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How neural stem cells promote the repair of brain injury through immunoregulation

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Brain injury, such as traumatic brain injury and stroke, are important causes of death and long-term disability all over the world.[1] Inflammation may play an essential role in secondary injury after brain injury. Neural stem cells (NSCs) are pluripotent cells with self-renewal ability. It is initially assumed that NSCs transplantation promotes tissue restoration by direct structural cell replacement. However, the view has been recalibrated over the past decade. Bystander effect providing immunomodulatory, neurotrophic, and reparative support may play a major role instead.[2] However, the specific action mode of NSCs to exert immunomodulatory effect is still a lack of knowledge. On the one hand, the secretion of CD200, CX3 chemokine ligand 1 (CX3CL1), NSC-derived exosomes (NSC-Exos), and the regulation of nucleotide oligomerization domain (NOD)-like receptor protein 3 (NLRP3) can inhibit the inflammatory effects of microglia. On the other hand, changes in the systemic immune response also function in the immunomodulatory process. This article will focus on these aspects to discuss the potential immunoregulation mechanisms of NSCs.

CD200-CD200R Axis

CD200 is a member of the immunoglobulin superfamily expressed on most immune cells, including T and B cells, as well as on NSCs. The ligand-receptor interaction between CD200 and CD200R on microglia helps to maintain a relatively static state of microglia. In CD200-deficient mice, the long-term effects between neurons were reduced, microglia responded more strongly to Toll-like receptor 2 and 4 agonists, interferon (IFN)-gamma, and other inflammatory factors, suggesting that CD200 enhances synaptic plasticity and inhibits the inflammatory activity of microglia.[3] Conversely, activation of CD200R with CD200 fusion protein reduced the expression of microglial activation markers such as CD40.[4] The co-culture of NSCs and microglia leads to overexpression of CD200 in NSCs and CD200R in microglia, suggesting that there is a significant interaction between them, in which interleukin (IL)-4 may play an important role.[5] These phenomena suggest that NSCs may participate in the maintenance of the resting state of microglia through interaction with CD200R [Figure 1A].

CX3CL1-CX3C Chemokine Receptor 1 (CX3CR1) Axis

CX3CL1 is a cytokine containing 373 amino acids and is primarily expressed in NSCs and neurons in the central nervous system. It can be used as a membrane-binding protein or as a soluble ligand decomposed by a disintegrin and metalloproteinase 10 (ADAM10) and ADAM17 to bind to CX3CR1, which is expressed only by microglia in the brain.[6] When excitotoxicity or inflammatory injury occurs, the secretion of soluble CX3CL1 in the extracellular fluid increases. Soluble CX3CL1 acts on microglia to inhibit their activity under inflammatory conditions.[7] In the physiological state, after interruption of the CX3CL1-CX3CR1 axis, the secretion of interleukin (IL)-1-beta in microglia increased, leading to increased nuclear factor kappa-B-dependent transcription of pro-inflammatory cytokines including tumor necrosis factor (TNF)-alpha, IL-6 and, IFN-gamma.[8] In the pathological state, when brain tissue is damaged, CX3CL1 levels decrease, leading to microglial activation.[7] In a word, NSCs may secrete CX3CL1 that interacts with CX3CR1 on microglia to inhibit the excessive production of inducible nitric oxide synthase, IL-1, TNF-alpha, and IL-6, thereby promoting the repair of damaged brain tissue [Figure 1B].

