Spinal cord injury; Microglia; pro-inflammatory cytokines; CNS; Immune cells

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Introduction

The spinal cord is a complex structure composed of interconnecting neural circuits that transmit sensation, produce reflexes, and control motor functions. It is covered by layers of protection from the vertebrae, spinal meninges, and cerebrospinal fluid. The ventral surface is formed by two ridges named sulci that run longitudinally, as well as the dorsal surface, whose wide ventral surface is formed by the thickened areas of the spinal cord termed enlargements. Their function is to facilitate innervation of the upper and lower limbs. These structures are continually disrupted by various types of injuries throughout life, and it has been estimated that millions of people are disabled due to spinal cord injuries worldwide. In addition to leading to the necrosis and apoptosis of large populations of neurons, oligodendrocytes, and astrocytes, spinal cord injuries elicit a spatiotemporally regulated inflammatory response.

The spinal cord is composed of a central butterfly-shaped gray matter region that comprises neuronal cell bodies and their processes covered by a well-developed myelin sheath, and the white matter that is constituted by axons and glial cells interspersed in fine connective tissue and blood vessels. A transverse section of the mammalian spinal cord shows distinct spinal cord segments along the rostrocaudal axis: cervical, thoracic, lumbar, sacral, and caudal.

Most spinal nerves are mixed with two roots: the sensory dorsal root and the motor ventral root. The latter has a simple three-neuron reflex arc comprising primary sensory neurons, central projection of the primary afferents to the spinal circuits, and efferent motoneurons that comprise the pyramidal tract and innervate skeletal muscles for movement. The spinal cord dorsal horn is the entry for sensory information from prospective injuries. Afferent input from sensory organs is received by peripheral sensory nerves that are composed of low-threshold mechanoreceptors and high-threshold nociceptors. The convergence of several cutaneous afferent inputs to second-order neurons in the dorsal horn leads to the emergence of a network of spinal circuits that can gate sensory flow and form complex spinal responses such as central

Importance of Inflammatory Response in Spinal Injury

The spinal cord, a structure that contains neurons and support cells, is critical for regulating physiological functions such as heart rate, blood pressure, and temperature, as well as movement, touch, and pain. The spinal cord is protected by bony vertebrae and is bathed in cerebrospinal fluid. However, the spine is one of the more vulnerable areas of the central nervous system. Accidents such as falls, sports injuries, and car accidents, as well as diseases, can damage the spinal cord, causing dysfunction. The spinal cord is susceptible to injury due to its confinement within the bony vertebrae of the spine. Within millimeters of an initial injury, secondary damage develops that could lead to increased degrees of dysfunction.

An important component of secondary injury is the activation of supportive glial cells in the spinal cord, particularly microglia. Microglia are small glial cells and resident immune cells of

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the brain and spinal cord, and they have distinct morphological and transcriptomic states, which often vary by region. In their most common state, microglia participate in the homeostasis of the central nervous system through processes such as monitoring the interstitial fluid and clearing dead cells. Microglia become activated following spinal injury through processes such as morphological and transcriptional alterations, as well as increased migration and proliferation. Upon activation, microglia are able to participate in inflammatory processes. Activation can be beneficial for clearing debris and secreting trophic factors, but it can also be temporarily or chronically detrimental to the central nervous system.

After injury, harmful inflammatory factors such as cytokines, proteases, reactive oxygen species, and excitotoxic glutamate can be released that exacerbate further degeneration and dysfunction. Therefore, the inflammatory response is paramount in the aftermath of spinal injury, as it can have both beneficial and harmful effects. Understanding the mechanisms by which the inflammatory response is activated and the effects it has is critical to understanding the full picture of injury pathology and should help in the design of therapeutic interventions. After injury, the spinal cord becomes inflamed, and a pro-inflammatory state is adopted that supports further degeneration and dysfunction. It is important to understand the critical role of microglia in injury-induced inflammation, and it is necessary to have a grasp on the anatomy and function of the spinal cord, as well as the cellular and molecular aspects of the inflammatory process, in order to fully appreciate the post-injury state of the spinal cord.

