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REVIEW

Is Immunomodulation a Principal Mechanism Underlying How Cell-Based Therapies Enhance Stroke Recovery?

Nikunj Satani[1] · **Sean I. Savitz**[1]

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Abstract Inflammation within the brain and in peripheral tissues contributes to brain injury following ischemic stroke. Therapeutic modulation of the inflammatory response has been actively pursued as a novel stroke treatment approach for decades, without success. In recent years, extensive studies support the high potential for cell-based therapies to become a new treatment modality for stroke and other neurological disorders. In this review, we explore different types of cellular therapies and discuss how they modulate central and peripheral inflammatory processes after stroke. Apart from identifying potential targets for cell therapy, we also discuss paracrine and immunomodulatory mechanisms of cell therapy.

Keywords Stroke · Inflammation · Immune response · Cell-based therapy · Microglia · Spleen

Introduction

Although there have been significant advances in acute stroke care, the majority of patients with stroke have long-term disability. Cell-based therapies represent a new modality that offers high potential to enhance stroke recovery [1–3]. Many different cell types derived from a variety of tissues, including brain, bone marrow, umbilical cord, and adipose tissue, have advanced from the bench to clinical trials [4]. In this review, we discuss the different types of cell therapies that have been studied in animal stroke models and taken forward to clinical studies. Modulation of the immune responses after stroke both within the brain and peripheral tissues is likely an important mechanism underlying how many types of cellular therapies enhance stroke recovery. We review some of the pivotal studies that support an immunomodulatory effect of cell-based therapies in animal stroke models.

✉ Nikunj Satani
nikunj.b.satani@uth.tmc.edu

[1] Stroke Program, McGovern Medical School, UTHealth, Houston, TX, USA

Central and Peripheral Mechanisms of Inflammation

We begin by discussing those aspects of the inflammatory response after stroke that are affected by cell-based therapies. The brisk inflammatory response that begins immediately after stroke can broadly be classified as central or peripheral, depending on whether immune responses originate within the brain or peripheral tissues (Fig. 1). In the brain, resident microglia become activated and migrate to injured areas. Depending on the phenotype they assume, microglia either release proinflammatory cytokines (M1 phenotype polarization) or they can release neurotrophic factors preventing neuronal death and aiding in brain repair (M2 phenotype polarization) [5]. Loane and colleagues [6, 7] showed that microglia in brain can even be chronically activated following brain injury. In parallel to events within the brain, lymphocytes, neutrophils, and monocytes traffic to the brain from the periphery and contribute to further neuronal damage. The spleen is a principal reservoir that reduces in size after stroke, releasing inflammatory cells and cytokines into the circulation [8–15]. In addition, the bone marrow mobilizes a subpopulation of multipotent stem cells into the peripheral blood following stroke, which is then directed towards the brain, as well as peripheral organs such as the spleen [16].

