Mesenchymal Stromal Cells (MSC) : phenotypical and functional characterizations as tools for immunomodulation in Myasthenia Gravis

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Background

Myasthenia Gravis (MG)

- MG is a rare neuromuscular disease.
- Pathogenicity is due to autoantibodies directed against proteins of the neuromuscular endplate.
- Common treatments (corticosteroids and azathioprine) trigger severe side-effects, mandating the set-up of novel therapies.

Mesenchymal Stromal/Stem Cells (MSC)

- MSC are non hematopoietic multipotent progenitor cells.
- They can modulate the immune system via soluble mediators and cell-cell contacts.
- Previous studies in our new MG animal model, show that the transfer of MSC conditioned by peripheral blood mononucleated cells (PBMC) improved the clinical status of the animals. *(Sudres et al., JCI Insight 2017).*

Materials & Methods

MSC Samples

- Adipose derived MSC were obtained from ongoing collaborations: H. Rouard (EFS); J. Larghéro, V. Vanneaux (Hop. St Louis); D. Noël (INSERM UMRS 1183); C. Martinaud (CTSA) .

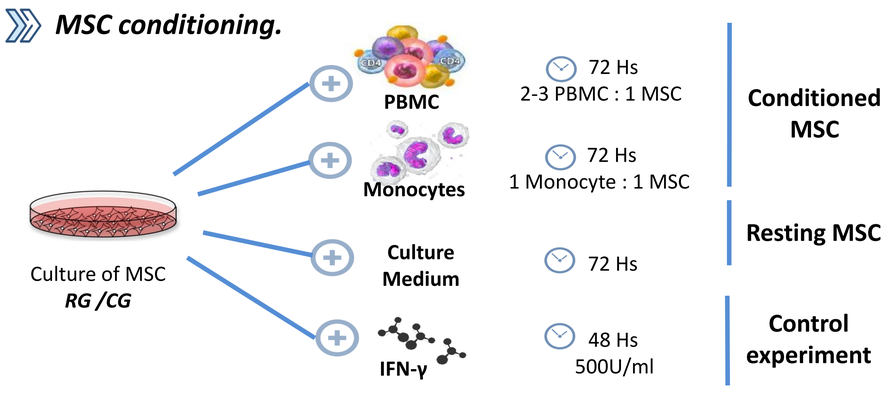
PBMC/ Monocytes samples

- PBMCs were obtained from venous blood from healthy volunteer donors (EFS, Rungis, France) using the Lymphoprep density gradient centrifugation protocol.
- Purified monocytes were sorted from PBMC by immunomagnetic negative selection using anti-human CD14 antibody complexed to magnetic particles.

MSC thawing, cell culture and expansion.

- Cells were seeded at a density of 4,000/cm² cells/
- Media culture for:
  - RG : α-MEM + 10% Fetal Bovine Serum + 1ng/ml bFGF + antibiotics
  - CG : α-MEM + 5% by platelet lysate + 2U/ml heparin + antibiotics
- Cells were kept in a 37 °C humidified incubator containing 5% CO2.

MSC conditioning.



Assessment of markers expression by Flow cytometry and CyTOF

- Resting and conditioned cells (flow cytometry: all conditions; CyTOF: only IFNγ) were trypsinized.
- Cells were incubated with monoclonal fluorochrome-conjugated or metal-conjugated primary Ab against Human antigens.
- 61 antibodies were tested by flow cytometry and 31 by CyTOF.

Category	Antibody	Alternative Name
Miscellaneous molecules	CD10	CALLA
	CD13	Aminopeptidase
	CD34	Hematopoietic progenitor cell Ag.
	CD45	Lecucocyte common Ag. (LCA)
	CD45RA	LCA isoform
	CD45RO	LCA isoform
Cell adhesion molecules (varied)	CD9	MRP-1
	CD36	Platelet glycoprotein 4
	CD44	HCAM
	CD47	Neurophilin
	CD54	ICAM-1
	CD56	NCAM
	CD57	HNK1
	CD62E	E-selectin
	CD62L	L-selectin
	CD62P	P-selectin
	CD81	Tetraspanin 28
	CD106	MadCAM
	CD146	MCAM
	CD166	ALCAM
Integrins	CD29	Integrin-β1
	CD49a	Integrin-α1
	CD49b	Integrin-α2
	CD49c	Integrin-α3
	CD49d	Integrin-α4
	CD49e	Integrin-α5
	CD49f	Integrin-α6
	CD49g	Integrin-α7
	CD61	Integrin-β3