Microglia

Microglia are the resident immune cells that monitor and maintain the environment of the healthy CNS. These myeloid-lineaged macrophages are specialized cells that enter the developing brain early in life and are maintained throughout life by local self-renewal 1. Microglia are critical for homeostasis within the neural environment and thus neuronal health. Under physiological conditions, microglia constantly survey their environment by extending and retracting their processes. They remain in a highly ramified state and have a small cell body. This “resting” morphology is established early in development and is maintained throughout life. At this state, microglia can be activated rapidly in response to “danger” signals from dead or dying cells, misfolded proteins, and pathogens. Upon activation, microglia switch to a highly amoeboid morphology with retracted processes and an enlarged cell body. Microglial immunoreactivity for “activation markers,” such as CD68 and ED1, is often used to demonstrate the presence of pathological conditions.

Many microglial functions have been uncovered during health and after pathological conditions. Microglial activation is typically accompanied by the upregulation of genes related to proinflammatory cytokines and chemokines, reactive oxygen species, complement proteins, and proteases. Some of these secreted factors can be neurotoxic and exacerbate damage. For example, activated microglia may cause tissue damage or death by releasing proinflammatory

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cytokines such as IL-1β, IL-6, and TNF-α, which can invoke the death cascade apoptosis pathway. Therefore, certain microglial responses to injury or disease can be damaging as well as protective. To better understand how microglia function in the healthy CNS, appreciation of both their protective role and their capacity to become harmful is required. These numerous and diverse functions of microglia underlie their critical importance in mediating tissue responses following injury or inflammation in the CNS. Understanding how they behave after these events is central to discerning their role in the innate immune response to spinal injury and the ongoing progression of tissue damage.

Characteristics of Microglia

As the resident immune cells of the CNS, microglia play an important role as active participants in the inflammatory response after SCI. Under homeostatic conditions, microglia are characterized by a small cell body with long, highly branched processes that extend into the parenchyma where they interact with neurons, astrocytes, and blood vessels in a surveillance capacity. This morphology allows them to continually sample their environment and detect pathological changes. Microglia are defined by a unique morphological state (ramified), as well as a distinct gene expression profile from other cell types including antigen presenting cells, as defined by the expression of Iba1, TMEM119, P2RY12, CX3CR1, and others.

In the healthy CNS, microglia perform numerous essential functions involved in neuronal development, maintenance of homeostasis, and support trophic functions for neurons. In early postnatal development, microglia prune synapses and neuromuscular junctions, resulting in the maturation of neuronal networks. Under homeostatic conditions, microglia respond to local neurochemical changes or injury by rapidly mobilizing large processes toward the area of interest and retracting their peripheral processes to form a more compact structure. In response to a more global change in homeostasis, such as during disease, this morphological transformation is accompanied by alterations in gene expression and cellular function, collectively categorized as microglial activation. Generally, activated microglia are characterized as a hypertrophic and amoeboid shape, as well as an upregulation of MHC II and a basal level of TNF-α release. In vitro, M1-like microglia, often referred to as pro-inflammatory, can be generated by treatment with LPS, IFN-γ, or TNF-α, and they are associated with the release of pro-inflammatory cytokines like IL-1β, IL-6, TNF-α, and IL-12. Conversely, M2-like microglia resemble a more anti-inflammatory phenotype, with a pro-resolving cytokine response, generating IL-4, IL-10, and IL-13. However, it is essential to remember that a clear demarcation and categorization of such states is an oversimplification of the complex biological system that encompasses multiple intermediate states.

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Role of Microglia in the Central Nervous System

Microglia, the immunological resident cells of the central nervous system (CNS), differentiate from progenitors of the yolk sac. Throughout life, these cells exert numerous essential homeostatic functions in the healthy CNS and modulation in response to neurodegenerative diseases, insults, and injuries. Microglia maintain the health of the CNS parenchyma by monitoring their environment, responding to cellular dysfunction, promoting cell survival, and removing cellular debris. In addition to sentinel duties, under homeostatic conditions, microglia are actively involved in developmental processes. It has been suggested that they partake in the pruning and maintenance of synaptic connectivity, hence influencing the formation and shaping of neural circuits. Microglia also regulate neuronal excitability and different aspects of the electrophysiological properties of successive age-dependent neural networks, hence influencing the developmental window of neuroplasticity. Moreover, microglia have been shown to restrict the spread of neurodegeneration in an activity-dependent fashion in a domain-dependent manner. A more protective role of microglia has also been suggested in the context of neurodegenerative diseases and aging. There is an increasing body of literature implicating microglial involvement in the modulation of neuroplasticity and sensitive restructuring of behavioral responses 1.

