Role of inflammation on the therapeutic efficacy of mesenchymal stem cell (MSC) in preclinical models of autoimmune and inflammatory disorders

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M2 Biologie Santé - October 13th 2017
Basic principles of autoimmunity

- AD are characterized by inflammatory disorders
- AD, such as T1DM, IBD or RA, are estimated to affect 7.6–9.4% of the population
- The prevalence of autoimmune diseases is typically higher in women than in men; SLE is an extreme example, with a 10:1 female to male ratio.
- The two major cellular components of the adaptive immune system are B cells and T cells.
Why the immune system attacks the self?

- **Modifications in self-antigens due to:**
  - Smoking, pollution
  - Medical treatment
  - Viral infection
  - Bacterial infection....

- **Modifications in the immune response:**
  - Development of pathogenic auto-antibodies
  - Abnormal lymphocyte development and differentiation
Two types of autoimmune diseases: organ-specific and systemic

**Organ-specific**

- Immune system attack self-antigens in a particular organ or tissue
- It leads to an impairment of the structure and function the targeted organ or tissue

**Systemic**

- Self-anti-gens are widespread for autoimmune attack
- Treatment used aimed at repressing the excessive activation of the immune system
Organ- and non organ-specific autoimmune diseases

- **Organ-specific:**
  - Hashimoto thyroiditis
  - Thyrotoxicosis
  - Addison’s disease
  - Atrophic gastritis
  - Juvenile diabetes mellitus
  - Multiple sclerosis

- **Non organ-specific**
  - Systemic Lupus Erythematosus
  - Rheumatoid Arthritis
  - Crohn
  - Scleroderma
  - Dermatomyositis
Organ- and non organ-specific autoimmune diseases

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Impaired immune response in SLE

- Defective apoptotic clearance allows deposition of immune complexes which can stimulate B and T cells.

- Hyperactive B cells then produce auto-antibodies which activate complement, causing tissue damage.

- Plasmacytoid dendritic cells (pDCs) activated by immune complexes then release excessive interferon α/β (IFNα/β), again causing tissue damage.
Organ- and non organ-specific autoimmune diseases

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Rheumatoid arthritis is a systemic autoimmune disorder

- Women are more likely to be affected than men

- RA has been associated with certain of the HLA class II molecules.

- HLA-DR1 and DR4 occur in 70% of patients with RA
Organ- and non organ-specific autoimmune diseases

➢ Organ-specific:
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  - Scleroderma
  - Dermatomyositis
Crohn’s disease: distribution

- Upper GI
- Ileocolic (most common)
- Small Intestine
- Colon
Common symptoms of Crohn’s disease

- Diarrhea
- Abdominal pain and tenderness
- Loss of appetite and weight
- Fever
- Fatigue
- Rectal bleeding and anal ulcers
- Stunted growth in children
Impaired immune response in Crohn’s disease

Where function is completely lost and cannot be substituted by hormones, as many occur in lupus nephritis or chronic rheumatoid arthritis, tissue grafts or mechanical substitutes may be appropriate.

Conventional immunosuppressive therapy can be used to damp down the immune response but, because of the dangers involved, tends to be used only in life-threatening disorders such as SLE and dermatomyositis.

Anti-inflammatory drugs are prescribed for RA with the introduction of selective cyclo-oxygenase-2 (COX-2) inhibitors.

For those with more established disease, attention has been focused on the synergistic treatment with anti-TNFα plus methotrexate.

Most of autoimmune diseases are treated with medications that alleviate specific symptoms.
T cell mediated autoimmune disease e.g. RA, MS

