Mesenchymal stem cell-macrophage choreography supporting spinal cord repair

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Abstract

Spinal cord injury results in destructive events that lead to tissue loss and functional impairments. A hallmark of spinal cord injury is the robust and persistent presence of inflammatory macrophages. Mesenchymal stem cells (MSCs) are known to benefit repair of the damaged spinal cord often associated with improved functional recovery. Transplanted MSCs immediately encounter the abundance of inflammatory macrophages in the injury site. It is known that MSCs interact closely and reciprocally with macrophages during tissue healing. Here, we will review the roles of (transplanted) MSCs and macrophages in spinal cord injury and repair. Molecular interactions between MSCs and macrophages and the deficiencies in our knowledge of underlying mechanisms will be reviewed. We will discuss possible ways to benefit from the MSC-macrophage choreography for developing repair strategies for the spinal cord.

Key words Stem cells, Bone marrow, Immune cells, Healing, Recovery, Paralysis, SCI.

Introduction

Traumatic spinal cord injury (SCI) typically results in immediate loss of nervous tissue followed by a phase of secondary damage. The initial trauma destroys neural cells and ruptures blood vessels in the injury epicenter, while in the surrounding tissue (penumbra) the spinal cord-blood barrier (ScBB) of blood vessels is often breeched and neural cells become necrotic. Secondary damage is perpetuated by the many cytotoxic factors associated with neural and epithelial cell death as well as by immune cells. The loss of nervous tissue ultimately leads to the formation of cystic cavities, which can be found at the injury epicenter and in adjacent segments.

A hallmark of SCI is a robust and persistent inflammatory response. The destructive events initiated by an injury to the spinal cord lead to a plethora of damage signals which cause a massive infiltration of immune cells that initiate the inflammatory response [1-3]. In most tissues, intrinsic mechanisms regulate the evolution of macrophages from an inflammatory to an anti-inflammatory, pro-reparative phenotype which supports recovery of homeostasis and tissue repair. However, in the damaged spinal cord, the majority of macrophages remain in their inflammatory phenotype resulting in persistent inflammation [4-8].

Mesenchymal stem cells (MSCs) have been investigated for treatment of SCI [9-11]. MSCs secrete growth factors and chemo- and cytokines which mediate paracrine actions that support anatomical repair and functional recovery [12-16]. Their potential to create a reparative environment is the main motivation for exploring MSCs for repair of many types of tissues [17-21]. In animal models of SCI, MSC transplantation demonstrated promise for promoting repair [9-11, 22]. The mechanisms by which transplanted MSCs execute their reparative actions in the damaged spinal cord remain incompletely understood.

In many types of tissues, including skin and muscle, MSCs home into an injury and start communicating with immune cells thereby supporting tissue repair and remodeling. MSCs exert

dual roles as ‘sensors’ and ‘modulators’ of the inflammatory response [23-26]. Macrophages, in turn, are known to mediate MSCs polarization to pro- or anti-inflammatory profiles [27-31]. These reciprocal actions between MSCs and macrophages have significant influence on the overall healing process. Homing of MSCs into the damaged spinal cord does not occur which prevents such repair-promoting MSC-macrophage interactions.

In the injured spinal cord a transplant of MSCs will encounter the abundantly present inflammatory macrophages. In this review, we focus on the role of transplanted MSCs, macrophages, and their reciprocal interactions in spinal cord injury and repair. We will give special attention to their roles in the formation of new blood vessels (i.e., angiogenesis) after SCI. Understanding the contributions of these two cell types and their interactions may be beneficial for the development of effective repair approaches for the spinal cord. We will also discuss potential approaches to benefit from their interactions for promoting repair and recovery after SCI.

Inflammation after SCI

An injury to the spinal cord causes immediate and secondary destructive events which together cause nervous tissue damage and, consequently, functional impairments. These destructive events, which include neural cell death, tissue destruction, and blood vessel rupture and leakage, result in an immune response which is characterized by enduring inflammation.