Recent studies have provided new insights into microglial roles during neurodegeneration, injuries, and multiple pathologies of the CNS. However, the functional consequences of dysfunctional microglial activity and the net outcomes can result in diverse pathological conditions. The CNS is a distinct immunological compartment separate from the peripheral immune system. The net outcome of the damaging inflammatory response is determined by the balance between protective and harmful roles. Characterization of microglia and understanding their complex interactions with other neural cells in both health and diseased states of the CNS will shed light on the best ways to maintain or restore CNS health after spinal injury. This will facilitate the development of future therapies that seek to give similar advantage to the endogenous cellular machinery of recovery while limiting secondary damage.

Inflammatory Response After Spinal Injury

A common pathophysiological consequence of spinal cord injury (SCI) is an inflammatory cascade following the primary insult. The nature of this inflammatory response is complex and variable, including responses that are both beneficial and detrimental. An injury to the spinal cord results in a myriad of molecular and cellular events, including necrotic cellular death, apoptosis, and the release of pro-inflammatory cytokines, chemokines, and reactive oxygen species (ROS) that recruit immune cells. Dysregulated inflammation contributes to secondary injury and tissue degeneration, while coordinated inflammatory responses can enhance tissue preservation and repair. In mammals, spinal cord injury triggers the opening of the blood-spinal

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cord barrier (BSCB) and the relocation of blood-derived leukocytes into the injury site. The BSCB is an endothelial structure that serves as a barrier to protect the spinal cord from pathogens or blood-derived neurotoxic/immune factors while also mediating nutrient transport. Disruption of the BSCB in SCI has been demonstrated in both rodents and non-human primates, facilitating the infiltration of blood-derived leukocytes into the spinal cord injury site. After injury, there is an initial upregulation of intracellular adhesion molecule-1 (ICAM-1) on the endothelium within the rostral and caudal hypoxia-ischemia injury sites of the BSCB after 24 hours. Since ICAM-1 is a ligand for the leukocyte integrin LFA-1, these mechanisms imply that dysregulation of the BSCB after experimental SCI may facilitate the recruitment of leukocytes into the injured tissue.

Inflammation is a tightly regulated immunological response to tissue injury that serves to initiate repair processes and restore homeostasis. Tissue damage in the spinal cord, following either traumatic injury or pathological conditions, elicits an inflammatory response, which is characterized by the production and release of pro-inflammatory and anti-inflammatory cytokines, chemokines, and other mediators. The inflammatory response can be classified into three distinct phases: acute, sub-acute, and chronic inflammation. During the acute phase of inflammation following injury, glial cells such as microglia and astrocytes are rapidly activated and proliferate, exhibiting an injury-induced morphological transition. Microglia develop from a branched and ramified resting state into an activated amoeboid shape with upregulated major histocompatibility complex (MHC) class II and surface receptors. In the days following injury, blood-derived immune cells such as neutrophils and monocyte-derived macrophages infiltrate into the site of injury, coincident with the phagocytosis of debris from damaged neurons and oligodendrocytes. There is a parallel and dynamic release of pro-inflammatory cytokines and neuroprotective factors secreted by a variety of cellular players involved in the inflammatory response. In addition to mediating macrophage polarization and the subsequent cellular processes of secondary degeneration, pro-inflammatory cytokines also influence astrocytes and oligodendrocytes, resulting in the secretion and release of neurotoxic mediators that contribute to the spread of neuroinflammation dysfunction.