NSCs Reduce the Expression of NLRP3 in Microglia

NLRP3 is an inflammasome that regulates the activation of caspase-1 and promotes the maturation and secretion of pro-IL-1-beta and pro-IL-18 in innate immune defence. It also induces caspase-1-dependent programmed cell death associated with inflammation, which is called pyroptosis.[9] The selective blockade of the NLRP3 inflammation pathway by MCC950 (NLRP3-specific inhibitor) alleviates the

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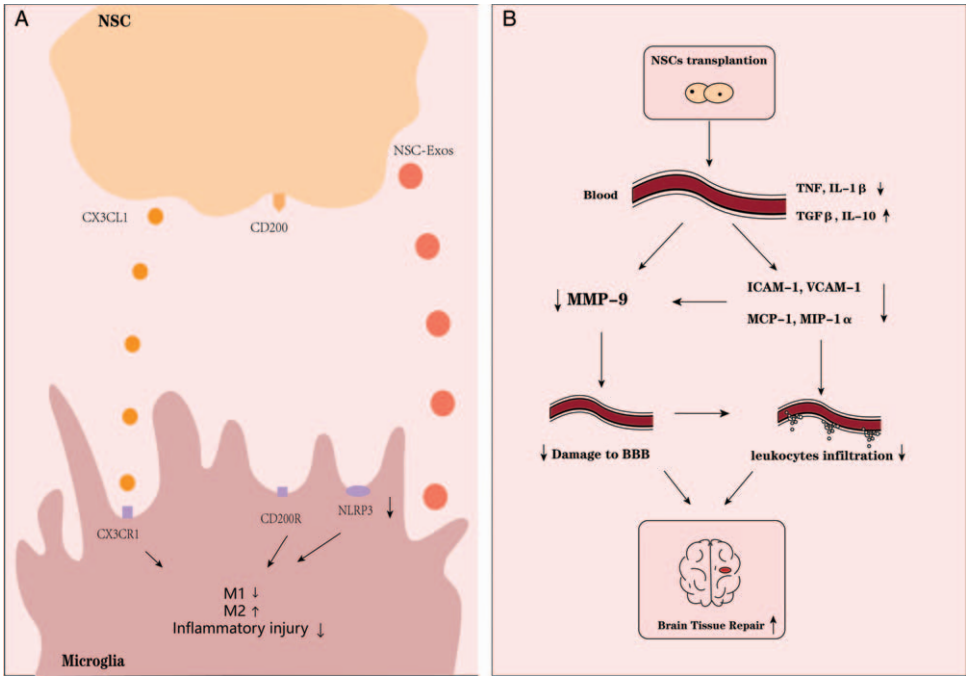

Figure 1: NSCs inhibit the inflammatory effects of microglia (A) and regulate systemic immune responses (B). BBB: Blood-brain barrier; CX3CR1: CX3C chemokine receptor 1; ICAM: Intercellular adhesion molecule; IL: Interleukin; MCP: Monocyte chemoattractant protein; MIP: Macrophage inflammatory protein; MMP: Matrix metalloproteinase-9; NLRP3: Nucleotide oligomerization domain-like receptor protein 3; NSC: Neural stem cell; TGF: Tumor growth factor; TNF: Tumor necrosis factor; VCAM: Vascular cell adhesion molecule.

behavioral deficits and inflammatory injuries in the injured sites.[10] Therefore, NLRP3 plays a negative role in tissue repair. NSC transplantation can attenuate the expression of NLRP3 inflammasome, reduce levels of caspase-1 and IL-1β in BV2 microglia stimulated by phosphate polysaccharide, thereby preventing microglia from releasing neurotoxic inflammatory factors and exerting neuroprotective effects.[11] Besides, NSC transplants could confer neuroprotection to alleviate tissue loss via inhibiting microglial pyroptosis and promoting phagocytosis by surviving activated microglia, which may be related to the decreased NLRP3 activation.[12]

NSC-Exos Mediate the Signals from NSCs to Microglia

Exosomes are membrane-bound nanovesicles that originate from the endocytic pathway and recently have emerged as a new avenue for cell-to-cell communication. They usually consist of proteins, lipids, microRNA, and messenger RNA. Exosomes generated by stem cells are involved in tissue repair theoretically. The effects of NSC-Exos have been shown to promote neuroprotection in the models of stroke. Signals from NSC-Exos to microglia may be associated with the beneficial effects. NSC-Exos can significantly reduce neuronal apoptosis, microglial activation, and neuroinflammation in rats. MiR-26b-5p exosomes enriched in NSC-Exos can relieve nerve injury after cerebral ischemia/reperfusion in mice by suppressing microglial activation.[13]

