Regulatory T-Cells (T_{regs}) Within Bone Marrow-Derived Stem Cells (BMSCs) Actively Confer Immunomodulatory and Neuroprotective Effects Against Stroke

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Abstract

Stroke is the second leading cause of death worldwide and the third major cause of adult disability in adults. Regulatory T-cells (T_{regs}) may exert a neuroprotective effect on ischemic stroke by inhibiting both inflammation and effector T-cell activation. Transplantation of human bone marrow-derived stem cells (BMSCs) in ischemic stroke affords neuroprotection that results in part from the cells' anti-inflammatory property. However, the relationship between T_{regs} and BMSCs in treatment of ischemic stroke has not been fully elucidated.

Immunocytochemistry (ICC) and flow cytometry were used to identify cells expressing phenotypic markers of T_{regs}: CD4, CD25, and FoxP3 protein. T_{regs} were isolated using magnetic sorting from murine spleens. Primary rat neuronal cells (PRNCs) were subjected to an oxygen-glucose deprivation and reperfusion (OGD/R) condition. The cells were re-perfused and co-cultured with T_{regs} and/or BMSCs. We measured neuronal cell viability using ICC with Hoechst and MAP2.

We detected a minority population of T_{regs} within BMSCs with both ICC and flow cytometry. PRNCs were protected from OGD/R when co-cultured with BMSCs containing varying proportions of T_{regs}. The BMSC treatment containing the native population of T_{regs} conferred maximal neuroprotection compared to the treatment conditions containing 0%, 10%, and 100% relative ratio T_{regs}. Increasing the T_{reg} population resulted in increased IL6 secretion and decreased FGF-β secretion by BMSCs.

BMSC transplantation stands as a potent treatment for ischemic stroke. Modulation of the immune system is a key mechanism by which BMSCs confer neuroprotection. This study shows that a minority population of T_{regs} exists within the therapeutic BMSC population, and those T_{regs} are robust mediators of the immunomodulatory effect provided by BMSC transplantation. The ratio of T_{regs} found naturally in BMSCs correlates with the highest level of neuroprotection after ischemic stroke.

Introduction

Rescue of the peri-infarct region after ischemic stroke has been linked to inflammatory response. T_{regs} and BMSCs have been independently shown to confer neuroprotection after stroke by reducing inflammation [1,2]. The mechanism of BMSC's anti-inflammatory effect has not yet been fully elucidated. Since BMSCs are harvested from bone marrow, we hypothesized that a yet-unidentified subpopulation of bone marrow derived cells exists that is partially responsible for the anti-inflammatory effect of BMSCs.

Methods and Materials

ICC and flow cytometry were used to identify $CD4^+/CD25^+/FoxP3^+$ T_{regs}. Magnetic isolation techniques were used to both enrich and deplete cell populations of T_{reg}, as previously described [3]. PRNCs were subjected OGD/R to simulate ischemic stroke. The cells were re-perfused and co-cultured with T_{regs} and/or BMSCs. Cell viability was measured using ICC.

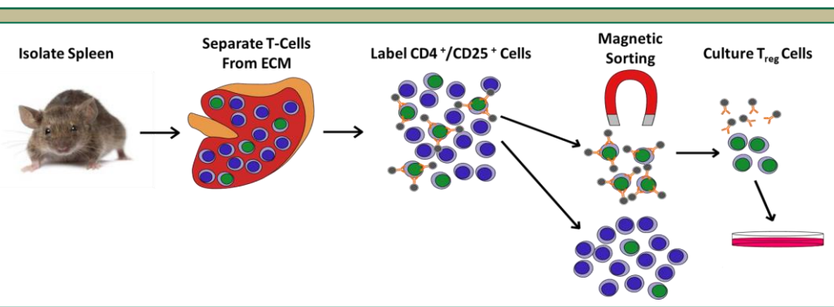


Figure 1. T_{reg} magnetic cell isolation procedure. Spleens were isolated from mice and the cells were incubated with magnetic bead conjugated antibodies and isolated by exposure to a magnetic field to yield purified CD4+/CD25+ T_{regs}.

Results

A subpopulation of cells expressing characteristic T_{reg} protein markers CD4, CD25, and FoxP3 was identified in human BMSCs. The native population of T_{regs} in BMSCs increases neuroprotection after OGD/R and reduces IL-6 production relative to the same BMSC population depleted of native T_{regs}. Supplemental T_{regs} isolated from mice spleens were added to co-culture after OGD/R. Increasing ratios of T_{regs} decreases neuroprotective capacity of BMSC treatment. Increased ratios also increase IL-6 production and reduce FGF-β production by BMSCs.