Different phases in inflammation after SCI

Damage to neural cells induces the expression of damage-associated molecular patterns (DAMPS), which are sets of small molecules and proteins released by cells present in the injury

site that are responsible for several injury-related aspects of SCI, including homing of inflammatory cells [32, 33]. Neutrophils start accumulating within 6 h after injury, lymphocytes within 6-12 h, and macrophages within 12-24 h [7, 34, 35]. After a peripheral nerve injury, dorsal root ganglion neurons secrete CCL2, an attractant chemokine that mediates macrophage migration and activation for repair [36]. After SCI, increased concentrations of macrophage chemoattractant protein (MCP-1) and other cytokines are present in the damaged tissue [37, 38]. Immune cells also infiltrate an injury site through ruptured blood vessels to encounter the DAMP-positive microenvironment and exacerbate the inflammatory milieu [3]. The initial inflammatory wave of the immune response is essential for debris clearance, phagocytosis of dying cells, and initiation of angiogenesis through the secretion of growth factors [1, 2, 6, 39].

In many types of tissue, the initial inflammatory phase is followed by a regulated induction of an anti-inflammatory, reparative, phase, which is governed by regulatory T lymphocytes (Tregs) and tissue remodeling/reparative macrophages [40]. For so far unknown reasons, wound healing and inflammation-mediated clearing of cellular debris in the damaged spinal cord differs dramatically from that in regenerative tissues, such as muscle and skin. After SCI, there is no efficient regulated induction of a reparative and anti-inflammatory phase, resulting in a chronic cytotoxic inflammatory state that contributes to secondary degeneration thereby limiting repair and functional recovery [4, 5, 41-43]. In the contused adult rat spinal cord, reparative macrophages are found early after injury, during the initial inflammatory phase, but disappear within a few days, while the inflammatory macrophages remain chronically present [5, 6]. In people with SCI, increased inflammatory markers were found in blood (44) and cerebrospinal fluid [45, 46]. Post-mortem analysis of human spinal cord tissue showed the persistent presence of inflammatory cells [4].

It has been shown that both systemic and local chronic inflammation are characterized by aggressive immune cell behavior in the injury site accompanied by a continuous production of

cytotoxic molecules, including reactive oxygen species (ROS), nitric oxide (NO), and apoptosis-inducing molecules, which are likely to contribute significantly to secondary nervous tissue degeneration. It is possible that suppression of inflammation in the chronic phase could allow remodeling cells to intervene and promote tissue repair more efficiently, after the beneficial acute inflammatory role has been executed [47].

Macrophage phenotypes

Macrophages are the most prevalent immune cell present in the spinal cord during the later phase of inflammation after injury. The macrophages originate initially from resident activated microglia, which tend to dissipate in the chronic phase, and from infiltrated monocytes, which remain present in the chronic phase [1, 39, 48]. Macrophages sense the cellular and molecular composition of the microenvironment and in response alter their gene expression profile to secrete the appropriate effector molecules and express the necessary receptors on their surface [49, 50]. This dynamic shift in the phenotype of macrophages is known as ‘polarization’ [51]. Two main categories of macrophage polarization are used to classify them into inflammatory, cytotoxic, M1-like macrophages or anti-inflammatory, reparative, M2-like macrophages. However, a wide spectrum of polarization is found *in vivo* in each of these two categories, which complicates repeating studies and understanding the actual mechanisms of macrophage polarization during wound healing [51-53].

Numerous groups have characterized the macrophage phenotype evolution in damaged spinal cord nervous tissue in different injury models [6, 7, 41, 42, 47]. M1-like macrophages are mainly characterized by the secretion of inflammatory cytokines, such as interleukins (IL) 1β, 6, 12, tumor necrosis factor alpha (TNFα), interferon gamma (IFNγ), and expression of the inducible nitric oxide synthase (iNOS), among other markers. M2-like macrophages are characterized by

the secretion of anti-inflammatory molecules, such as IL4, IL10, transforming growth factor beta (TGFβ), and multiple growth factors that induce tissue remodeling [50, 51, 54, 55]. Evidence in the literature demonstrate that interventions resulting in depletion of M1-like macrophages in other tissues than spinal cord caused impairment of the healing process and lack of functional recovery [56, 57]. Interestingly, approaches that induce the shift from M1- to M2-like macrophages earlier than the natural timeline increase the chances of excessive fibrosis and, ultimately, failed tissue remodeling [58, 59]. The absence of reparative (M2-like) macrophages was also shown to result in a lack of neural tissue repair [60]. Together, these studies demonstrate the relevance of the role of each macrophage phenotype for successful tissue repair.