Phases of Inflammation

Following a spinal cord injury, the inflammatory response is crucial for host defense and tissue repair. Microglia, the resident immune cells of the CNS, are rapidly activated within hours of injury, leading to morphological and phenotypic changes. This activation of microglia is tightly regulated in both time and space, forming a classic arc from the injury epicenter to the periphery of the damage. Microglial activation can range from neuroprotective to neurodegenerative states, and they have the ability to phagocytose debris and secrete cell signaling molecules. Accumulating evidence indicates that activated microglia can promote beneficial and detrimental effects after injury, highlighting the importance of understanding microglial function

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to develop new therapeutic strategies for CNS diseases. Inflammation occurs as a dynamic two-phase cascade after SCI, with an acute phase characterized by the infiltration of immune cells into the injury site and prolonged neurodegenerative responses mediated by local glial cells. Reactive astrocytes and activated microglia/macrophages form a glial scar bridge around the injured site, which may inhibit axonal regeneration, while other glial cells secrete pro-inflammatory cytokines and chemokines leading to the propagation of a secondary degeneration cascade. Some bridge-forming astrocytes release neurotrophic factors to provide a supportive environment for axonal regrowth, but this is normally insufficient to overcome the glial scar formation. Additionally, some classically activated macrophages may engulf myelin debris and promote remyelination and tissue repair. SCI-induced inflammation can be divided into an acute and a chronic phase. An enormous release of inflammatory mediators occurs within minutes to hours after injury (acute phase), and phagocytes including neutrophils and macrophages infiltrate the damage site and remove cellular debris, dying neurons, and oligodendrocytes through phagocytosis and the secretion of proteases, while macrophages also secrete inflammatory cytokines. Microglia, the resident immune cells of the CNS, are rapidly activated and proliferated, sensing the injury and immediately changing morphology to an activated state. In the same time window, the death of oligodendrocytes leads to the collapse of the myelin sheath coating axons, and then the secondary degeneration cascade is propagated through senescent oligodendrocytes that release chemokines and mediators of neuroinflammation to recruit microglia and macrophages, and surrounding astrocytes that release pro-inflammatory cytokines to activate nearby astrocytes and oligodendrocytes, leading to a spreading wave of apoptosis for surrounding oligodendrocytes, axons, and neurons.

Cellular Players in the Inflammatory Response

The acute stage of the inflammatory response after spinal cord injury (SCI) is characterized by a rapid increase in the blood level of several pro-inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and others, which is consistent with the classical notion of acute inflammation induced by trauma. Due to the loss of integrity of the blood-spinal cord barrier (BSCB) after the injury, circulation-derived leukocytes and plasma proteins extravasate into the tissue in a greatly elevated manner, and activated phagocytes modulate the early inflammatory response. On the other hand, a series of signaling events induced by damage-associated molecular patterns (DAMPs) released from the injured cells provoke inflammatory signaling pathways with cell-autonomous action in non-myelinating glial cells, particularly in a prominent subpopulation of cells of mesodermal lineage—microglia.

Microglia, as resident immune cells of the central nervous system (CNS), play a dual role in modulating the inflammatory response after SCI. Within a few hours post-injury, parenchymal microglia retract their processes, swell, and change to ameboid morphology, becoming the first responders to the focal traumatic events. Besides microglia, the injury-induced activation of a

plethora of progenitor and/or precursor cells of totally different lineages (neural, mesenchymal, epithelial, etc.) and ages turning into astrocytes, oligodendrocytes, or gliomas fate confounds the interpretation of data if not strictly considered. Otherwise, comments on neuroinflammation would need to cover these evolving players.

In this view of principally the role of microglia in neuroinflammation, the cell-autonomous response of non-myelinating dysfunctional glia is considered thus at the outset. Damaged tissue aggregates de novo in plasma domains enveloped by swollen processes of activated microglia, named glial scars. These unidirectional multi-faceted scar formations and their underlying mechanisms were investigated in great detail, providing scientists with a significant body of knowledge at the cellular and molecular levels. Generally, microglia eliminate cell corpses through phagocytosis and degrade surplus proteins through proteolysis and phagosome-lysosome intracellular pathways.

