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R E V I E W

Anti-inflammatory effect of stem cells against spinal cord injury via regulating macrophage polarization

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Abstract: Spinal cord injury (SCI) is a traumatic event that involves not just an acute physical injury but also inflammation-driven secondary injury. Macrophages play a very important role in secondary injury. The effects of macrophages on tissue damage and repair after SCI are related to macrophage polarization. Stem cell transplantation has been studied as a promising treatment for SCI. Recently, increasing evidence shows that stem cells, including mesenchymal stem, neural stem/progenitor, and embryonic stem cells, have an anti-inflammatory capacity and promote functional recovery after SCI by inducing macrophages M1/M2 phenotype transformation. In this review, we will discuss the role of stem cells on macrophage polarization and its role in stem cell-based therapies for SCI.

Keywords: stem cells, macrophages, spinal cord injury, polarization

Introduction

According to the National Spinal Cord Injury Statistical Center, there are ~54 cases per million population or ~17,000 new cases of spinal cord injury (SCI) annually in the US. SCI usually results in the loss of neurons and axonal damage, leading to loss of functions.[1] The pathology of SCI can be divided into two distinct phases. First is the acute physical injury, which involves compression and/or contusion to the spinal cord, resulting in axonal damage and tissue destruction. This is followed hours to days later by secondary injury mechanisms.[2] These secondary injuries involve persistent inflammation, glial scar formation, demyelination of surrounding neurons, and substantial cellular death.[3,4] Among all aspects of secondary injury, the inflammatory response is the major cause and leads to widespread cell damage and expansion of the lesion.

Macrophages accumulate within the epicenter of the injured spinal cord and play a very important role in neuroinflammation, which is the most important pathological process and affects the local microenvironment.[5,6] With increasing evidence confirming that M1 and M1 macrophages have differential contributions to tissue damage and repair, reprogramming M1 macrophages to adopt the M2 or regulatory phenotype may be helpful for controlling and resolving inflammation after SCI.[7] Stem cell transplantation is a promising candidate to treat SCI.[8–12] Recent work showed that stem cells, such as mesenchymal stem cells (MSCs),[13–15] neural stem/progenitor cells (NS/PCs),[16,17] and embryonic stem cells (ESCs),[18–20] have immunoregulatory capacity and anti-inflammatory effects and could enhance functional improvement through inducing macrophages M1/M2 phenotype transformation post SCI.

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This review will discuss 1) the general feature of macrophages in response to SCI, 2) the phenotype and function of macrophages in SCI, and 3) the effects of stem cells on macrophage polarization and its role in stem cell-based therapies for SCI.

Macrophage response to SCI
Sources and distribution of macrophages after SCI

There are two types of macrophages, which participate in the inflammatory response after SCI, monocyte-derived macrophages and microglia-derived macrophages. After SCI, microglial cells are activated quickly within a few minutes and part of them differentiates into macrophages. Then, blood monocytes are recruited by chemokines and cytokines such as macrophage chemotactic protein 1 (MCP-1) to the injury area where they differentiate into macrophages 2–3 days postinjury and bone marrow (BM)-derived macrophages persist at the injury site indefinitely.[21–25] Macrophages are the major inflammatory effector cells, and the dual role of macrophages during SCI is demonstrated by two different type of macrophages, classical macrophages M1 and alternative macrophages M2.

Dual effect of macrophages after SCI

There are both positive and negative effects of macrophages on tissue repair and regeneration after SCI. On the one hand, some studies show that infiltrating macrophages have harmful effects. For example, the spinal cord mRNA levels of interleukin-1β (IL-1β) increase 12 hours after SCI;[26] 72 hours after injury treated with IL-1 receptor antagonist, the contusion-induced apoptosis was significantly reduced.[27] This result showed that IL-1β is harmful to tissue at the early stage. Macrophages are the main source of IL-1β. Popovich et al[28] found that macrophage infiltration was markedly reduced and the hindlimb function was significantly improved after the experiment animals were treated with liposome-encapsulated clodronate to deplete peripheral macrophages. Meanwhile, behavioral recovery was paralleled with more myelinated axon and decreased cavitation and enhanced sprouting. On the other hand, there are some results indicating that macrophages may have beneficial effects on tissue repair. Macrophages were transplanted into adult rat-injured spinal cords and then the regrowing axons were detected in the adjacent spinal cord sections.[29] The result indicates that activated macrophages at the injured site could provide a beneficial microenvironment, which is good for regeneration of sensory axons, possibly due to the release of transforming growth factor-β (TGF-β).[30] Mitrasinovic et al[31] showed

that macrophages/microglia, with increasing expression of the macrophage colony-stimulating factor receptor, have a protective effect on neurons subjected to excitotoxic and teratogen-induced injury, together with macrophage colony-stimulating factor.

