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

Judith Lorant, Marine Charrier, Rafael Contreras Lopez, Christophe Blanquart, Blandine Lieubeau-Teillet, Cindy Schleder, Isabelle Leroux, Tejedor Gauthier, Candice Babarit, Yann Pereon, et al.

To cite this version:
Judith Lorant, Marine Charrier, Rafael Contreras Lopez, Christophe Blanquart, Blandine Lieubeau-Teillet, et al.. Demonstration of immunomodulatory properties for the human MuStem cell population, a promising candidate for cell therapy of muscular dystrophies. 26th Annual Congress of the ESGCT, Oct 2018, Lausanne, Switzerland. 2018. hal-01927456

HAL Id: hal-01927456
https://hal.archives-ouvertes.fr/hal-01927456
Submitted on 19 Nov 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
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 recipients 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 these diseases 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 subjected to continuous IS lead to muscle regeneration and long-term clinical status stabilization. Interestingly, an IS restricted to the transplantation period was shown to be sufficient to sustain their transplantation benefits and prevent host immune response 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 stromal myogenic potential, positioning them as a promising candidate for muscle-dedicated regenerative medicine.

The aim of the present study is to explore the immunological-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 cells isolation and culture: Human muscle-mderived cells were isolated from skeletal muscles of 9 to 15-year-old patients free of known muscle disease. To isolate MuStem cells, MSC were subjected to a modified version of prepping protocol described by Rouger et al. in 2011. MuStem cells were then cultivated on collagen-coated flasks in proliferation medium containing 15% FBS and human recombinant growth factors. For pre-inflammatory stimulation, 75% confluent cells were cultured 24 to 48h in medium supplemented with 5% FBS, 1% R10 (4%)

Lymphocyte immunosuppression assay: Either MuStem cells or bone marrow mesenchymal stem cells (BM-MSC) were cultured with Cell Trace Violet (CTV)-labeled allogeneic peripheral blood mononuclear cells (PBMC) (1:1 ratio, cells/PBMC), during 2 to 3 days, under potential immunosuppressive (IS) stimulation. Analysis of T-cells proliferation, activation and cytokine profiles were performed by flow cytometry. For inhibitory experiments, either anti-Interleukin-10 (IL-10) or anti-Interleukin-12p70 (IL-12p70) antibodies were added to the co-cultures at 100 ng/mL and 10-fold, respectively. Stimulation of MuStem cells was analyzed from 1 to 3 days in culture with human CD14+ monocytes or bone marrow-derived dendritic cells (BM-DC) in the presence of lipopolysaccharide (LPS) 1 μg/mL.

Impact of hMuStem cells on T-lymphocyte features

Inhibition of T-lymphocyte proliferation and activation

MuStem cells directly inhibit T-lymphocyte proliferation in a dose dependent manner.

Expression of immunosuppressive molecules

MuStem cells express CD59 that is known to prevent C3 convertase assembly and to accelerate foraminated complement decomposition.

Impact of hMuStem cells on complement activation

Inhibition of complement-mediated hemolysis and factor H depletion

MuStem cells secrete Factor H that inhibits alternative pathway by preventing C3 convertase assembly.

MuStem cells partially inhibit complement mediated lysis through regulation of membrane attack complex (MAC) formation.

Schematic summary

MuStem cells are able to inhibit 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 IL-4 production

MuStem cells act on T-lymphocytes through paracrine factors

- Inhibit regulatory T cells production (indicatively from BM-MSC)
- MuStem cells display a membrane profile that suggest a direct interaction with CD30 and CD80 expressing cells
- Inhibit iNOS production (indistinguishable from BM-MSC)
- MuStem cells express the complement inhibitory molecule CD55, that suggest a possible role in the complement pathway.

MuStem cells exhibit interesting immunomodulatory properties that could be useful for a future application in dystrophic muscle tissue context.

Conclusions

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

Marine Chartier1,2, Judith Lorant1, Rafael Contreras Lopez3,4, Christophe Blanquart5, Blandine Liebesch6, Cindy Schleder1, Isabelle Leroux1, Gautier Téjedor1, Candice Babarit1, Yann Percron1, Patricia Luz-Crawford1, Guillaume Lamirault1, Farida Djoud1 and Karl Rouger1

1 Unité de REI-A INRA UR701, École nationale vétérinaire, agroalimentaire et de l’umunumité-Nantes-Atlantic (Oriris), Université Bretagne Loire (UBL), Nantes, F-44407, France; 2 INSERM UM174 INRS URMICS, UMR 6269, Université de Nantes, Nantes, F-44072, France; 3 INRAE, Unité d’Essai à L’Insu, Poitiers, F-86034, France; 4 LGC, Laboratoire de Chimie Cellulaire, Université de Nantes, F-44078, France; 5 LAMH, Unité de Référence Météorologie Numérique, CNRS, Laboratoire d’Expériences Contrôlées, Centre Hospitalier Universitaire Nantes Est, Nantes, F-44409, France