Different macrophage phenotypes have specific and crucial roles in tissue repair and remodeling. It has been proposed that modulating macrophages to a pro-reparative phenotype is a promising strategy to promote repair after SCI [8, 26, 47, 61, 62]. Their specific roles in the inflammation process need to be considered when designing macrophage-modulating strategies. Even though M1-like macrophages might appear to be the ‘bad’ players in the immune response after injury being uncooperative of repair, they are essential for removing cellular/tissue debris before reparative events can successfully be executed. At the same time, M2-like macrophages appear to be the ‘good’ players in the immune response after injury supporting repair, but when promoting this phenotype in the injured spinal cord, these will need to be precisely regulated to avoid counter-productive effects. The ultimate goal would be to design strategies to modulate the immune response after injury that respects the natural timeline of macrophage phenotype evolution. Promoting the M2-like phenotypes after the window of necessary inflammation has passed is a promising strategy to enhance the natural immune-mediated tissue healing process that is lacking in the spinal cord [47].

Macrophages affect angiogenesis

Angiogenesis is an essential process for wound healing. Cells involved in tissue repair are in need of oxygen and nutrients to survive and successfully contribute to the complex tissue repair process. For this, the formation of new blood vessels from existing capillaries at and near the injury site needs to be synchronized with the dynamic cellular aspects of repair. Macrophage polarization is tightly connected with the regulation of angiogenesis after injury [56, 63-65]. M1-like macrophages secrete enzymes that modify the extracellular matrix (ECM), as well as vascular endothelial growth factor (VEGF) which promotes proliferation of the vascular endothelial cells. M2-like macrophages secrete platelet-derived growth factor (PDGF) and various other factors that promote the proliferation of smooth muscle cells and pericytes which are needed to stabilize newly formed blood vessels [35, 62, 63, 66].

The literature clearly shows that the effects of macrophages on angiogenesis after injury is essential and in need of a precise orchestration to facilitate proper repair. Thus, the sequential evolution of macrophage phenotypes needs to be considered cautiously when designing and exploring novel treatments for repair of the spinal cord. Clinically, treatments implemented in the acute/subacute phase of SCI will certainly need to consider the different roles of the macrophage phenotypes in angiogenesis. When treatments are given to people in the chronic phase of SCI, the focus should be on reducing (persisting) inflammation and promoting the activation of the reparative macrophage phenotypes.

MSCs for spinal cord repair

An injury to the spinal cord causes tissue loss and functional impairments [67]. Tissue loss is reversely correlated with functional recovery after SCI [10, 63, 68]. One of the approaches to elicit repair after SCI is the transplantation of stem cells which are known to secrete paracrine factors that influence repair and/or differentiate into neural cells to replace those that were lost [14, 21,

69, 70]. Using models of SCI, studies have shown that stem cell-based approaches elicit anatomical repair often accompanied by functional recovery [10, 71, 72]. An extensively explored type of stem cell for spinal cord repair is the MSC [10, 20, 73-75]. MSCs can be harvested from different types of (mesenchymal) tissues, including bone marrow, adipose tissue, and muscle. Obtaining the cells from these sources can be accomplished with relatively minor side-effects, which adds to their clinical relevance. It has been suggested that MSCs can transdifferentiate into the neural lineage, which would support their use for cell replacement approaches. However, some studies have not been able to conclusively confirm their ability to transdifferentiate. In most studies, the MSCs used are a heterogeneous population of cells, which deserves some caution because different subpopulations may have different repair potential [76].

Reparative properties of MSCs in the injured spinal cord

MSCs are known to secrete an abundance of (paracrine) factors that initiate and regulate specific actions which contribute to the overall anatomical repair and functional recovery seen after their transplantation into the injured spinal cord [14, 16, 18, 74, 77, 78]. Many of these MSC-mediated events result in neuroprotection which often is associated with functional recovery [10, 78, 79]. Evidence in the literature shows that an intraspinal MSC transplant leads to a decrease in apoptotic neural cell death [16, 80] and in overall neurotrophic support of the damaged tissue [74, 81]. Other studies demonstrated that a transplant of MSCs results in angiogenesis within the injured spinal cord segment and stabilization of the ScBB of breeched blood vessels in the penumbra [10, 15, 82]. Also, MSC transplants were found to result in reduced breakdown of ECM, which typically occurs after SCI and contributes significantly to tissue disorganization and destruction [83].

