Progenitor cell therapies as a novel treatment for traumatic brain injury: a pathway towards neuroprotection

"*Understanding the pathophysiological disturbances resulting from TBI as well as potential mechanisms of progenitor cell therapies may afford significant promise in advancing treatment for TBI.*"

KEYWORDS: inflammation ■ mesenchymal stromal cells ■ neuroprotection ■ progenitor cells ■ traumatic brain injury

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Traumatic brain injury (TBI) places a tremendous burden upon the American healthcare system and is associated with significant long-term patient morbidity [1]. In addition, all acute monotherapies focused on maintaining cerebral perfusion have failed to reverse the neuronal injury observed with TBI [2,3].

Preliminary research has shown potential neuroprotection after the intravenous injection of adult tissue progenitor or stem cells after TBI. By definition, adult tissue progenitor cells have the capacity for self-renewal and are multipotent (able to differentiate down multiple cell lines) [4]. Progenitor cells are maintained in select microenvironments throughout the body, which include bone marrow, adipose tissue, umbilical cord blood and within neural tissue (dentate gyrus and hippocampus). Within such niches, progenitor cell depletion, proliferation and activation are tightly regulated [5].

Early preclinical work has shown functional improvement after the intravenous injection of bone marrow-derived mesenchymal stromal cells (MSCs) for the treatment of TBI. Many such studies hypothesized that the transplanted MSCs were engrafting at the site of injury and adopting neuronal cell markers indicating differentiation into neurons [6]. However, the role of 'transdifferentiation' is now largely disregarded. Much debate remains about both the frequency and clinical significance as well as the validity of neural marker expression with most investigators believing this to be erroneous [7–9].

A more recent hypothesis to explain the observed neuroprotection is progenitor cell engraftment at the site of injury with modulation of the locoregional inflammatory response. Work completed in the Cox laboratory has detailed the proinflammatory mircroenvironment of both the direct injury and penumbral regions of the brain after TBI, highlighting a potential target for progenitor cell therapy [10]. Additional *in vitro* studies investigating direct contact cultures of MSCs and immunologic cells have shown a decrease in proinflammatory cytokine production (IFN-γ) with a concordant increase in anti-inflammatory cytokine production (IL-4 and -10) [11]. Unfortunately, results have been inconsistent with *in vivo* models. Such inconsistency is potentially secondary to limited progenitor cell engraftment and the pulmonary first pass effect.

Classic biodistribution studies that track MSCs after intravenous injection demonstrate that the overwhelming majority of cells are sequestered in the lungs [12]. Harting *et al.* demonstrated in a rodent TBI model that only 0.001% of intravenously transplanted cells engraft in the brain parenchyma with significant sequestration within the lung parenchyma (>96%) when evaluated 2–3 days after TBI. Furthermore, virtually no MSCs were found to remain in the parenchyma 2 weeks after transplantation [13]. Additional work completed in the Prockop laboratory has shown similar results with less than 0.001% of transplanted MSCs bypassing the pulmonary microvasculature [14]. Therefore, the observed pulmonary first-pass effect limits the number of MSCs that come into contact with the area of injury, thereby decreasing the likelihood that the neuroprotection is derived from a locoregional response alone.

Secondary to the limitations inherent to the intravenous delivery of progenitor cells, we believe that the observed neuroprotection could be due to modulation of the systemic inflammatory response ultimately affecting resident intracerebral microglial cell differentiation. As previously mentioned, the majority of transplanted

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progenitor cells remain sequestered within the lung microvasculature and parenchyma after intravenous delivery. Nemeth *et al.* have shown interaction between such sequestered progenitor cells and resident lung macrophages leading to increased anti-inflammatory cytokine production [15]. Additional studies completed in our laboratory have shown that a small percentage of MSCs that bypass the lungs become entrapped within the spleen [12]. The splenic white pulp, rich in immunologic cells (e.g., naive T cells), is located adjacent to the capillary beds within the spleen. Indeed, additional studies completed in the Cox laboratory have shown transplanted bone marrow-derived multipotent adult progenitor cells (MAPCs) within the white pulp. A concordant increase in anti-inflammatory cytokine production was found leading to preservation of the blood–brain barrier [16]. We hypothesize that such interactions between transplanted progenitor cells and distant organ systems lead to modulation of the systemic inflammatory response representing a critical step in the pathway towards neuroprotection.

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Furthermore, the observed increase in anti-inflammatory cytokine production is potentially due to direct interaction between transplanted progenitor cells and naive T cells within the splenic white pulp, leading to an increase in $CD4^+$ T cells. A subset of $CD4^+$ T cells ($CD4^+CD25^+FoxP3^+$) known as T-regulatory cells have anti-inflammatory properties, partly secondary to the production of IL-4 and IL-10. T-regulatory cells have been shown to be important in recovery from stroke. Through a series of *in vivo* experiments we have shown that the intravenous injection of bone marrow-derived MAPCs after TBI leads to an increase in T-regulatory cells in both the spleen and plasma leading the aforementioned increase in anti-inflammatory cytokine production.