Regulation of Microglia Phenotypic Polarization by NSCs

Present studies divide microglia into a classical pro-inflammatory state (M1) and an alternative anti-inflammatory state (M2). Numerous studies have confirmed that the co-culture of microglia and NSCs *in vitro* reduces the number of M1 microglia and increases the number of M2 microglia. In closed craniocerebral injury model in mice, induced NSC transplantation decreased the levels of ED1 and TNF-alpha-positive microglia in the injured cortex and increased levels of insulin growth factor-1-positive microglia.[14] The mechanism of NSC regulation of microglial polarization is unclear. Inflammatory inhibitors referred above and other signaling pathways such as the CXCL12-CXCR4 axis may play a role in this effect.[14]

Regulation to Systemic Immune Responses

NSCs not only regulate microglia but also regulate systemic immune responses. Systemic application of stem cells through the intravenous injection can help reduce the expression levels of pro-inflammatory factors in ischemic cerebral hemisphere and blood, and increase the expression of anti-inflammatory factors such as tumor growth factor (TGF) beta and IL-10 in blood. Furthermore, a large number of stem cells were found in the spleen, and the contraction of spleen was blocked, suggesting suppression of the peripheral immune response after intravenous transplantation.[15]

Inflammatory reactions caused by brain injury lead to the accumulation of leukocytes in the damaged area, and the inflammatory effect of leukocytes further aggravates brain injury. Intracerebral infiltration of peripheral blood leukocytes requires interactions between vascular endothelium and leukocytes, mediated by cell-surface proteins such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1. Besides, monocyte

chemoattractant protein-1 and macrophage inflammatory protein-1α secreted by inflammatory cells recruit monocytes and neutrophils after inflammatory stimulation. Decreased expression of monocyte chemotactic protein, reduced level of adhesion molecules, and amelioration of blood-brain barrier damage was observed after stem cell transplantation, which contributes to lower infiltration of white blood cells and better repair of injured brain tissue.[16]

Matrix metalloproteinase-9 (MMP-9) is a member of the metzincin family and has recently emerged as a significant and unique player in brain physiology and pathology. Various complications, including excitotoxicity, neuronal damage, apoptosis, oxidative stress, interference with oxidative DNA repair mechanisms, and most importantly blood-brain barrier opening, are associated with MMP-9. The overexpression of MMP-9 increases the permeability of the blood-brain barrier by degrading tight junction proteins.[17] Transplanted stem cells can reduce both microvascular inflammation and damage to the blood-brain barrier (BBB) by inhibiting the expression of MPP-9. The effect is may be mediated by adenosine monophosphate-activated protein kinase-dependent ICAM-1 downregulation potentially, thus alleviated neutrophil infiltration and inflammatory cytokines release.[16]

Conclusions

Different types of stem cells such as NSCs and mesenchymal stem cells (MSCs) have been researched in the treatment of patients with brain injury. For both SC types, the bystander mechanisms play an important role in the therapeutic effect rather than simple cell substitution. However, there are some differences between NSCs and MSCs. NSCs exert their immunoregulatory effects mainly through the regulation of microglia around the lesion site, while MSCs are prone to control the immune response in the periphery. Jointly, NSC-based therapy is an attractive approach to promote the repair of brain injury. Although further efforts should be made to understand the biological roles of NSCs in immunoregulation, the basic concept about the function of NSCs is becoming clear. With the development of research on the mechanism of NSC-based therapy, the curative effect of NSCs can be better exploited, and this is expected to bring a new dawn for the repair of brain injury.

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Conflicts of interest

None.

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