Besides the above-mentioned events, MSCs are known to affect the profile of the inflammatory response through modulation of the macrophage phenotype, which has important consequences for repair [25, 84-87]. MSCs have dual roles as 'sensors' and 'modulators' of the inflammatory response [23]. Inflammatory cues in an injury microenvironment are sensed by MSCs that in response produce cytokines that modulate macrophages to express their anti-inflammatory, pro-reparative, phenotype, which supports restoration of homeostasis and promote tissue repair [23, 24, 88] (Fig. 1).

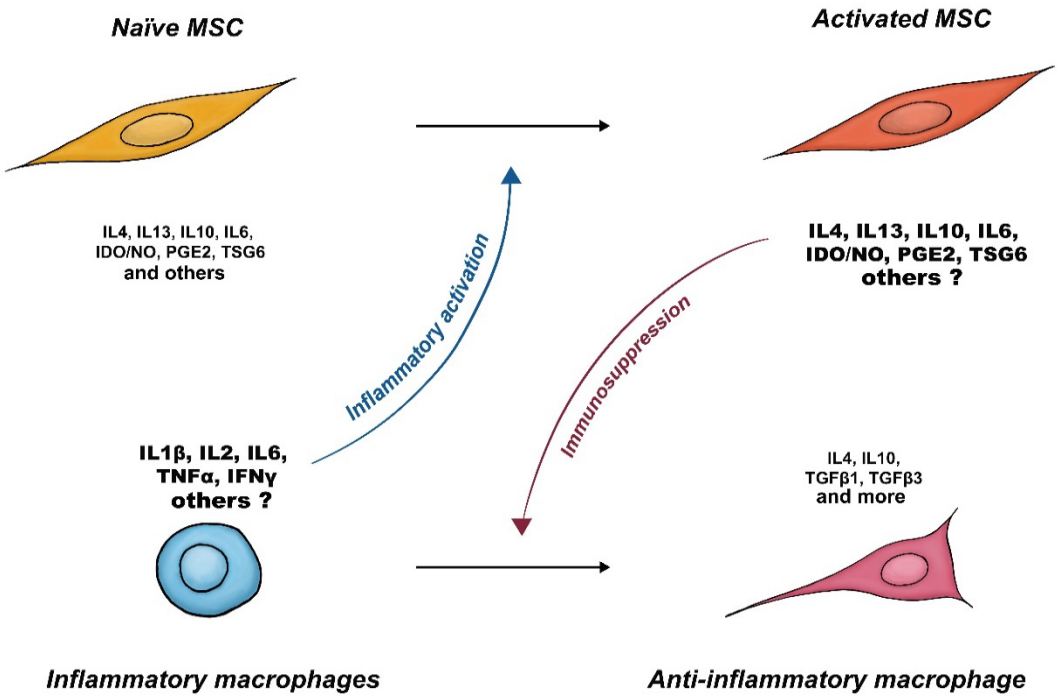

Fig. 1. MSCs and Macrophages reciprocally influence each other. Naïve MSCs secrete basal levels of anti-inflammatory cytokines [13, 83]. Inflammatory macrophages secrete numerous molecules that act as inflammatory stimuli modulating a shift from naïve MSC to 'activated' MSC ('inflammatory activation'). The activated MSCs have switched on their immunomodulatory program which results in the secretion of similar molecules as naïve MSCs but in much higher levels. These high levels of anti-inflammatory cytokines drive the switch from inflammatory to anti-inflammatory macrophages ('Immunosuppression'), which are important in tissue repair and remodeling. The specific stimuli and molecular hierarchy involved in these cells regulation is still incompletely characterized [11, 23, 27, 87, 90-94].

