2010 Research Grant Program Winning Abstract

T Regulatory Cells as a Therapeutic Target for Progenitor Cells in Traumatic Brain Injury (TBI)

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The total annual economic impact of traumatic brain injury (TBI) is approximately 60 billion US dollars. Annually, 50,000 patients die from TBI and a large portion of the 6.5 million patients affected by TBI suffer from resultant physical, cognitive and psychosocial deficits. 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 novel therapies may afford significant promise in advancing treatment for TBI.

A major focus of our laboratory involves understanding the pathophysiology of TBI and the potential role of a variety of adult progenitor (stem) cells. Progenitor cells have shown promise in TBI, including preserving the blood brain barrier and improving cognitive function. The mechanism of stem cell benefit has not been clearly elucidated.

We and others have demonstrated that the large majority of stem cells (after intravenous administration) do not accumulate in the brain and/or injury cavity in any significant number. Additionally, there is a significant first pass pulmonary sequestration of stem cells. Therefore, adult progenitor cells may exert a loco-regional effect by modulating systemic or extra-organ processes. An increasing body of work in other neurological injury models (i.e., stroke) indicates a significant potential role of the immune system. After stroke in animal models, splenic mass decreases. The spleen is a large reservoir of lymphocytes. In particular, T-regulatory cells (CD4+CD25+FoxP3+) may provide protection by modulating pro-inflammatory processes. Our hypothesis is that adult stem cells may function by altering the levels of pro and/or anti-inflammatory cytokines through a T-regulatory cell mediated effect.

Specific Aim One: Determine the pattern of T-regulatory cells up/down regulation after TBI. We have demonstrated in previous work that there is significant induction of pro-inflammatory cytokine production following TBI. Additionally, following injury, it has been shown that the T-regulatory cell population increases at approximately 72 hours, potentially as an innate protective response. Little work has been done, however, to clarify the T-regulatory cell profile in TBI at this time point. Using a controlled cortical impact (CCI) rodent model of traumatic brain injury, we propose to characterize the population of T-regulatory cells by flow cytometry. 72 hours following TBI, whole blood will be collected into BD Preservative Free Sodium Heparin Tubes. Red blood cells will be lysed with BD FACS™ Lysing Solution. The resultant lymphocyte population will be stained with CD4, CD25, and FoxP3 antibodies from BD and flow cytometry will be utilized to characterize the percentage of T-regulatory cells.

Specific Aim Two: Determine if adult progenitor cells alter T-regulatory cell function in vitro. 72 hours following CCI, whole blood will be collected into BD Preservative Free Sodium Heparin Tubes and red blood cells lysed with BD FACS Lysing solution. The

resultant lymphocyte population will be stained with CD4 and FACS separation will be performed to isolate the CD4+ fraction of T-cells. The resultant T-cells will be co-cultured with adult progenitor cells as well as brain supernatant from the rodent in which the cells were collected. 72 hours following co-culture, the lymphocytes will be stained with CD4, CD25, and FoxP3 antibodies to characterize the percentage of T-regulatory cells. Additionally, endpoints of T-regulatory cells including IL-4 and IL-10 will be quantified using BD FCM cytokine beads. Controls will include CD4 T-cells with adult progenitor cells only and CD4 T-cells alone.

Specific Aim Three: Determine if adult progenitor cells alter T-regulatory cell function in vivo. Rodents will undergo CCI and injection with adult progenitor cells at 2 and 24 hours following TBI. At 72 hours, the rats will be sacrificed and whole blood collected in BD Preservative Free Sodium Heparin Tubes. A portion of the blood will be centrifuged at 250 *g* for 5 minutes and the plasma collected and snap frozen. The plasma will be analyzed for IL-4 and IL-10 using BD FCM cytokine beads. Additionally, whole blood will be lysed with BD FACS Lysing solution and the resultant lymphocyte population stained for CD4, CD25, and FoxP3 antibodies to characterize the percentage of T-regulatory cells.

This project will allow us to determine if a potential mechanism of adult progenitor cells in TBI is via a T-regulatory cell mediated pathway. 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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