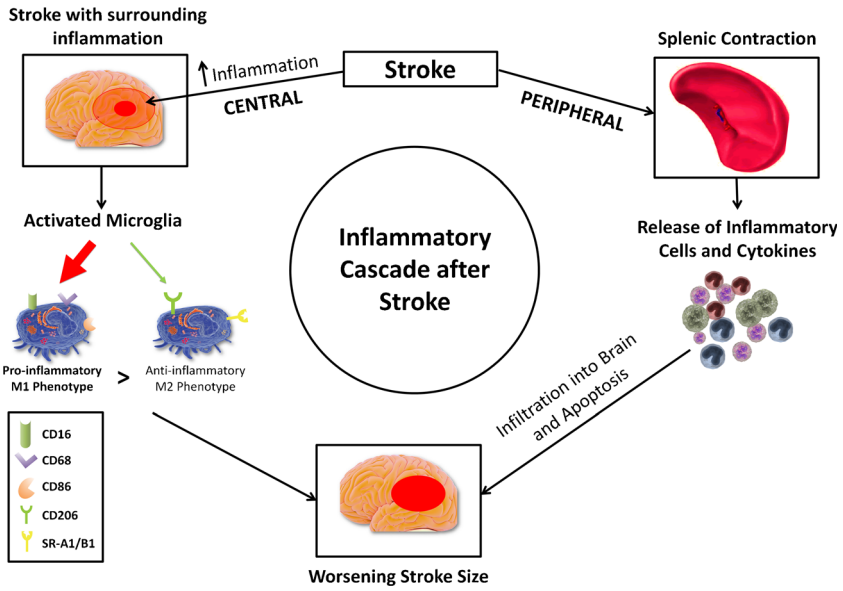

Fig. 1 *Inflammatory cascade following stroke showing central and peripheral mechanisms.* In brain, resident microglia are activated predominantly towards the proinflammatory M1 phenotype. Spleen contracts, releasing inflammatory cells and cytokines. Collectively, they contribute in worsening stroke size

A Brief Review of Cellular Therapies Under Active Investigation

Different types of cell-based therapies have been studied for years and even decades in animal models of stroke. Included in the following subsections are specific examples of cell therapies that have been extensively studied in animal models and taken forward to clinical trials.

Bone Marrow

As early as the 1980s, autologous bone marrow injection was used to stimulate healing in tibial fractures, suggesting the reparative effects of marrow cells in large bone defects [17, 18]. Over the last 2 decades, marrow cells have been extensively studied for a range of medical disorders beyond their established uses for transplantation in oncology.

Bone Marrow Stromal Cells

Marrow stromal cells (MSCs) represent a very small population of the bone marrow which adhere to tissue culture plates making them relatively easy to isolate but need to be grown and passaged in cell culture. MSCs have been shown to exert profound immunomodulatory properties and have gained approval as a treatment in certain countries for graft *versus* host disease. Several meta-analyses have consistently demonstrated their treatment effects in various animal models of stroke [19–24]. In fact, MSCs are likely the most widely studied type of cell therapy in the preclinical stroke literature [1–3].

Multipotent Adult Progenitor Cells

The bone marrow also contains another subpopulation of primitive progenitor adherent cells called multipotent adult progenitor cells (MAPCs). They have gene expression and population doubling times distinct from MSCs and have also been found to exert immunomodulatory effects and improve outcome in stroke animal models [20].

Bone Marrow Mononuclear Cells

The mononuclear fraction within the bone marrow contains MSCs and other stem cells but principally contain many types of mature cells of various lineages. They do not require growth in cell culture and can be easily isolated from bone marrow within hours thereby making autologous testing much easier than more purified cell types [25]. Our previous studies showed that rats treated with autologous mononuclear cells (MNCs) showed significant reductions in lesion size and neurological deficits up to 28 days after stroke [25]. A recent meta-analysis shows the pooled effect size of MNC treatment in animal stroke models [26].

Human Umbilical Cord Blood Stem Cells

Umbilical cord blood contains a large number of immature progenitor cells and have been studied extensively in stroke animal models because of their limitless supply and simple collection procedure [27]. Various types of cell populations have been isolated from umbilical cord for applications in stroke. Numerous studies have shown neuroprotective effects of human umbilical cord blood stem cells comparable with that of bone marrow stromal cells [1, 28–33].

Adipose Tissue-Derived Stromal Cells

Human adipose tissue is also known to contain pluripotent stromal cells and serves as a well explored alternative to bone marrow and umbilical cells [34–39].

Neural Stem Cells

Neural stem cells (NSCs) are multipotent and self-renewing cells that can differentiate into a wide array of specialized cells in the nervous system [40, 41].

Commonality of Mechanisms: Paracrine Hypothesis

Over a decade of research indicates that many types of cell-based therapies release biological factors that target key aspects of brain injury and repair, including: 1) stimulation of endogenous neurogenesis and angiogenesis; 2) white matter preservation and reorganization; and 3) reduction in cell death of surviving yet vulnerable tissue around the infarct (Fig. 2). There have been many recent studies that demonstrate the role of these trophic factors in recovery after stroke [42–44]. Indeed, the secretomes of cellular therapies have become an active area of research in the hopes of identifying the factors produced by cell therapies that are critical to their effects. Many cell therapies also release microvesicles such as exosomes, which themselves may penetrate and re-engineer the biological properties of target tissues [45–47].