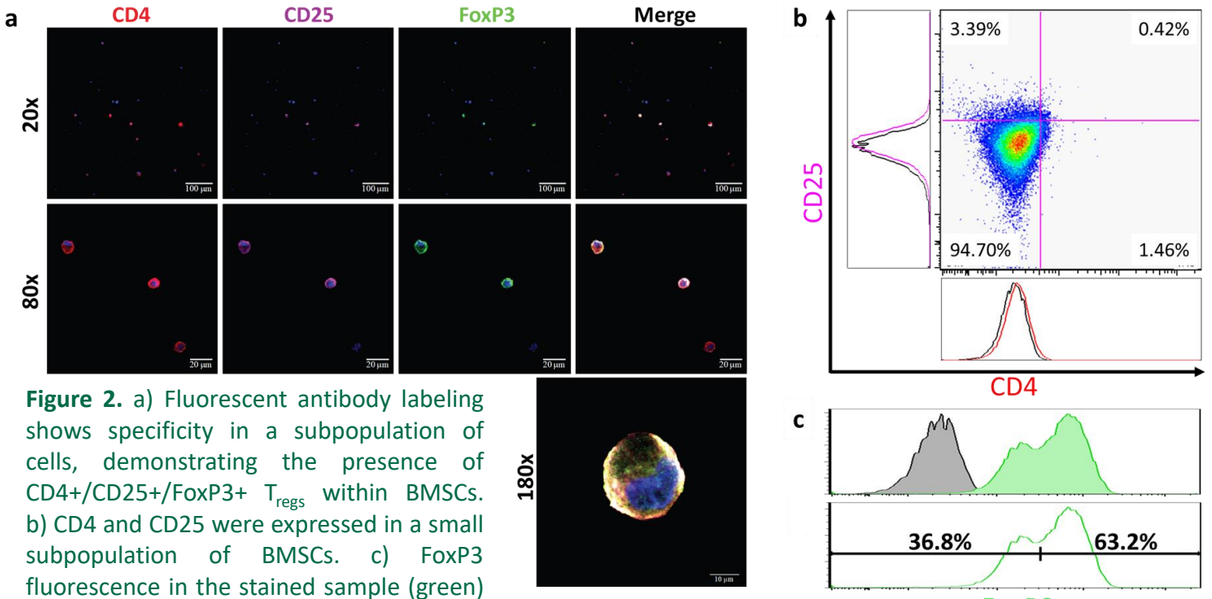
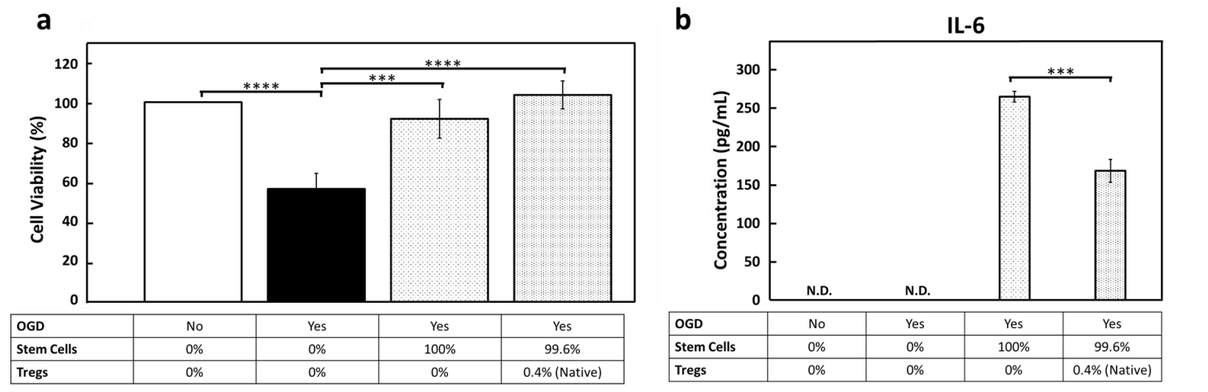
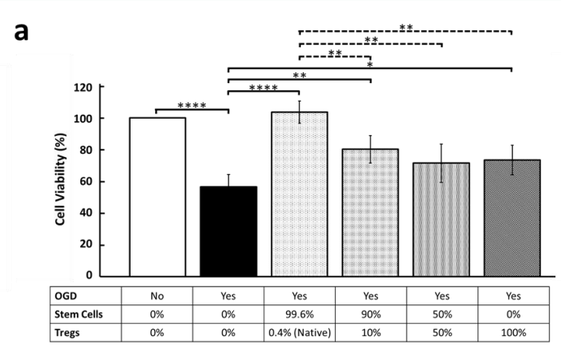


Figure 2. a) Fluorescent antibody labeling shows specificity in a subpopulation of cells, demonstrating the presence of CD4+/CD25+/FoxP3+ T_{regs} within BMSCs. b) CD4 and CD25 were expressed in a small subpopulation of BMSCs. c) FoxP3 fluorescence in the stained sample (green) shows bimodal distribution. Bimodal distribution suggests there is a FoxP3+ and a FoxP3- subpopulation in BMSCs. Scale bars: 20x-100μm; 80x-20μm; 180x-10μm