Microglial Activation in Spinal Injury

After CNS injury, microglia have the potential to activate, proliferate, or transform into a reactive state. Pristine resting microglia possess small cell bodies, long thin processes, and elaborate branched processes that continuously survey the CNS environment. Following acute CNS injury, microglia retract their processes, increase cell body size, express upregulated or newly synthesized genes, and migrate toward the injury site. This classic phenomenon is termed microglial activation. Activated microglia are capable of heightened cytokine production, phagocytosis of cellular debris, and proliferation. Phagocytosis of cellular debris is essential for axon regeneration, and complete elimination of apoptotic neurons drastically impairs regeneration. Emerging evidence suggests the existence of several alternative activation states in addition to the classical M1 and M2 states. Microglial activation after spinal cord injury is substantial and persistent. Neuroinflammatory processes are highly regulated, and microglial function is tightly controlled. The subsequent outcome of microglial activation ultimately depends on the proper milieu, the balance between pro-inflammatory and anti-inflammatory signals, microglial polarization, and the variety of activated microglial functions.

Shortly after a spinal cord insult, there is a rapid and sustained increase in the density of microglial cells in the vicinity of the lesion site, and activated microglia have a typical morphologic appearance with thickened and retracted processes, enlarged cell bodies, and increased immunoreactivity for major histocompatibility complex class II antigens and other pro-inflammatory molecules. Although activated microglia proliferate and enhance their production of certain neuroprotective factors, they also release several neurotoxic substances, including pro-inflammatory cytokines, free radicals, and excitatory amino acids, which can exacerbate neuronal injury. Cytokines released onto resident cells may alter the microglial response, and a late, sustained rise in the levels of pro-inflammatory cytokines may correlate with an unregulated, chronic degenerative state. Microglial cells, the resident immune cells of the CNS,

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are capable of migrating to sites of injury and undergoing profound phenotypic changes in response to a variety of insults. Studies regarding the dual role of the immune response after CNS injury have focused largely on the infiltration of peripheral leukocytes across the blood-brain barrier during the ongoing CNS inflammatory cascade. However, penetration of this barrier is not the initial event following CNS trauma, and the first wave of cellular response occurs in the absence of their involvement. It is now recognized that the cells that populate the CNS during the initial phase of the inflammatory response to CNS injury are the parenchymal microglia.

Mechanisms of Microglial Activation

Microglial activation after spinal cord injury (SCI) occurs. The characterization of this response and its mechanisms remains incomplete. It is empirically known that lumbar glucocorticoids are anti-inflammatory, and based on this and other pharmacological experiments, some mechanisms have been proposed. The characterization of microglial activation after SCI and the mechanisms controlling this activation are important steps to understand the processes underlying inflammation and injury in the spinal cord. Earlier studies showed that non-steroidal anti-inflammatory drugs could reduce microglial activation and spinal injury. They are indirect inhibitors of cyclooxygenases, the enzymes converting arachidonic acid into prostaglandins, and they are known to alter the synthesis of cytokines, including IL-1β and IL-6. It has been shown that microglia in culture, after stimulation with lipopolysaccharide, carrageenan, or stress input, produced prostaglandins and cytokines. The systemic, peripheral, or central administration of prostaglandins or cytokines mimicked those effects, inducing the production of other prostaglandins and cytokines. On the contrary, the application of dexamethasone, a glucocorticoid, to these cultures inhibited the signals that induced the activation of microglia, suggesting that there are mechanisms controlling the activation of microglia after SCI, in addition to the known damaging effects of microglial activation.

Microglia are the resident immune-like cells of the central nervous system. These motile cells act to survey and respond to insults, removing cellular debris and secreting neuroprotective factors, acting to maintain homeostasis in the CNS. In pathology, such as following traumatic spinal cord injury, microglia are activated, adopt an amoeboid morphology, proliferate, and migrate toward the injury site. There they secrete pro-inflammatory factors, including cytokines and free radicals, causing an inflammatory response. When there is damage to the brain or spinal cord, the local microglia become active very quickly. They enlarge their cellular bodies and retract their processes. This amoeboid form of microglia can be distinguished from the ramified resting phase. Various models have investigated the transition of microglial cells from resting state to activated state. The overall effect of activation of microglia depends on the nature of the injury and the mode of activation. They can be broadly classified into two groups:

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1) classically activated, pro-inflammatory microglia activated by IFN-γ and lipopolysaccharide;
2) alternatively activated, neuroprotective microglia activated by IL-4 and IL-13.

