

Phenotypical and functional characterizations of conditioned Mesenchymal Stromal Cells MSC, as tools for immunomodulation in Myasthenia Gravis

Alexandra Bayer Wildberger¹, José Villegas¹, Julien Verdier¹, Mariette Giannini¹, Solène Maillard¹, Frédérique Truffault¹, Aurélien Corneau², Nadine Dragin¹, Valérie Vanneaux³, Hélène Rouard⁴, Danièle Noël⁵, Christophe Martinaud⁶, Rozen le Panse¹, Sonia Berrih-Aknin^{1*}, Jean-Thomas Vilquin^{1*}.

1: Sorbonne Université, INSERM UMRS 974, Centre de Recherche en Myologie, Institut de Myologie, groupe hospitalier Pitié-Salpêtrière ; Paris, France.

2: Sorbonne Université, INSERM UMRS 1135, Centre d'Immunologie et des Maladies Infectieuses (CIMI) ; Plateforme de Cytométrie (CyPS), UMS30-LUMIC, Faculté de Médecine Pierre et Marie Curie, groupe hospitalier Pitié-Salpêtrière ; Paris, France.

3: Cell Therapy Unit, CIC de Biothérapies (CBT-501), INSERM U1160, Institut Universitaire d'Hématologie, Hôpital Saint-Louis, AP-HP ; University Paris Diderot, Sorbonne Paris Cité ; Paris, France.

4: Université Paris-Est-Créteil (UPEC), INSERM/IMRB-U955, laboratoire BCTSVT ; Etablissement Français du Sang d'Ile-de-France, Unité d'Ingénierie et de Thérapie Cellulaire ; Créteil, France.

5 : INSERM U1183, Institut de Médecine Régénératrice et Biothérapies (IRMB), Hôpital Saint-Eloi ; Montpellier, France.

6 : INSERM UMRS-MD 1197, Unité des Médicaments de Thérapie Innovante, Centre de Transfusion Sanguine des Armées ; Clamart, France.

*: corresponding authors.

Introduction and Objective

Myasthenia Gravis (MG) is an autoimmune disease whose pathogenicity is due to autoantibodies directed against the neuromuscular endplate. The present treatments are symptomatic and trigger severe side-effects, mandating the set-up of novel therapies. Mesenchymal Stromal/Stem Cells (MSC) are multipotent progenitors that can modulate the immune system via soluble mediators and cell-cell contacts. Our team recently demonstrated that the transfer of research grade (RG-) MSC conditioned by a co-culture step with peripheral blood mononucleated cells (PBMC) improved the clinical outcomes in a humanized MG mouse model (*Sudres et al., JCI Insight 2017*).

To shift towards a clinical perspective, RG-MSC (grown in fetal bovine serum) are replaced by clinical grade (CG-) MSC (grown using human platelet lysate). Here, we aim to characterize the phenotype of resting and conditioned CG-MSC, as compared to RG-MSC, as well as to validate their functional capacities in vitro.

Methods

Adipose tissue derived RG- and CG-MSC were compared for their growth kinetics. They were also conditioned by co-culture with PBMC or monocytes, by activation with IFN- γ , or left untreated (resting state). Their phenotype was assessed by classical flow cytometry using 61 antibodies, and complemented by powerful single-cell mass cytometry (CyTOF), using a dedicated panel of 31 antibodies targeting extracellular and intracellular antigens.

Immunomodulating capacities of CG-MSC were evaluated by PBMC inhibition assay (CFSE test), and the productions of key players cytokines (IL-6, TGF- β , IDO and PGE2) were quantified by ELISA in culture supernatants.

Results

- Despite some differences in growth kinetics, RG- and CG-MSC showed close phenotypes. A strong correlation has been observed in markers expression between CG-primary cultures from different donors, and the same was true for RG-cultures.
- Comparing conditioning treatments, some markers were modulated by all of them, while others were exclusively modulated by one of them, thus conferring conditioning signatures.
- Both resting and conditioned MSCs blocked the proliferation of activated PBMC in vitro.
- The conditioning treatments enhanced the secretion of immunomodulatory molecules by MSC, but they differed in their molecular footprint.

Conclusion

This study shows that RG- and CG-MSC share close phenotypes, it confirms CG-MSC immunomodulation capacities and unveils phenotypic and functional signatures for the different treatments.