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Demonstration of immunomodulatory properties for the human MuStem cell population, a promising candidate for cell therapy of muscular dys dystrophies

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Introduction

Over the last eighteen years, the identification of stem cells in adult tissue opened new opportunities in cell-based therapy strategy. However, allogenic cell transplantation protocols are highly limited by graft rejection.

To overcome this issue, long-term immunosuppression (IS) are classically used, resulting in improved cell engraftment but also major adverse effects. Recently, many in vitro studies demonstrated pleiotropic immunomodulatory properties for adult stem cells, especially mesenchymal ones that have been shown to modulate the behavior of many immune cells through paracrine secretion or direct contact. These features could increase their ability to engraft in allogeneic recipient despite the lack of strong IS, thus improving their therapeutic efficiency. In addition, delivery of cells with immune privilege behavior may be beneficial in the context of degenerative disorders to limit chronic inflammation that characterizes tissues and interfere with the repair process.

In the lab, we isolated muscle-derived stem cells (termed MuStem cells) from healthy dogs and demonstrated that their systemic delivery in dystrophic dogs submitted to continuous IS lead to muscle regeneration and long-term clinical signs stabilization. Interestingly, an IS restricted to the transplantation period was shown to be sufficient to sustain their transplantation benefits and to prevent host immunity responses in allogeneic context, suggesting a possible immune privilege behavior for the MuStem cells. Recently, human MuStem cells were isolated and characterized as exhibiting in vitro some myogenic, chondrogenic, osteogenic and adipogenic potential. The aim of the present study is to explore the immunomodulatory-related features of hMuStem cells and more specifically the interaction with T-cells features and the complement system activation, two key effectors of allograft rejection.

Materials & Methods

MuStem cell isolation and culture: Human muscle-derived cells were isolated from skeletal muscle of 9 to 15-year-old patients free of known muscle diseases. To isolate hMuStem cells, MSC were subjected to a modified version of previous protocol initially described by Rouger et al. in 2011. Human cells were then cultivated on coated plastic: peaks in proliferation medium containing 15% FCS and human recombinant growth factors. For pro-inflammatory stimulation, 75% confluent cells were cultured 24 to 48h in medium supplemented with 5% of human serum (FBS). Four different batches of hMuStem cells were used.

Lymphocyte immunosuppression assay: Either hMuStem cells or bone marrow mesenchymal stem cells (BM-MSC) were cultured with Cell Trace Violet (CTV)-labeled allogeneic peripheral blood mononuclear cells (PBMC) (1:10 ratio, cells/PBMC), during 2 to 3 days, under pro-inflammatory (PI) stimulation. Analysis of lymphocyte proliferation, activation and regulatory profile were performed by flow cytometry. For inhibitory experiments, either Isotype control or 1:10 mouse anti-human IL-4 (Abcam) were added to the co-cultures at 0.5 and 1.0 IFU, respectively. For Mixed Lymphocyte Reaction (MLR), treated (50%) hMuStem cells were added in graded ratios to human CD3+ T cells and mixed with irradiated PBMC co-cultures. After 5 days, T proliferation was evaluated by 3H-thymidine uptake (1.05 µCi/ml).

In vitro complement-mediated hemolysis: Sheep red blood cells were incubated with 1% human serum as source of complement and (i) without hMuStem cell supernatant (positive control), (ii) with hMuStem cell supernatant (negative control), (iii) with Factor H-depleted hMuStem cell supernatant, (iv) with Factor H-depleted human serum. Lysis of red blood cells was measured by OD reading at 460nm.

Evaluation of MuStem cells immune-phenotype: hMuStem cell supernatants were collected after 48h of culture in native or stimulated condition and stored at -20°C. Prostaglandin E2 (PGE2) and Factor H were measured using total urinary detection kit (Guyton Chemical) according to manufacturer's procedure. FACs analysis was made by counting at least 15,000 events after CDS1, CDS2, CDS4, CDS4, IFN, PD1 and PD2 immuno-staining.

Impact of hMuStem cells on T-lymphocyte features

MuStem cells directly inhibit CTV+ T-cell proliferation in a dose dependent manner

Modulation of CD4+ T-lymphocytes subsets

- CD4+ cells equally inhibit CD4+ and CD8+ lymphocyte proliferation and activation in PBMC population
- Inhibitory capacity of MuStem cells is similar to those of BM-MSC
- As BM-MSC, hMuStem cells have no impact on pro-inflammatory IFNγ and IL-17 subsets

Implication of PGE2 and NO in inhibition of T-lymphocyte proliferation

MuStem cells constitutively express NO donors

MuStem cells do not express the co-stimulatory molecule CD11b and CD86

MuStem cells express the co-stimulatory molecule CD86

MuStem cells are selectively secreted PGE2 and respond to TNFα stimulation

MuStem cells constitutively express iNOS, unlike BM-MSC

MuStem cells secrete NO independently of pro-inflammatory stimulation

MuStem cells secrete NO in BM-MSC supernatant (ELISA and NO analysis)

Pattern recognition molecules expression

MuStem cells are able to modulate lymphocyte feature

- By inhibiting lymphocyte proliferation under MLR activation
- By specifically inhibiting both CD4+ and CD8+ lymphocyte proliferation and activation in PBMC population
- By promoting IL-10 and COX-2 expression

MuStem cells act on TNFα-producing pathways through paracrine factors

PGE2 secretion

NO production (dissociated from BM-MSC)

MuStem cells display a membrane profile that suggest a possible direct inhibitory effect on T-cells

Hemolytic activity of hMuStem cells supernatants

- hMuStem cells inhibit CTV+T-cell proliferation in a dose dependent manner

Conclusion

hMuStem cells are able to inhibit complement pathway

- In a paracrine manner, by factor H secretion

- hMuStem cells express the complement inhibitory molecule CD68 and CD69 that suggest a possible direct inhibitory effect on T-cells

MuStem cells exhibit interesting immunomodulatory properties that could be exploited in preclinical setting in dystrophic muscular tissue context.