Category	Antibody	Alternative Name
Immunomodulation	CD24	Heat Stable Ag.
	CD55	DAF
	CD59	MAC-IP
	CD119	IFNγ Receptor α-chain
	CD273	PD-L2
	CD274	PD-L1
	CD276	B7-H3
other molecules with immunological implication	CD7	gp40
	CD14	LPS Receptor
	CD40	TNFRSF5
	CD69	Very early activating factor
	CD80	B7-1
	CD86	B7-2
	CD184	CXCR4
	CD200	OX-2
	HLA-ABC	-
	HLA-DR	-
Receptors	CD36	Platelet glycoprotein 4
	CD140a	PDGFRa
	CD140b	PDGFRb
	CD172a/b	SIRPβ
	CD221	IGF-R3
	CD271	NGFR
	CD309	VEGFR
Negative MSC markers	CD14	LPS Receptor
	CD11b	Integrin αM
	CD45	Lecucocyte common Ag. (LCA)
positive MSC markers	CD73	Ecto-5'-nucleotidase
	CD90	Thy-1
	CD105	Endoglin

PBMC inhibition assay (CFSE test)

- Activated PBMC were labeled with carboxyfluorescein succinimidyl ester (CFSE), and put in contact with resting or conditioned CG MSC (1:5 ratio)
- After 3 days, the percentage of proliferating PBMC out of the total live cells was assessed by flow cytometry.

Study purpose

To develop an immunomodulating approach in clinical perspective, we compared:

- **Phenotype**
- **Functional capacities**

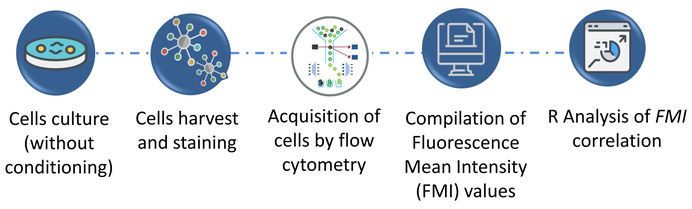
Research Grade MSC vs Clinical Grade MSC

Conditioning: PBMC, Monocytes, IFNγ

Grown in Fetal Bovine Serum → Research Grade MSC (RG) / Clinical Grade MSC (CG) → Grown in Platelet Lysate

Results

1 *Phenotype comparison between resting Research Grade and Clinical Grade MSC.*



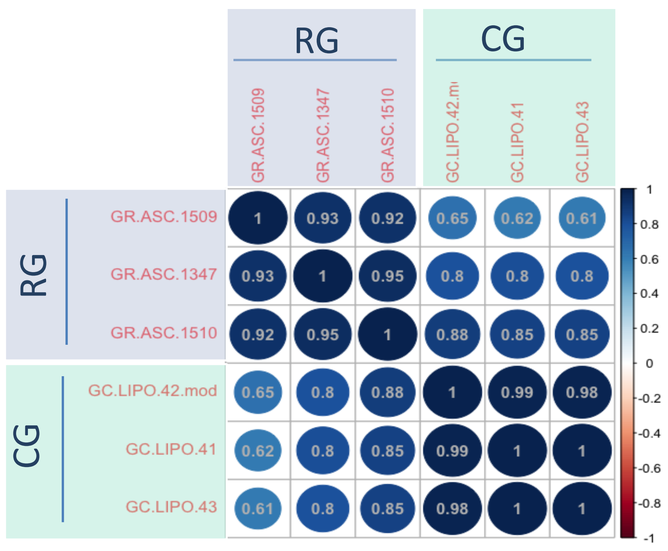


Figure 1: Strong correlation between different RG and CG MSC markers. Correlation between each pair of culture is visualized through a colored circle and the correlation coefficient (color defines the direction of relationship and the correlation coefficient indicates degree of association, ± 1 indicates a perfect association)

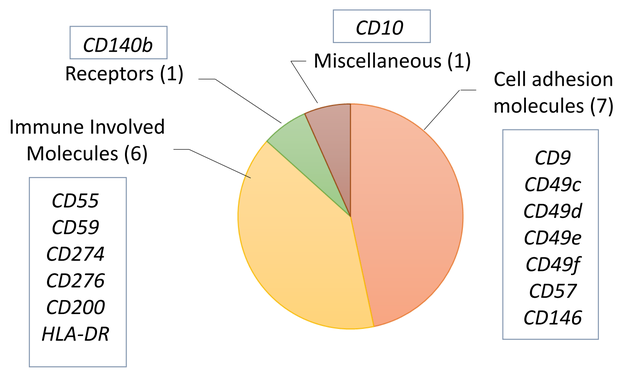


Figure 2: Markers that show significant differential expression between RG and CG cultures

- **Strong correlation in markers expression for different CG cultures. Idem for RG cultures**
- **RG and CG are close in phenotype**
- **15/61 markers distinguish CG from RG**

