Abstract

Stroke is the second leading cause of death worldwide and the third major cause of adult disability in adults. Regulatory T-cells ($T_{reg}$) 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 $T_{reg}$ and BMSCs in treatment of ischemic stroke has not been fully elucidated.

Immunomodulatory (ICC) and flow cytometry were used to identify cells expressing phenotypic markers of $T_{reg}$, CD4, CD25, and FoxP3 protein. $T_{reg}$ 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 $T_{reg}$ and/or BMSCs. We measured neuronal cell viability using ICC with Hoechst and MAF2.

We detected a minority population of $T_{reg}$ within BMSCs with both ICC and flow cytometry. PRNCs were protected from OGD/R when co-cultured with BMSCs containing varying proportions of $T_{reg}$. The BMSC treatment containing the native population of $T_{reg}$ conferred minimal neuroprotection compared to the treatment conditions containing 0%, 10%, and 100% relative ratio $T_{reg}$. 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 $T_{reg}$ exists within the therapeutic BMSC population, and those $T_{reg}$ are robust mediators of the immunomodulatory effect provided by BMSC transplantation. The ratio of $T_{reg}$ 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. $T_{reg}$ 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+$T_{reg}$ 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 treatment. The cells were re-perfused and co-cultured with $T_{reg}$ and/or BMSCs. Cell viability was measured using ICC.

Results

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

Discussion

- Positively identified $T_{reg}$ 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 $T_{reg}$.

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

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