Consequences of Microglial Activation

Microglial cells act as resident immune cells in the central nervous system (CNS), playing a central role in the inflammatory response following spinal cord injury. They are originally derived from yolk-sac progenitors and are distributed throughout the CNS during embryonic development. Prior to injury and under physiological conditions, microglia have a ramified morphology characterized by numerous long, slender processes and small cell bodies, which assess the local environment by continuously sampling the surrounding extracellular space through dynamic process motility. Following various CNS insults, their morphology and function undergo inherited changes through activation, resulting in the typical amoeboid phagocytic morphology. After spinal injury, the kinetics of microglial activation are biphasic, with the first wave peaking at 1–3 days post-injury and the second wave occurring 2–3 weeks later. In addition to phagocytosis, the activation of microglia is associated with the chronic release of numerous neurotrophic and pro-inflammatory factors.

As the first immune cells to be activated in response to CNS insults, microglia participate in the removal of cellular debris through phagocytosis, thus supporting tissue repair and restoration of homeostasis. This classic neuroprotective function immediately following the injury helps to minimize damage and the spread of neuroinflammation. Microglia correlate with neurologic motor recovery after spinal cord injury. An enhanced activation state, characterized by upregulated MHC-II and CD68, correlates with better axonal regeneration after full transection. Selective removal of a subgroup of activated microglia leads to a reduction in the sprouting of axonal and collateral connections. Additionally, microglial depletion reduces angiogenic sprouting by blocking the release of pro-angiogenic factors, which promote the formation of new blood vessels and enhance neurogenesis after SCI.

In parallel to the beneficial response, substantial evidence indicates that activated microglia can exert a variety of toxic effects, aggravating the damage, promoting cell death through the release of neurotoxic mediators, and exacerbating the inflammatory process. For example, the release of pro-inflammatory cytokines, nitric oxide, proteases, or excitatory amino acids can induce neuronal and oligodendrocyte cell death. Conversely, the release of neurotrophic factors can promote neuronal survival and glial proliferation. Recent studies have suggested that microglia can also adopt an M2-like activation profile and promote neuroprotection after SCI. Although M2 activation is thought to suppress pro-inflammatory responses and promote neuroprotection, there is also evidence that different M2 states can be associated with different pro-inflammatory profiles. Therefore, a careful balance between detrimental and protective mechanisms, modifiable by microglia, may be fundamental to establishing the net cell fate after spinal injury.

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Modulation of Microglial Response

Modulation of microglial response after spinal injury can be accomplished through a variety of different means. For many injuries modulation of the injury response may be particularly beneficial, where in some cases immune suppression may result in beneficial outcomes and far more often than not should cells actually be removed from the area of injury. In other circumstances enhancement of microglial activation may restore homeostasis and facilitate recovery 2. Broadly, there are a variety of different pharmacological agents available that target microglial response which could be harnessed in further study. For instance, fractalkine, a chemokine expressed both in neurons and glia, is one widely studied target of intervention. Following CNS injury fractalkine signaling contributes positively to disease process through upregulating pro-inflammatory cytokine expression, chemokine production and phagocytic activity. In light of this the fractalkine receptor has been targeted in studies of ischemia and SCI with favorable results, where blockade of this pathway led to decreased lesion size and increased functional recovery also through reducing myeloperoxidase activity. Similarly, colony stimulating factor 1 receptor (CSF1R) has emerged as another prominent target in neurodegenerative disease and injury. As the microglial counterpart of macrophage colony stimulating factor CSF1R has considerable implications in the proliferation and function of these cells both in homeostasis and injury. Genetic and pharmacological inhibition of CSF1R has been found to reduce activation and proliferation of microglia/macrophages following neural injury. In particular, blockade of this pathway in rodent models of SCI using GW2580 had broad implications in the reduction of white matter degeneration and secondary cell death as well as improved fine motor recovery. Gabapentin, a 2-subunit ligand of voltage-gated calcium channels, has specifically been used in the context of SCI due to its ability to bind to the α2δ subunit of VGCCs in order to block synaptogenic excitotoxicity. As such, subunit binding has broad relevance for curtailing maladaptive activity and combating excitotoxicity inherent to injury. Ion channel blockers which inhibit microglial activation such as minocycline and other similar antibiotics which inhibit the activity of either MMP-9 or COX-2 have been examined as preventative measures. These pharmacotherapeutic agents nevertheless offer potential approaches to target microglial consistently following SCI. In addition to the adverse effects predicted in pathogenesis these drugs have offered potential avenues to study the fate of microglia as well as assess the complexity of their functions after injury. In keeping with alternative strategies to prevent prolonged activation approaches to halt the ADAM10-TNFα pathway with blockers have yielded further insight targeting C33a, a peptide derived from APP, in CHO cells and in in vivo models of neurodegeneration and has been found to bias microglial toward an alternative, immunoregulatory phenotype in a CHIT1-dependent manner. To this end, through CHIT1 inhibition of the ADAM10-TNFα pathway resulted in an upregulatory switch from M1-like to M2-like expression and this biasing towards an anti-inflammatory phenotype was recapitulated in the context of injury studies examining tissue response to transplantation