Exposure to IFNγ induces MSCs to enhance their secretion of indoleamine 2,3-dioxygenase which inhibits the proliferation of activated natural killer (NK) cells and T lymphocytes [89]. The innate ability of MSCs to modulate inflammation could be a powerful tool to limit secondary nervous tissue degeneration and thus providing a larger tissue platform for recovery strategies. For developing such approaches a better understanding of the mechanisms by which transplanted MSCs execute these immunomodulatory functions and communication with the macrophages is needed.

The literature so far provides ample evidence that the paracrine activities of MSCs transplanted in the injured spinal cord cause direct or indirect events that lead to neuroprotection. The direct events are through releasing anti-apoptotic and neurotrophic molecules that rescue neural cells from injury-induced death. The indirect actions are through the release of molecules that limit ECM breakdown, promote angiogenesis, stabilize breeched ScBB, and modulate the immune response. Together these events can exert neuroprotection and therefore limit the loss of neural tissue. Evidence has shown that limiting the (secondary) loss of nervous tissue after SCI is associated with functional recovery [10].

Reciprocal communication between MSCs and macrophages

It is well known that MSCs modulate the immune response, while macrophages influence the behavior of MSCs [27, 91, 95]. The directionality of the interactions between MSC and macrophages suggests a tightly-controlled reciprocal cooperation in regulating repair in regenerative tissues. In fact, extensive evidence in the literature showed that macrophages establish a bidirectional crosstalk with stem cells promoting healing in various tissue types [91, 92, 96].

MSCs modulate macrophage polarization

MSCs constitutively secrete immunosuppressive cytokines, such as TGFβ, IDO, NO, TNF-inducible gene-6 (TSG-6), prostaglandin-E2 (PGE2), and anti-inflammatory ILs that induce modifications in the metabolism of macrophages, resulting in a switch to the anti-inflammatory macrophage phenotypes [23, 24, 87, 92, 97] (Fig. 1). When cultured with MSC-conditioned medium, macrophages polarize showing increased expression of M2-like surface markers and reduced secretion of the inflammatory cytokines, IL1β, IL6 and TNFα [87]. Macrophages in culture with MSC spheroids, which secrete enhanced levels of PGE2, or with conditioned medium thereof, polarize from M1-like to M2-like macrophages [98, 99]. Systemic administration of umbilical cord-derived MSCs to an animal model of an inflammation-related disorder, showed an increase in anti-inflammatory macrophages and alleviation of the associated symptoms [94]. Clearly, the accumulated evidence in the literature so far reveals that MSCs support a shift among macrophages from the M1- to M2-like phenotype (Fig. 2).

Transplantation of MSCs into the contused spinal cord in adult rats was found to result in a decrease in TNFα, IL6 and IL1β and an increase in IL4 and IL13 accompanied by tissue sparing, axon preservation, decreased scar formation, and improved functional outcomes [11]. Treatment of the adult mice contused spinal cord with conditioned medium of embryonic MSCs resulted in restoration of the macrophage phenotype after the inflammatory phase and improved locomotor recovery, indicating that soluble factors are important in MSC immunomodulation [100]. In general, promoting the shift to M2-like macrophages, via MSCs, may support repair after SCI [26].

Macrophages influence MSCs

Inflammation and macrophage-derived cytokines influence the secretory profile of MSCs [12, 24, 101]. It was found that in vitro exposure to macrophage-conditioned media, as well as co-culture

with different phenotypes of macrophages, modifies the secretome of MSCs and their viability for cardiac tissue repair [27]. Crisostomo and colleagues used inflammatory stimuli, such as TNFα and lipopolysaccharides (LPS), to condition MSCs and found an increase in the secretion of growth factors with known activities supporting tissue repair [12]. Other types of stem cells, including oligodendrocyte precursor cells [60] and hair stem cells [102], can also be modulated by macrophage-secreted molecules and this is known to result in improved tissue regeneration. Considering the sequence of macrophage phenotypes during wound healing, it is clear that the immunomodulatory crosstalk between MSC and macrophages within the injury site is crucial for the overall repair process and recovery [91]. Further investigations into these immunomodulatory mechanisms could support the development of therapies for the spinal cord that harness the reparative and modulatory potential of MSCs and macrophages to create a reparative milieu in the injury site.

