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

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The aim of the present study is to explore the immunological-related features of hMuStem cells in order to modulate the immunomodulatory properties for adult stem cells engraftment but also for skeletal myogenesis. Recently, human MuStem cells were isolated and characterized as exhibiting an inflammatory stimulation, 70% confluent cells were cultured 24 to 48h in medium supplemented with 50 ng/mL of TNFα, PGE2, and human recombinant growth factors. For pro-inflammatory stimulation, 75% confluent cells were cultured 24 to 48h in medium supplemented with 50 ng/mL of TNFα and 500ng/mL of IFN-γ. Four distinct batches of sheep red blood cells were used.

Lymphocyte immunosuppression assay: Either hMuStem cells or bone marrow non-myeloid stem cells (BM-MSC) were cultured with Cell Trace Violet (CTV)-labeled allogenic peripheral blood mononuclear cells (PBMC) (1:10) and PBMC cells, incubating 2 to 3 days, under phytohemagglutinin (PHA) stimulation. Analysis of lymphocyte proliferation, activation and regulatory profile were performed by flow cytometry. For inhibitory experiments, either IFNγ- or TNFα-monomeric or humanized (hMABM) were added to the co-cultures at 10, 50 and 100, respectively. For Mixed Lymphocyte Reaction (MLR), irradiated (35Gy) hMuStem cells were added in graded ratio to human CD3+CD25−PBMC cultures in 24-well plates at 1:1:1, 1:1:2 and 1:1:3 ratios. After 5 days, T-cell proliferation was evaluated by [3H]-thymidine uptake (0.52μCi/mL).

In vitro complement-mediated hemolysis: Sheep red blood cells were incubated with 15% human serum as source of complement and (i) without hMuStem cell supernatant (positive control) (ii) with native hMuStem cell supernatant or (iii) with Factor H-depleted hMuStem cell supernatant. Lysis of sheep red blood cells was measured by OD reading at 405nm.

Evaluation of hMuStem cells immunophenomena: hMuStem supernatants were collected after 48h of culture in native or stimulated condition and stored at -20°C. Phospho(serine) p38 (p38) and Factor H secretion were measured by ELISA, NO concentration was measured using total nitrite detection in sheep red blood cell supernatant or by [3H]-thymidine uptake (0.52μCi/mL).

**Impact of hMuStem cells on T-lymphocyte features**

- hMuStem cells equally inhibit CD4+ and CD8+ lymphocyte proliferation and activation in PBMC population.
- Inhibitory capacity of hMuStem cells is similar to those of BM-MSC.
- hMuStem cells reduce formation of CD4-6.10+ (T-reg-like) and CD4+FoxP3+ (T-reg-like) lymphocytes to a lesser extent than BM-MSC.
- hMuStem cells have no impact on pro-inflammatory (IFNγ) and IL17+ subsets.

**Impact of hMuStem cells on complement activation**

- hMuStem cells secrete Factor H that inhibits alternative pathway by preventing C3 convertase assembly.
- hMuStem cells partially inhibit complement mediated cytolysis through Factor H secretion when depletion results in hemolytic reactivation.

**Materials & Methods**

- **hMuStem cell isolation and culture:** Human muscle-derived cells were isolated from skeletal muscles of 9 to 15-year-old patients free of known muscle disease. To isolate hMuStem cells, MSC were isolated by a modified version of pre-plating protocol initially described by Roget et al. 2011. hMuStem cells were then cultured on coated-plateau fibronectin in proliferation medium containing 15% FBS, PGE2 and human recombinant growth factors.
- **In vitro complement-mediated hemolysis:** Sheep red blood cells were incubated with 15% human serum as source of complement and (i) without hMuStem cell supernatant (positive control) (ii) with native hMuStem cell supernatant or (iii) with Factor H-depleted hMuStem cell supernatant. Lysis of sheep red blood cells was measured by OD reading at 405nm.

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