Macrophage polarization and SCI
Macrophage phenotype

The opposite effects of macrophages on the pathological process of SCI may be due to different phenotypes of macrophages. There are two main subsets of macrophages, M1 (classical activation) and M2 (alternative activation).[32]

Classical activation involves the induction of M1 macrophages by toll-like receptor (TLR) ligands or proinflammatory cytokines, such as interferon-γ (IFN-γ). These cells produce high levels of oxidative metabolites (e.g., superoxide and nitric oxide), inducible nitric oxide synthase (iNOS), chemokine (C-C motif) ligand 12 (CCL12), and proinflammatory molecules such as tumor necrosis factor-α (TNF-α), IL-1β, interleukin-6 (IL-6), and interleukin-12 (IL-12) that are essential for host defense and tumor cell killing but that also cause damage to healthy cells/tissue.[33–37]

M2 macrophages are characterized by expression of Ym1, arginase-1 (Arg-1), CD206, CD209, and IL-1 receptor antagonist (IL-1Ra), found in inflammatory zone 1 (FIZZ1) and mannose receptor, and based on their high production of anti-inflammatory cytokines such as IL-12 and low production of proinflammatory cytokines such as IL-1β and IL-12. These cells also have high phagocytic activities.[38–44] The differentiation of M2 macrophages is promoted by different molecules such as IL-4, IL-6, IL-10, IL-13, vascular endothelial growth factor, and prostaglandin E2 (PGE2). M2 macrophages play a major role in resolving inflammation, degrading scar, and remodeling tissue by secreting trophic factors and releasing IL-10.[45] M2-polarized macrophages can be further divided into M2a (promoted by IL-4 or IL-13),[44,46] M2b (elicited by immune complexes in the presence of a TLR ligand),[47] and M2c (following stimulation by anti-inflammatory factors such as glucocorticoid hormones, IL-10, and TGF-β).[48,49]

Macrophage polarization after SCI

Genes associated with M1 and M2 macrophages detected by cDNA microarrays were rapidly induced in the injured spinal cord. M1 gene expression maintained for up to 1 month after SCI. However, the expression of M2 genes was transient and returned to preinjury levels by 7 days post-SCI. Meanwhile, the immunofluorescence staining result showed that CD86-positive M1 macrophages and Arg-1 (a typical marker of

M2 macrophages)-positive macrophages coexisted within injury site at 3 days after SCI, but only M1 macrophages had persisted until 28 days postinjury. The expression of Arg-1 returned to normal level 7 days after SCI.[50] Therefore, it is well known that the rapid response and maintenance of M1 and the transient existence of M2 at the injured spinal cord area lead to the secondary damage.[51] In rat, the adoptive polarization of macrophage to M2 is beneficial for facilitating the recovery after SCI.[52]

Following SCI, macrophages express cytokines such as TNF-α, IL-1β and IL-6 and chemokines including CCL8, CCL15, and CXCL9 to alter macrophage phenotype to M1.[53] At the same time, IFN-γ released by Type 1 T helper (Th1) cells, microglia, and astrocytes plays a very important role in macrophage polarization. This indicates that macrophages could be polarized in both autocrine and paracrine manners. Kigerl et al[50] found that M2 macrophage response was very short and returned to the level of normal spinal cord. When BM-derived M2 macrophages with fluorescent dye were injected into injury site, the number of M2 macrophages was reduced by 20–40%, while the number of M2 macrophages transplanted into the normal spinal cord almost did not change.[50] So the microenvironment after SCI is more likely to induce macrophages to M1 cells and keep M1 state of macrophages.

Macrophage polarization in CNS injury could be affected by many factors, such as reactive oxygen species (ROS) and chondroitin sulfate proteoglycan.[54,55] For example, macrophage polarization is influenced by the degree of injury. chondroitin sulfate proteoglycans (GSPGs), a major component of the glial scar, is considered to be a major obstacle for tissue recovery after injury. GSPGs directly activated macrophages via the CD44 receptor and promoted M2 macrophage polarization after SCI.[55] Myelin debris may be an associated factor switching the macrophage phenotype from M2 to M1.[56] In addition, ROS are involved in the activation process of M1 macrophages partly by nuclear factor-kappa light-chain-enhancer of activated B cells (NF-kB) pathway. It still needs further study to understand how ROS, resulting from SCI, regulate the polarization of macrophages.[57,58]