Commonality of Mechanisms: Immunomodulation

There is also growing evidence and even convergence that the immune response is another important target of how some types of cellular therapies may exert their treatment effects. Some of the first speculations to suggest that exogenous cells change the immune response after acute neurological injury arose when it was found that intravenous delivery of human umbilical cord blood cells reduces the expression of proinflammatory cytokines, as well as CD45/CD11b^{-} and CD45/B220^{+} cells in the brain [48]. It was subsequently found that the intravenous delivery of many types of cultured stem cells such as MSCs and MAPCs lead to their trapping in peripheral organs—most notably in the lungs and to a lesser extent in the spleen [49, 50]. This entrapment of exogenous cells may be important to their immunomodulatory effects [51]. For example, MSCs lodging in the lungs may reprogram alveolar macrophages to release anti-inflammatory cytokines through cell-to-cell contact (Fig. 2) [52]. Increasing evidence suggests that MSCs may modulate the immune response after stroke. For example, Yoo et al. [53] found that transforming growth factor (TGF)-β secreted by MSCs play a key role in suppressing the immune responses in a rat stroke model [53]. Similarly, as described below, cells entrapped in the spleen could modify splenocytes to exert anti-inflammatory properties by producing interleukin (IL)-4 and IL-10 (Fig. 2) [51, 52]. The systemic administration of autologous bone marrow mononuclear cells has also been found to reduce the levels of proinflammatory molecules, while increasing anti-inflammatory cytokines within the brain and blood [54, 55]. Depending on the delivery route and temporal course of ischemic injury, some types of cell therapies may either

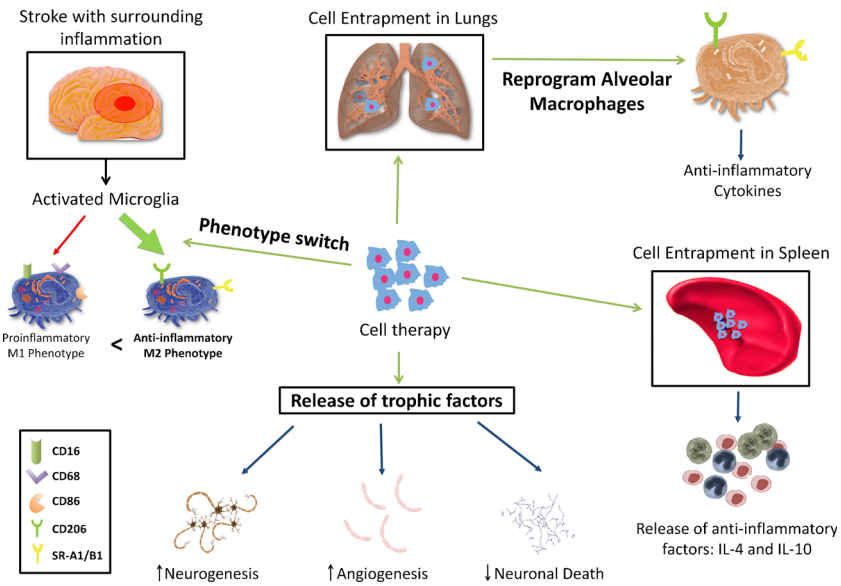

Fig. 2 *Cell therapy and its multitarget immunomodulatory function.* In brain, cell therapy causes a phenotype shift of resident microglia towards an anti-inflammatory M2 phenotype. Entrapment in lungs reprograms alveolar macrophages, while in spleen they aid in release of anti-inflammatory factors, such as interleukin (IL)-4 and IL-10. Release of various trophic factors ensures increased neurogenesis and angiogenesis while decreasing apoptosis of neurons. Collectively, cell therapy reduces post-stroke inflammation and limits stroke expansion

modify immune cell trafficking to the brain, immune cell activation within the brain, or both [28].