Increased differentiation of naive T cells in to T-regulatory cells with an increase in anti-inflammatory cytokine production is a key portion of the pathway towards neuroprotection; however, investigation into a potential end effector cell within the brain parenchyma is required. Resident intracerebral microglial cells are known to play an essential role in the response to injury. Classic proinflammatory $CD86^+$ M1 macrophages are known to increase in concentration after acute injury. Conversely, $CD206^+$ M2 macrophages have been shown to secrete anti-inflammatory cytokines and have a neuroprotective effect. Kigerl *et al.* have shown a decrease in the M2 macrophage population with a concordant increase in M1 marcophages after acute CNS insult (ischemic stroke) [17]. Additional work completed in the Gendelmen laboratory has shown that T-regulatory cells may activate microglial cells to preferentially differentiate into the M2 phenotype, as has been shown in neurodegenerative disease processes [18]. Additional studies completed in our laboratory have shown that intravenous progenitor cell injection leads to a shift in the microglial cells to a predominantly M2 phenotype leading to stabilization of the intracerebral microvascular architecture and preservation of the blood–brain barrier.

While preliminary studies have shown potential neuroprotection associated with intravenous progenitor cell delivery for TBI, the mechanism of action remains intensely controversial. We believe that the transplanted progenitor cells interact with immunologic cells located in organ systems distant to the CNS, thereby modulating the systemic immunologic/inflammatory response. More specifically, direct interaction between transplanted progenitor cells and naive T cells within the white pulp of the spleen increases differentiation into T-regulatory cells leading to an increase in systemic anti-inflammatory cytokine concentrations. The observed increased levels of systemic anti-inflammatory cytokines activate resident intracerebral microglial cells to preferentially differentiate into neuroprotective M2 macrophages, ultimately leading to enhanced neuroprotection.

A large body of work has failed to show significant efficacy from single agent pharmacologic neuroprotective therapies. Understanding the pathophysiological disturbances resulting from TBI as well as potential mechanisms of progenitor cell therapies may afford significant promise in advancing treatment for TBI. The mechanism of progenitor cell benefit in TBI and neurologic injury is best described as constantly evolving. In an area in which all current monotherapies have failed to show significant benefit, laboratory studies that examine novel therapies for TBI and the mechanisms of benefit of these therapies offer significant promise.

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Bibliography

1 Thurman DJ, Alverson C, Dunn KA, Guerrero J, Sniezek JE. Traumatic brain injury in the United States: a public health perspective. *J. Head Trauma Rehabil.* 14, 602–615 (1999).

2 Gray DS, Burnham RS. Preliminary outcome analysis of a long-term rehabilitation program for severe acquired brain injury. *Arch. Phys. Med. Rehabil.* 81, 1447–1456 (2000).

3 Cowen TD, Meythaler JM, DeVivo MJ, Ivie CS 3rd, Lebow J, Novack TA. Influence of early variables in traumatic brain injury on functional independence measure scores and rehabilitation length of stay and charges. *Arch. Phys. Med. Rehabil.* 76, 797–803 (1995).

4 Weiner LP. Definitions and criteria for stem cells. *Methods Mol. Biol.* 438, 3–8 (2008).

5 Scadden DT. The stem-cell niche as an entity of action. *Nature* 441, 1075–1079 (2006).

6 Lu D, Mahmood, A Wang L, Li Y, Lu M, Chopp M. Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome. *Neuroreport* 12, 559–563 (2001).

7 Castro RF, Jackson KA, Goodell MA, Robertson CS, Liu H, Shine HD. Failure of bone marrow cells to transdifferentiate into neural cells *in vivo*. *Science* 297, 1299 (2002).

8 Wagers AJ, Sherwood RI, Christensen JL, Weissman IL. Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* 297, 2256–2259 (2002).

9 Roybon L, Ma Z, Asztely F *et al.* Failure of transdifferentiation of adult hematopoietic stem cells into neurons. *Stem Cells* 24, 1594–1604 (2006).

10 Harting MT, Jimenez, F, Adams SD, Mercer DW, Cox CS Jr. Acute, regional inflammatory response after traumatic brain injury: implications for cellular therapy. *Surgery* 144, 803–813 (2008).

11 Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* 105, 1815–1822 (2005).

12 Fischer UM, Harting MT, Jimenez F *et al.* Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first pass effect. *Stem Cells Dev.* 18(5), 683–692 (2009).

13 Harting MT, Jimenez F, Xue H *et al.* Intravenous mesenchymal stem cell therapy for traumatic brain injury. *J. Neurosurg.* 110, 1189–1197 (2009).

14 Lee RH, Pulin AA, Seo MJ *et al.* Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. *Cell Stem Cell* 5, 54–63 (2009).

15 Nemeth K, Leelahavanichkul, A, Yuen PS *et al.* Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat. Med.* 15, 42–49 (2009).

16 Walker PA, Shah SK, Jimenez F *et al.* Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: preserving the blood brain barrier via an interaction with splenocytes. *Exp. Neurol.* 225, 341–352 (2010).

17 Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG. Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. *J. Neurosci.* 29, 13435–13444 (2009).

18 Huang X, Reynolds AD, Mosley RL, Gendelman HE. $CD4^+$ T cells in the pathobiology of neurodegenerative disorders. *J. Neuroimmunol.* 211, 3–15 (2009).