of M2-biasing treated microglia achieved significant upstream benefits both in recovery of motor function and of the injury response at the cellular and molecular level. As such transplanted M2-biasing microglia produced less TNFα, IL1β, IL-6, CCL2, CXCL10, and IL-10 after injury and upregulated an inflamed homeostatic state more consistent to microglia in uninjured conditions. Taken together, microglia's active role in the innate immune response remains an emerging and active research priority with abundant and novel opportunities to enhance the therapeutic capacity of microglia so as to more effectively underpin neural repair. Beyond the dual regulation of neuroinflammatory processes functioning in a paracrine manner microglia also possess highly tightly-interconnected signaling pathways that can modulate synaptic connectivity in the adult CNS, indicating that these cells may actively participate in constructing cellular networks in the neural microenvironment. Therefore, in light of their integrated role in both immune and cellular networks in the CNS there remains considerable promise in further study into microglia as a therapeutic target in SCI and broader neural injury.

Therapeutic Approaches to Modulate Microglial Activation

The deregulation of microglia function after SCI exacerbates local inflammation; various therapeutic approaches have been proposed to restore controlled activation of this cell population, but few have reached clinical development 1. In this review, a spectrum of treatment options is described that aim to modulate microglial activation following SCI and the advantageous mechanisms involved.

Pharmacological approaches are the most widely researched strategies to tackle microglial involvement in SCI pathology. The 'gold standard' safety profile drugs of the analgesic-acetaminophen family are commonly taken to treat post-injury neuropathic pain; its mechanism of action to reduce production of TNF-α, IL-6, and IL-1β levels in spinal cord tissue has been associated to the restoration of microglial homeostasis. Other anti-inflammatory bioactive compounds such as curcumin, quercetin, and resveratrol are potent inhibitors of the NF-κB signaling pathway; these agents ameliorated microglial activation and improved motor function recovery in rats subjected to T8 tract transection.

A combination of therapeutic strategies has shown complementary effects in dampening the inflammatory response and supporting tissue health. For example, electrical stimulation of the optic nerve combined with intravitreal injections of minocycline effectively inhibited microglial activation and promoted neuronal and axonal survival after traumatic injury in rabbits. Other approaches include physical therapy combined with bioactive food supplements such as omega-3 and curcumin, as well as lifestyle changes like the consumption of a Mediterranean diet, which has shown neuroprotective effects against corticosterone-induced microglial inflammation 2. Preclinical models and clinical trials using such conjunction strategies remain scarce and warrant further investigations to evaluate their efficacy. Upon systemic administration, Ad-Mfn2 reduced microglial activation in SCI rats, decreased the concentrations

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of pro-inflammatory cytokines, and increased the levels of anti-inflammatory cytokines. A double-edged role during the inflammatory response observed in different studies tended to drive efforts toward neuroprotective applications of M2-transformed microglia and not clear punctually the machineries utilized by microglia which become type M1 and secrete virotic agents after SCI.