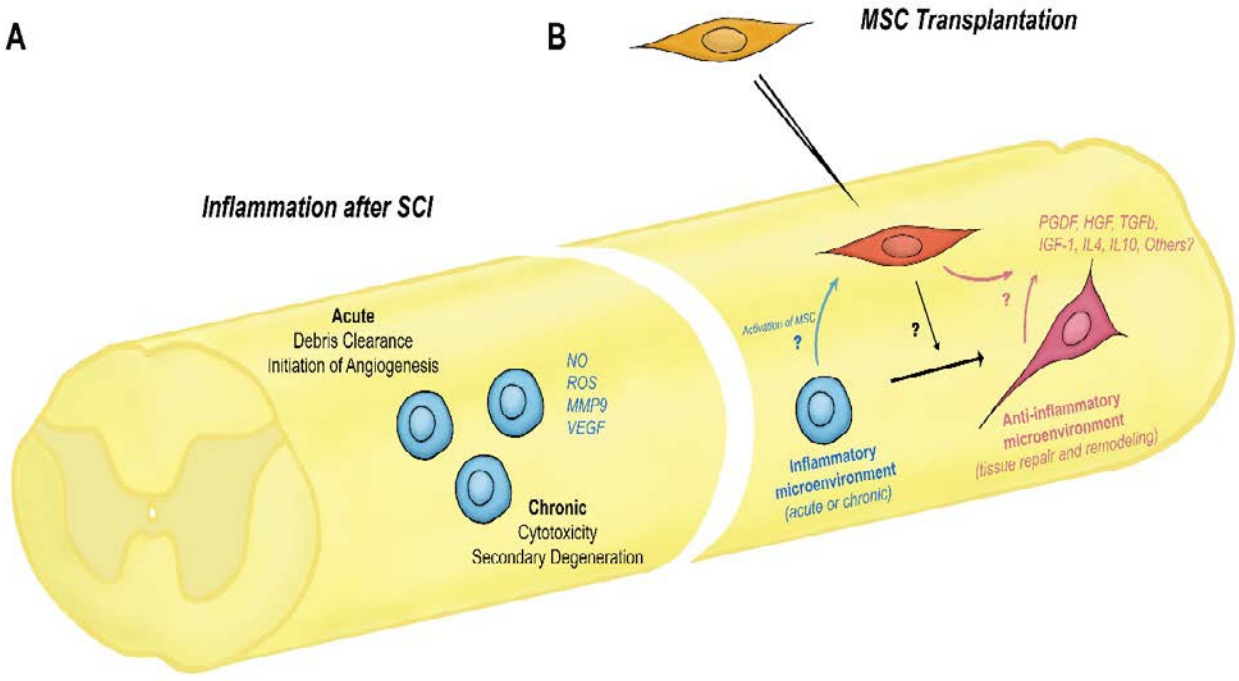

Fig. 2. MSC and Macrophages orchestrate spinal cord repair. A. Injury to the spinal cord is characterized by robust infiltration of immune cells. During the (sub-)acute phase of injury, inflammatory (M1-like)

macrophages help clearing debris by releasing NO, ROS and ECM-modifying enzymes, such as MMP9. M1-like macrophages also release proangiogenic factors that promote the formation of new blood vessels, such as VEGF. In the spinal cord, macrophage polarization fails to shift to the M2-like phenotypes, causing chronic inflammation and, consequently, a continuous exposure to cytotoxic molecules that impede recovery and induce secondary degeneration [1, 7, 103, 104]. **B.** Transplants of MSCs may promote the shift of macrophages to the reparative (M2-like) phenotype. Exposure of MSCs to the inflammatory microenvironment activates their immunomodulatory program; mechanisms underlying this activation are still incompletely understood. The transition to reparative macrophages and the paracrine effect of MSCs supports repair through released reparartive molecules, such as PDGF, HGF, IGF-1, or TGFb. The regulatory mechanisms need further study in order to benefit the development of targeted MSC therapies for spinal cord repair [6, 11, 23, 26, 27].