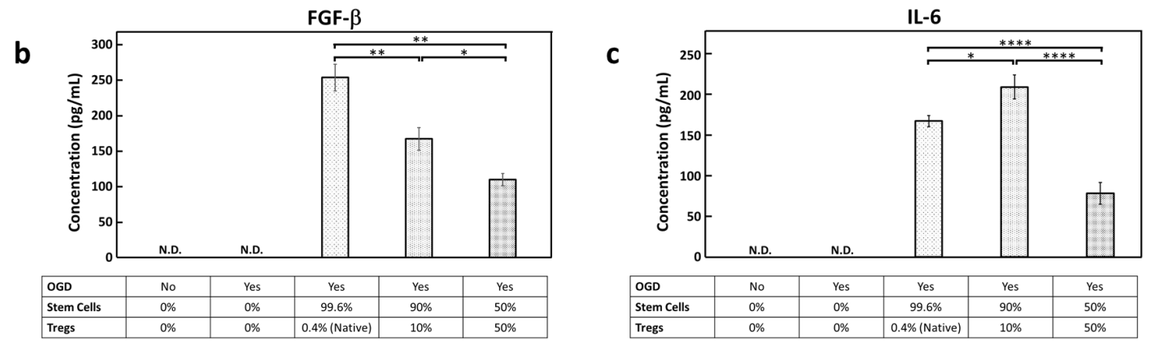


OGD	No	Yes	Yes	Yes
Stem Cells	0%	0%	100%	99.6%
Tregs	0%	0%	0%	0.4% (Native)

Figure 3. a) OGD/R results in significant decrease in cell viability. BMSCs with T_{regs} exhibit greater neuroprotective capacity than BMSCs without T_{regs}. b) Interleukin-6 secretion was significantly increased by depleting BMSC cell transplant of T_{regs}. T-test significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ****$p < 0.0001$



OGD	No	Yes	Yes	Yes	Yes	Yes
Stem Cells	0%	0%	99.6%	90%	50%	0%
Tregs	0%	0%	0.4% (Native)	10%	50%	100%



OGD	No	Yes	Yes	Yes	Yes
Stem Cells	0%	0%	99.6%	90%	50%
Tregs	0%	0%	0.4% (Native)	10%	50%

OGD	No	Yes	Yes	Yes	Yes
Stem Cells	0%	0%	99.6%	90%	50%
Tregs	0%	0%	0.4% (Native)	10%	50%

Figure 4. a) Treatment with BMSCs and the native ratio of T_{regs} confers significant neuroprotection after OGD/R. Supplemental T_{regs} in ratios of 1:10 and 1:1 T_{regs} : BMSCs shows significant reduction in neuroprotection relative the native 1:100 ratio. b) Increasing the ratio of T_{regs} correlates to a dose-dependent decrease in FGF-β secretion. c) IL-6 production was significantly increased in the 1:10 T_{reg} ratio treatment group, while it was significantly decreased in the 1:1 ratio treatment group. T-test significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ****$p < 0.0001$

Discussion

- Positively identified T_{regs} in BMSCs, and observed a neuroprotective effect that was dependent on T_{reg} concentration.
- Increasing or decreasing the T_{reg} population ratio decreased the neuroprotective effect of BMSC treatment.
- Cytokine secretion related to BMSC immunomodulation, differentiation, and survival was dependent on the proportion of T_{regs}.

Conclusion: BMSC transplant is a powerful treatment following ischemic stroke. This study showed that a minority population of T_{regs} exists within the therapeutic BMSC population, and those T_{regs} are independent modulators of the immunosuppressive effect provided by BMSC transplantation. The ratio of T_{regs} found naturally in BMSCs correlates with the highest level of neuroprotection after ischemic stroke.

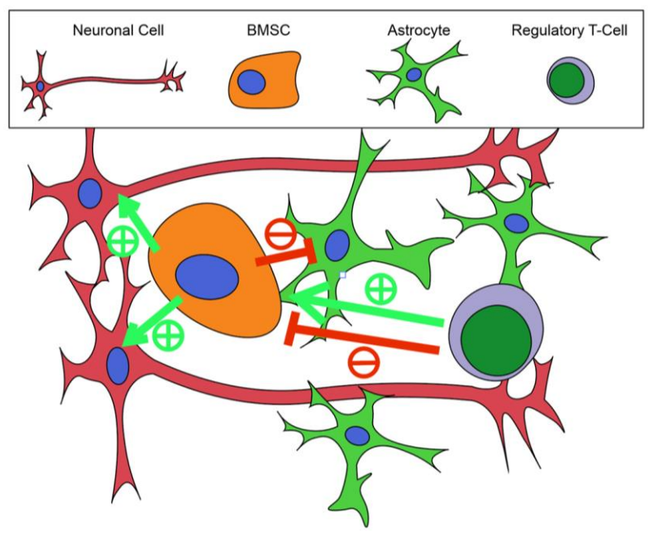


Figure 5. A graphical depiction of cell culture is depicted showing the interaction between T_{regs}, BMSCs, and neural cells (astrocytes and neurons). BMSCs are shown to be neuroprotective by promoting neuron survival (green arrows, +) and attenuating astrocyte activation (red arrows, -). T_{regs} are shown to potentially have a dualistic, concentration-dependent effect on BMSCs. At the native concentration, T_{regs} relatively decrease BMSC IL-6 production, a potentially deleterious pro-inflammatory cytokine. BMSC FGF-β production, a cytokine related to BMSC survival, proliferation, and differentiation, is reduced in a concentration dependent manner with T_{reg} co-culture.

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