Potential Future Directions in Microglial Modulation

Despite significant progress in understanding microglial biology and the challenges involved in identifying developmentally sensitive and receptor-agonistic strategies to modulate microglial activation, improvement of neuropathology often necessitates the development of novel approaches to enhance the neuroprotective transitions of microglial responses to spinal cord injuries and related diseases. Although the harmful roles of microglial activation following a physical or chemical insult are intensively studied, as well as the underlying molecular mechanisms involved in such processes, it remains to be determined whether the activated, inflammatory microglia, bearing the M1 phenotype, could be reverted back to an anti-inflammatory and neuroprotective M2-like phenotype. Induced pluripotent stem cells, generated from patient fibroblasts, are considered a further step toward personalized medicine and have put forth new opportunities to model human neurological diseases and study their pathogenesis. Three independent studies originating from different laboratories reported the successful generation of pure, homogeneous populations of human pluripotent stem cell-derived microglia. Deriving human microglia from induced pluripotent stem cell lines can potentially overcome the limitations of studying human-related immunity and disease pathology in rodent microglial models. Further studies are warranted regarding the applicability of this technology in the spinal cord and its potential for developing patient-specific microglial models for familial forms of neurodegenerative disorders. There is ample evidence that innate immune mechanisms are activated upon traumatic or degenerative stress in the central nervous system. While the activation of scavenging and phagocytic functions of macrophages is considered to be beneficial, inflammatory activation of macrophages often proceeds to tissue destruction. A balance between M1 (pro-inflammatory) and M2 (anti-inflammatory) responses is thus crucial for tissue repair and neuronal survival. Following traumatic or degenerative insults, it is hypothesized that non-obese diabetic-type macrophages, characterized by M1 activation, would be recruited to contused spinal cord tissue, where they undertake scavenging or phagocytic roles. On the other hand, BALB/c-type macrophages, characterized by M2 activation, would accumulate within uninjured spinal cord tissue, promoting recovery and tissue preservation. Failure to attain the appropriate balance of M1:M2 would cumulatively exert neurotoxic function, facilitating secondary damage as well as traumatic disease development.

Research over the past decades has focused, correctly, on elucidating cellular and molecular mechanisms that contribute to harmful effects on the central nervous system. Indeed, microglial

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activation has multiple paths and diverse effects on central nervous system homeostasis; some of which could be protective and beneficial. Recent studies demonstrated that microglia might have exhibited neuroprotective responses following cortical or spinal cord injuries during a certain time window in the acute phase; thus, further analysis throughout multiple time points is required to elucidate whether such events could be reproduced within other central nervous system insults.

Conclusion and Future Perspectives

Microglia play a critical role in the inflammatory response after acute spinal injury, acting as the resident immune system of the central nervous system (CNS). Following spinal cord injury (SCI), it is now appreciated that microglia are rapidly and dramatically activated by the trauma. The observation of the complex phenotype of microglial activation after SCI implies that microglial receptivity and responses, per se, involved in a multitude of biochemical cascades and physiological processes are high and time-dependent. Therefore, it would not be fully or accurately understood unless the temporal processes of the cascade events of SCI were closely monitored. Currently, 48 hours and a few days after SCI are better understood of the cascade events, yet there remains a decade-long time gap for the complex biology of microglia fundamental to chronic inflammation of SCI to be elucidated. In addition, it would also be very relevant to the ongoing endeavor of exploration of time-dependent therapeutic modalities to ameliorate the detrimental effects of inflammation after SCI 1.

The present understanding of the dual nature of microglial attributes in SCI and other CNS injuries reveals complexity as beneficial means to catabolyze debris, as well as harmful factors to prolong secondary injury states to perpetuate an unregulated gain of deleterious metalloglobins. Until such intricate interplays and cross talk are better understood, attempts to develop more efficacious targeted therapies would remain to be a daunting challenge. Given that mounting evidence has demonstrated both benign and detrimental consequences on nucleus activation of microglia after SCI, the timing of the intervention seems to be paramount. While the beneficial attributes of early intervention may rest on the surprising exhaustion of microglial ability to combat chronic inflammation, harmful effects of late intervention may result in escalation of inflammation instead of amelioration in the periphery. Therefore, understanding microglial biology in SCI over a continuum of time remains a prerequisite for the successful transition of basic research breakthroughs into clinical applications and translational development in the prevention or mitigation of secondary damage after CNS trauma. In the rejuvenated effort to harness the neurological recovery of SCI based on the modulation of glial receptors in microglia and astrocytes, experimental endeavors ought to complement currently failed antagonizing strategies with promising innovative modalities utilizing endogenous responses to trauma.

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Competing interests

The authors declare no conflict of interest.

Ethics Statement

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Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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