MSC immunomodulation requires activation

Effective immunomodulation by MSCs occurs upon exposure to activating stimuli. Under stress conditions, MSCs are programmed to increase their secretion of growth factors. For instance, MSCs in culture under hypoxic conditions produce enhanced levels of growth factors [12]. Cultured MSCs exposed to inflammatory molecules increase the expression of receptors known to respond to immune regulatory mediators and the production and secretion of anti-inflammatory factors [24, 105]. Despite the classical immunosuppressive potential of MSCs, it is now also widely accepted that MSCs exhibit a spectrum-type of phenotypic polarizations, similar as that seen among macrophages, depending on their microenvironment. MSCs express toll-like receptors (TLRs) which represent one of the gates for determining their immunomodulatory activities [30, 31, 105]. LPS-induced activation of TLR4 on MSCs results in activation of the inflammatory pathway and the secretion of pro-inflammatory mediators. On the other hand, activation of TLR3 on MSCs induces the production of anti-inflammatory mediators [31]. These data indicate that MSCs need activation to exert their immunomodulatory properties. At present, the mechanisms involved in MSC activation remain partially known and further investigations are needed to potentially involve them in the development of reparative strategies that involve conditioned MSCs.

In many types of regenerative tissues, an injury leads to increases in chemokines and cytokines which induces the progressive homing and exposure of MSCs to activating stimuli that elicit their regulatory activities [101]. The SCI microenvironment contains many damage signals and inflammatory mediators that are also found in circulating cerebrospinal fluid and blood (44, 45). Transplanting naïve MSCs in the injured spinal cord may therefore imply a stressful shock for the cells which could compromise their survival and, thus, their effects on repair. Priming MSCs in vitro using inflammatory stimuli prior to transplantation could kick-start their machinery for increased production of anti-inflammatory cytokines before being introduced to the injury microenvironment. Preconditioning of MSCs may enhance their survival and immunomodulatory potential potentially resulting in more efficient repair of the injured spinal cord.

MSC preconditioning

The preconditioning strategy has been explored in various models of disease or trauma. In a model of pyelonephritis, an infectious disease that derives from an excessive inflammation disorder, MSCs were injected intravenously after preconditioning in vitro with activated leukocytes. This approach resulted in primed MSCs secreting enhanced amounts of TGFβ, matrix metalloproteinase-2 (MMP2), and glycogen synthase kinase-3β (GSK3β), which all are inflammation suppressors, and improving the disease outcome [106]. Another study compared the immunomodulatory potential of MSCs from bone marrow of human donors by measuring their secretion of PGE2 and capacity to induce the shift in macrophage phenotype, after pre-activating them with various inflammatory stimuli. Preconditioned MSCs showed an increase in their secretion of PGE2 compared to controls and showed a stronger ability to induce M2-like macrophages in culture. Interestingly, not all inflammatory stimuli resulted in the same outcome; preconditioning with IFNγ resulted in MSC-mediated induction of more inflammatory macrophages [76].

An alternative approach to improve MSC immunomodulation may be using genetic modification of the cells; this approach has been designated as ‘intrinsic preconditioning’. Transplantation of MSCs overexpressing IL13 in a mouse model of SCI resulted in a significant improvement in anatomical repair and functional recovery compared to transplantation of unmodified MSCs. In addition to the anatomical and functional effects, transplanting the modified MSCs also resulted in an increase in the population of M2-like macrophages, demonstrating successful immunomodulation [107].

The abovementioned results contribute to the evidence that inflammatory priming can result in more efficient MSC-mediated immunomodulation. However, these studies also raise awareness about the need to unravel the mechanisms and pathways involved in the different situations. When designing the preconditioning and transplantation experiments, considering the inflammation window within the immunomodulation time-line is necessary to allow the crucial actions of all inflammatory cells.

Conclusions and remarks

Unraveling the mechanisms underlying the interactions between macrophages and MSCs in the context of wound healing may provide tools to modify spinal cord nervous tissue to improve repair. Using transplanted MSCs to target inflammation provides the opportunity of combining therapeutic approaches that so far have mostly been addressed separately. MSCs may modulate inflammation as well as secrete growth factors that elicit neuroprotection. It needs to be kept in mind that MSC preconditioning may be an integral aspect of these potentially powerful repair approaches. Future mechanistic studies are necessary to unravel the true potential of MSC preconditioning and MSC-mediated immunomodulation for spinal cord repair.

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