Phenotype-specific roles of macrophages in SCI repair and regeneration

Oligodendrogenesis and remyelination

Recent studies indicate that M2 macrophages are important for efficient remyelination after CNS injury. During the relapsing phase of experimental autoimmune encephalomyelitis, there is an M1-dominant phenotype, while a dominant M2 response at the lesion site is observed in the remyelination phase.[59] Meanwhile, oligodendrocyte differentiation is also affected by macrophages. Conditional medium from M2 macrophages enhances the differentiation of NS/PCs into oligodendrocytes in vitro, but oligodendrocyte differentiation was significantly blocked by M1 macrophages via a TNF-α-dependent mechanism.[59] Further, M1 macrophage-conditioned medium was found to aggravate oxygen glucose deprivation (OGD)-induced oligodendrocyte death, whereas M2 macrophages protected oligodendrocyte from OGD.[60,61]

Neurogenesis

Macrophages also exert opposite effects on neurogenesis after injury. Proinflammatory M1 macrophages hinder neurogenesis by secreting destructive factors including IL-1β, IFN-γ, and TNF-α and aggravate long-term neurological deficits after injury.[48,62] In contrast, M2 microglia/macrophages promote basal neurogenesis.[63,64] Therefore, M1 and M2 macrophages have distinct effects on neurogenesis.

Axonal regeneration

Kitayama et al[65] show that M1 microglia/macrophages that are activated by lipopolysaccharide could inhibit neurite outgrowth and induce growth cone collapse of cortical neurons. M1 macrophages could also induce axonal retraction in adult dorsal root ganglion neuron through physical cell–cell interactions. On the other hand, M2 macrophages are critical for axonal regeneration.[66] Cytokines such as IL-10 secreted by M2 macrophages could promote axonal regrowth and functional recovery after SCI.[67] Taken together, M1 macrophages can block axonal regeneration.[65,68]

Effects of stem cells on polarization after SCI

Thus, polarization of macrophages to M2 is beneficial to facilitate the recovery after SCI. Recently, stem cell transplantation has been demonstrated to have a tremendous therapeutic promise by several different mechanisms. Stem cells, such as MSCs, NS/PCs, and ESCs, are multipluripotent cells existing in both embryonic and adult tissues. They have the capacity for asymmetric cell division and enhanced proliferation. Increasing evidence shows that stem cells have immunoregulatory capacity and promote functional recovery through regulating macrophage polarization.[69]

MSCs and macrophage polarization

MSCs represent a heterogeneous population of multipotent cells and are capable of secreting growth factor and differentiating into mesodermal, endodermal, and even ectodermal cells under appropriate culture conditions.[70] MSCs do not form teratoma and are safe for tissue regeneration and repair. MSCs possess the

immunomodulatory capacity to induce regulatory T cells,[71,72] regulate the differentiation and function of dendritic cells,[73] and inhibit lymphocyte proliferation.[74,75] Recently, it has become clear that MSCs also regulate the function and activation of macrophages.[76–79] There is a large body of evidence demonstrating that the effects of MSCs on macrophages are critical for inflammatory response and tissue repair after SCI.[80–84]

Nakajima et al observed that BM-MSCs altered macrophages into anti-inflammatory M2 and beneficially modulated the immune system.[80] When human BM-derived macrophages were cocultured with human BM-MSCs, these macrophages showed high expression of a well-known marker of alternatively activated M2 macrophages (CD206) and expressed low levels of TNF-α and IL-12 and high levels of IL-6 and IL-10.[85] And macrophages cocultured with MSCs showed increased phagocytic activity. Mouse BM-MSCs also had similar effects on macrophages.[86] Cho et al[87] also found that the M1 markers including IL-1β, IL-6, and iNOS of macrophages were significantly reduced, but the M2 markers such as Arg-1, IL-10, and CD206 of macrophages were markedly upregulated by treating with mouse BM-MSCs.

MSCs modulate macrophages to adapt a regulatory phenotype by cell contact and production of immunomodulatory and growth factors. The production of PGE-2 secreted by MSCs plays an important role in induction of MSC-educated macrophages.[86] Acetylsalicylic acid, a cyclooxygenase inhibitor, can impair the effects of MSCs on macrophages. In addition, Cao et al[88] found that M2 macrophages were recruited by the transplanted MSCs in a stromal cell-derived factor 1 (SDF-1)-dependent manner. A previous study showed that MSCs skewed macrophages toward an M2 phenotype through inhibiting NF-κB and activating STAT3.[89] Recent research has shown, exosomes have been implicated in many aspects of immune regulation.[90] MSCs exosomes have regulatory abilities for macrophage polarization and induce an anti-inflammatory M2-like phenotype via shuttling let-7b.[91,92]