2 *Phenotype change upon conditioning*

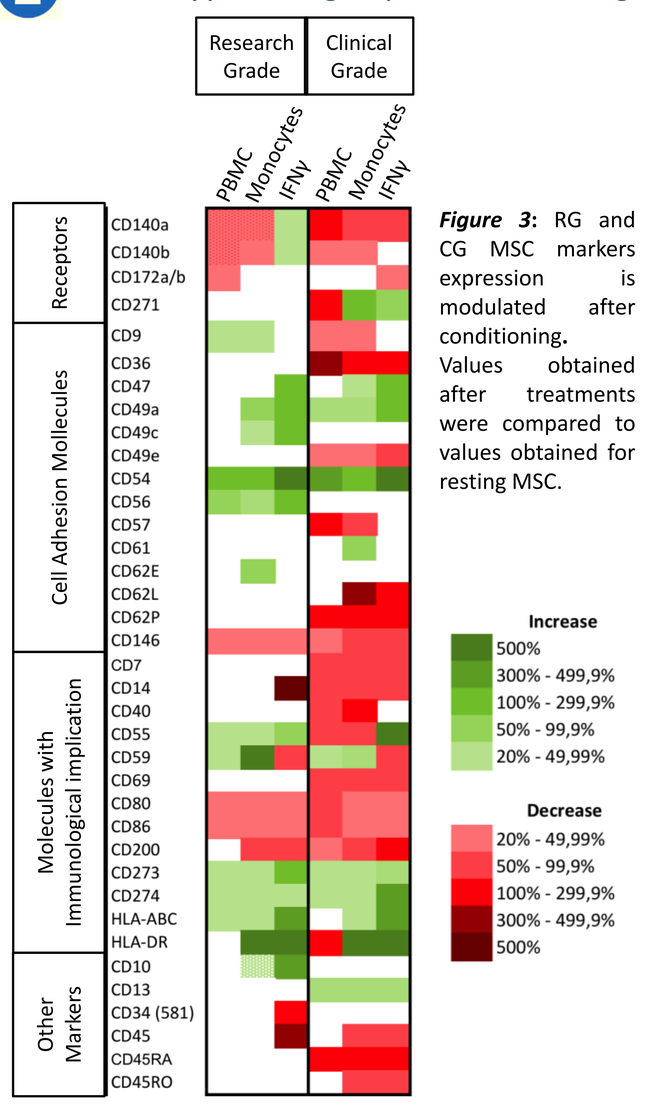


Figure 3: RG and CG MSC markers expression is modulated after conditioning. Values obtained after treatments were compared to values obtained for resting MSC.

- **CG MSC are more sensitive to activation than RG**
- **Some markers are modulated by all treatments**
- **Some markers are exclusively modulated by 1 tx.**

3 *Phenotype change upon conditioning (CyTOF analysis)*

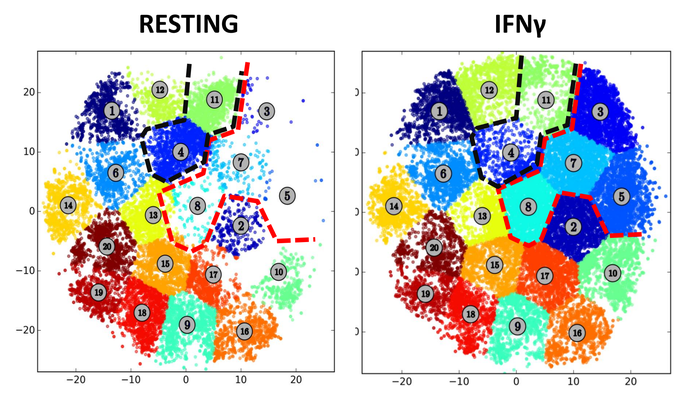
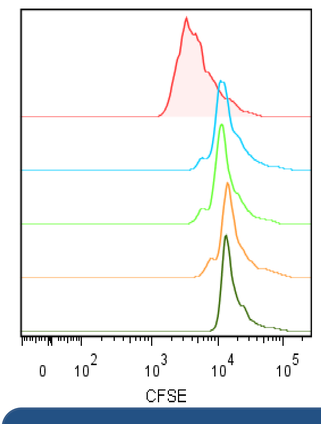


Figure 3. ACCENSE-generated clusters show 20 MSC subpopulations based on their different phenotypic profiles. After IFNγ treatment some clusters are enriched (red) and others impoverished (black)

- **IFNγ modulates sub-populations enrichment.**

4 *Functional Capacities Assessment*



Sample Name
PBMC without MSC
PBMC + Resting MSC
PBMC + PBMC conditioned MSC
PBMC + monocytes conditioned MSC
PBMC + IFN gamma conditioned MSC

Figure 4. Activated PBMC's proliferation is reduced in presence of MSC compared to PBMC placed alone in culture. Proliferation and CFSE FMI are inversely correlated.

- **Resting and Conditioned MSCs block PBMC activated in-vitro proliferation.**

Conclusions

This study first shows that research and clinical grade MSC share close phenotypes. This work also unveils phenotypic and functional markers of MSC along with their modulations according to different treatments. The CyTOF definition of new subpopulations may contribute to validate a cell therapy product for immunomodulation purposes.

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