It is, however, unknown whether the proregenerative environment created by cell therapy is the direct result of transplanted cells themselves, because of their immune cell activation or some other paracrine mechanisms. A very interesting hypothesis termed "The Dying Stem Cell Hypothesis" provides a different perspective on how cell therapy could exert immunomodulatory effects [56]. According to this hypothesis, at least a part of transplanted cells die either in circulation or after entrapment in the lungs or spleen. Phagocytosis of these apoptotic cells could act as a trigger for release of various anti-inflammatory cytokines [57–59]. It is quite possible that these different mechanisms synergize to create a favorable immunomodulation, which is why further research needs to be focused on finding the exact mechanisms behind how cell therapy enhances recovery through their effects on immune responses.

Targeting the Spleen as a Therapeutic Strategy

In addition to lungs, the spleen has become an important]focus to understand how entrapment of certain types of cell therapies modulates the immune response after stroke. Vendrame et al. [31] reported more than 10 years ago that the intravenous administration of umbilical cord cells leads to their migration to the spleen where they restore splenic mass and T cells back to the levels comparable in rats without stroke. These cells also downregulated proinflammatory gene expression and upregulated anti-inflammatory gene expression. Schwarting et al. [60] then found that systemically administered hematopoietic stem cells reduced the expression of proinflammatory cytokines and chemokine receptor gene transcripts. MAPCs also restore spleen mass and stimulate IL-4 and IL-10 production from the spleen in rodents with traumatic brain injury (TBI) [61]; our group has found similar results in a focal stroke model [62]. Even the intravenous delivery of neural stem cells interrupts splenic inflammatory responses in a model of intracerebral hemorrhage [40]. A study by Lee et al. [40] showed that intravenously administered NSCs reduced brain inflammatory infiltration and apoptosis. NSCs caused reduction in levels of both cerebral and splenic inflammatory mediators, most notably tumor necrosis factor-α. This study proves that even NSCs act by modulating immune responses in the brain, as well as in the spleen.

Collectively, these studies indicate that intravenous delivery of many types of cell therapies change the splenic response after stroke thereby altering inflammatory cell trafficking to the brain and the overall peripheral immune response [31, 60]. As a result, secondary injury within the brain is significantly attenuated, which, ultimately, may facilitate brain repair. How interfering with the splenic response can lead to recovery after stroke is poorly understood but may be associated with an upregulation of regulatory T cells, changes in microglia phenotypes, and repair of the blood–brain barrier (BBB) [63].

There are many studies establishing a role for the spleen in the inflammatory processes following ischemia–perfusion injury of various organs or trauma, with some even showing the importance of splenectomy in reducing the proinflammatory response [13, 64, 65]. As splenectomy is not feasible in patients with stroke, a novel method such as controlling poststroke inflammatory response by enhancing the $CD25^{bright}$ subpopulation of regulatory T cells could prove useful [66]. However, cell therapy may still have the best proregenerative potential because of its relatively broader immunomodulatory effect.

Targeting Microglia as a Therapeutic Strategy

In the brain, polarization of microglia towards M1 or M2 phenotypes appears to be a key step driving the inflammatory response and resulting injury in the brain after stroke. This activation occurs even before the inflammatory cells begin to infiltrate the brain parenchyma [67]. If we could use the mediators that drive this polarization as therapeutic targets, we could aim to convert activated microglia towards M2 phenotypes known to be neuroprotective. Utilizing this knowledge of microglial phenotypes, a therapeutic strategy could be devised whereby we can 1) drive the polarization of microglia towards an M2 phenotype after stroke; 2) inhibit M1 microglia from releasing proinflammatory factors; or 3) pharmacologically inhibit factors released by M1 microglia or augment those released by M2 microglia. There have been a number of studies that demonstrate the beneficial neuroprotective and neuroreparative effects of M2 switching [68–71]. These studies have focused on the importance of various M2 markers such as IL-4 [72, 73], IL-10 [72, 74, 75], IL-1RA [76], TGF-β [77, 78], CD206 [72, 79], arginase 1 [79, 80], granulocyte macrophage colony-stimulating factor [72], insulin-like growth factor 1 [81, 82], and peroxisome proliferator-activated receptor-γ [70, 71, 83]. Even though our understanding of the role of these individual factors have become more insightful, it is very difficult to achieve beneficial neuroprotective effects by modulating just one of these factors. It is more likely that a complex interaction between these factors is responsible for deciding the microglial phenotype.