Besides BM, MSCs can be isolated from adipose tissues, lung, gingiva, placenta, liver, cord blood, and umbilical cord.[93–96] There are some similar experiments showing that MSCs from the other tissues also can polarize macrophages to the alternatively activated M2 phenotype. In parallel observation with human placental MSCs, Abumaree et al[97] also found that the human placental MSCs also have potent anti-inflammatory and immunosuppressive actions via converting macrophages from a proinflammatory M1 phenotype to an anti-inflammatory M2 phenotype. At the same time, human gingiva-derived MSCs also have the same effects on the phenotype of macrophages.

More studies indicate that grafted MSCs significantly improve functional recovery in animal models of SCI by providing neural protection and support and angiogenic stimulation.[98] Nakajima et al[80] reported the activation of macrophages in the inflammatory environment after SCI was regulated by the grafted human MSCs. Transplantation of human undifferentiated MSCs after SCI promoted functional recovery and modified inflammatory environment by shifting the macrophages phenotype from M1 to M2 and increasing the levels of IL-13 and IL-4 and reducing the levels of IL-6 and TNF-α. With the alteration of macrophages phenotype, less scar tissue formation, increased myelin sparing, and more preserved axons were observed in MSC-transplanted group. Furthermore, there were no transplanted MSCs in the injured spinal cord differentiating into neurons, astrocytes, or oligodendrocytes. Therefore, MSCs suppress inflammation and enhance tissue repair by polarizing macrophages to the alternatively activated M2 phenotype after SCI.

NS/PCs and macrophage polarization

NS/PCs are capable of self-renewal and generating the main phenotypes of CNS cells. Transplantation of NS/PCs is promising treatment for SCI.[99–103] NS/PCs usual has been studied as a mean to replace the damaged neurons in SCI. However, increasing data showed that NSCs can promote motor functional recovery by modulating the host environment.

Cusimano et al[104] found that the transplantation of mouse NS/PCs only at the subacute phase of SCI could enhance the recovery of locomotor functions of mice with SCI. These cells skewed the inflammatory cell infiltrated in the injured area by reducing the proportion of M1 macrophages and promoted the injured spinal cord regeneration and repair. However, Nishimura et al[105] found that the transplantation of NS/PCs only at the subacute phase of SCI promotes functional recovery. Our unpublished data demonstrated that NSC-conditioned medium can reduce the expression of iNOS within the spinal cord and spleen of injured animals, indicating an ability to reduce systemic inflammation. Meanwhile, multipotent adult progenitor cells also induce a shift in macrophages from an M1 state to an M2 state, prevent macrophage-mediated axonal dieback, and promote regrowth after SCI.[106]

Embryonic stem cells

ESCs are a kind of pluripotent stem cell that can be derived from the inner cells mass of the early embryo. ESCs can restore function following transplantation into paralyzed rats.[18,107] Bottai et

al[18] injected undifferentiated ESCs through the tail vein 2 hours post-SCI and the Basso Beattie Bresnahan scores of ESCs transplantation group were significantly increased compared with the control group. The data in the same report showed that the number of invading neutrophils and macrophages was greatly reduced, and ESC transplantation may improve functional recovery through this inflammation inhibition effect. The other article reports that the level of TNF-α in injured spinal cord significantly decreases, and the inflammation following SCI is inhibited by ESCs transplantation. ESCs can recruit macrophage by secreting molecules, such as MCP-1 and Matrix metallopeptidase-9.[108] In addition, ESCs promote macrophage survival and polarize macrophages toward an M2-like phenotype by releasing factors such as IL-34 and activating STAT3 and STAT6 signaling pathways.[109] Transplanted ESCs may form teratomas, which are constituted by cells from endodermal, mesodermal, and ectodermal lineages.[110–112] Recently, Guo et al[113] show that ESC-conditioned media can effectively reduce lipid accumulation, promote an M2-like state, and improve functional recovery after SCI. ESC-conditioned medium is sufficient to promote functional recovery in a murine model of SCI and avoids teratoma formation.

Conclusion

Accumulating evidence suggests that the bias of M2 macrophages over M1 macrophages is highly correlated with the remission of SCI that was clearly augmented in cases receiving SCI interventions, including anti-inflammatory therapies and stem cells. Stem cells can alter the macrophage polarization and promote functional recovery postinjury. But the mechanism by which stem cells regulate macrophage polarization remains to be determined.

Disclosure

The authors report no conflicts of interest in this work.

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