One major advantage that cell therapy provides is that they do not have one target. They may modulate many aspects of the microglial activation process from M1 to M2 (Fig. 2). For example, Ohtaki et al. [84] found that intracerebral injection of MSCs leads to activation of M2 neuroprotective microglia in a model of global cerebral ischemia. Zanier et al. [85] then

found in a TBI model that direct injection of MSCs also induces M2 proregenerative traits made evident by downregulation of nitric oxide synthase and upregulation of Ym1, arginase-1, and CD206 mRNA [85]. Another similar study conducted by Hegyi et al. [86] found that MSCs caused polarization of microglia towards a phenotype overexpressing arginase-1, CD86, CD206, IL-10, and prostaglandin E2, and underexpressing tumor necrosis factor-α. This unique polarization could attenuate inflammation and enhance brain repair [86]. As some stem cells have the potential to drive microglia towards M1 and M2 phenotypes, depending on the trophic factors released by them, the approach of using stem cells for M1 to M2 polarization can be made even more specific by supplementing them with drugs, for example ones that involve peroxisome proliferator-activated receptor-γ activation [87–92]. Still yet another approach for some types of cell therapies is selectively pruning the proinflammatory microglia populations; for example, bone marrow MNCs have been found to promote apoptosis of M1 microglia in a TBI model [93]. Future studies are needed to decipher the mechanisms how cell therapies directly or indirectly (e.g., through the spleen) change the microglial population in the brain.

An important variable supported by many studies is the optimal timing of cell therapy to yield maximum therapeutic effect. Evidence shows that exogenous cells administered shortly after stroke are exposed to lesser hostile environments and are able to exert better neuroprotective effects as compared with longer time windows after stroke [94–96]. NSCs transplanted at 48 h showed better survival than those transplanted at 1 to 2 weeks, because of an increased exposure to well-established inflammatory milieu in latter group [95]. Pösel et al. [94] showed significant functional improvement in rat stroke models treated with monotherapy of granulocyte colony-stimulating factor (G-CSF), as well as combination therapy of G-CSF and bone marrow MNCs at 6 h poststroke. When bone marrow MNCs were given at 48 h, the beneficial effect of G-CSF therapy was completely abolished [94]. These studies show that timing of cell therapy can play a vital role in functional outcome.

Another viable approach to increase the potency of implanted stem cells is by creating stem cells overexpressing secretory molecules, which could modulate microglial/macrophage functions. Along the same lines, galectin-1 overexpressing neural stem cells when transplanted in stroke, were shown to reduce infarct volume, improve sensorimotor and cognitive functions, and ameliorated white matter injury. In addition, they modulated microglial function by reducing the secretion of proinflammatory cytokines in response to LPS stimulation and by enhancing the secretion of anti-inflammatory cytokines like IL-10 and TGF-β. This study indicates that galectin-1 aided in the release of molecules, which shifted microglia towards an M2 phenotype [97]. Future studies are important to identify methods to increase the potency of cell therapies to target microglia.

Preservation of BBB Integrity

BBB integrity plays a critical role in maintaining brain homeostasis and its disruption is among the initial steps in the evolving course of injury in stroke. Another anti-inflammatory mechanism for cell therapies may be a direct effect on the flow of inflammatory mediators entering the brain after stroke. Several studies have found that cell therapies reduce BBB disruption in models of acute neurological injury [98, 99]. Multiple pathways may be involved. Menge et al. [100] found that MSCs release tissue inhibitor of metalloproteinase 3, which leads to the preservation of adherens junctions and tight junctions of cerebral endothelial cells. Others have found that MSCs inhibit the upregulation of aquaporin-4 [101].

Conclusion

As an advantage over pharmacological agents, cell therapies very likely engage multiple biological targets. Their immunomodulatory effects appear more selective compared with other approaches that broadly suppress the immune response to injury. Selective modulation may promote a more proregenerative environment. In view of the central and peripheral immunomodulatory effects of various cell therapies, several key questions arise for future study. There is a need to identify what the key immune targets are, how long after injury these targets are available or modifiable, and whether immune targets may even be appropriate for the application of cell therapies for chronic stroke.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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