B cell mediated autoimmune disease e.g. RA, SLE, T1D, Crohn

Th17

Th1

B cell

MSC

MSC

Treg

Th3

Tr1

Breg

M2

ToIDC

Immunregulatory response

Soluble factors

Cell contact

PGE₂, IL10

ICAM1, VCAM1, CCL20

IL-6, IL-1β

PGE₂, IL10

IDO, HLAG5, Galectin, TGFβ, HGF

ICAM1/VCAM1, PD1/PDL1

CCL2, PD1/PL-L1

PGE₂, IL-6

PGE₂, IL10

TGFβ, IDO

PGE₂, IL10

IL10

MSC

IFNγ

TGFβ

IFNγ+TNFα

ICAM1/VCAM1

PD1/PDL1

CCL2

PD1/PL-L1

PGE₂, IL-6

PGE₂, IL-6

HO-1

IDO

PGE₂

IL10

IDO

PGE₂

IL6

IDO

HLAG5

Galectin

TGFβ

HGF

ICAM1/VCAM1

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CCL2

PD1/PL-L1

PGE₂, IL-6

PGE₂, IL-6

HO-1

IDO

PGE₂

IL10

IDO

PGE₂
<table>
<thead>
<tr>
<th>Disease model</th>
<th>Anti-inflammatory MSC effects</th>
<th>Negative/neutral studies?</th>
</tr>
</thead>
</table>
| Type 1 diabetes               | ↑ Regulatory T cells[^15-17,16]  
|                               | ↑ Inflammatory T cells[^14,17]  
|                               | Tw1 → Tw2[^13,16]  
|                               | ↑ Tissue repair[^18-20]  
|                               | |  
| Experimental autoimmune arthritis | ↑ Inflammatory cytokines[^27,29-31]  
|                               | ↑ Regulatory T cells[^27,30,34]  
|                               | ↑ T cell responsiveness[^27]  
|                               | ↑ IL-10[^22-25]  
|                               | ↑ Tr1/Tr17 cells[^29,30]  
|                               | ↑ Tr2 cells[^30,34]  
|                               | |  
| Graft-vs-host disease         | ↓ Auto-antibodies[^26]  
|                               | ↑ Inflammatory cytokines[^29,32,41,50]  
|                               | ↑ Regulatory T cells[^40]  
|                               | ↓ T cell proliferation[^41]  
|                               | ↑ Tr1 cells[^49]  
|                               | |  
| Systemic lupus erythematosus  | ↓ Anti-DNA antibodies[^50-55]  
|                               | ↑ Regulatory T cells[^54]  
|                               | ↑ T cell frequency[^50]  
|                               | ↑ Tr17 cells[^52]  
|                               | |  

[^15-17]: Klinker MW and Wei CH World J Stem Cells 2015
### Effect of MSC in mouse and rat models of arthritis

#### Reference, outcome

<table>
<thead>
<tr>
<th>Reference, outcome</th>
<th>Model</th>
<th>Transplant type</th>
<th>Number of animal used (N)</th>
<th>Dose of MSC</th>
<th>Route of administration</th>
<th>Time of treatment</th>
<th>Outcome of treatment/arthrits induction agent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MOUSE models</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Djouad, et al. [48], no change</td>
<td>CIA</td>
<td>Allogeneic</td>
<td>5–11 per group</td>
<td>$1 \times 10^6$ or $4 \times 10^6$</td>
<td>Tested IV, IP, IA, and IM</td>
<td>Day 0 or 21</td>
<td>In the CIA model of arthritis, MSC did not confer any benefit. IV injection of $4 \times 10^6$ MSC led to an increased incidence of arthritis, together with higher radiologic and histologic swelling scores ($P &lt; 0.01$). CIA-Bovine type II collagen in in Freund's incomplete adjuvant</td>
</tr>
<tr>
<td>Choi [47], delay onset—no change in severity</td>
<td>CIA</td>
<td>Syngeneic</td>
<td>10 per group</td>
<td>$1 \times 10^6$, 3 doses</td>
<td>IV</td>
<td>On days 21, 28, and 35 after primary immunization</td>
<td>This study demonstrated that systemic administration of IL-10-transduced MSC can delay the onset and reduce the clinical severity of CIA ($P &lt; 0.01$). However, naive MSC could delay arthritis onset they failed to reduce severity of arthritis. CIA--murine type II collagen in CFA</td>
</tr>
</tbody>
</table>

Hynes K. et al. Sem in Arth and Rheum 2016
### Effect of MSC in mouse models of arthritis

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Dose Details</th>
<th>Route</th>
<th>Outcome</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gonzalez et al. [50], improvement</td>
<td>CIA</td>
<td>(1) A single dose of $1 \times 10^6$ or 5 doses over 5 days</td>
<td>IP or IA</td>
<td>With and after disease onset</td>
<td>Human ADMSC completely abolished progression of arthritis at onset the disease ($P &lt; 0.001$). The beneficial effect of human ADMSC on CIA was not restricted to a xenogeneic system, since both syngeneic and allogeneic murine ADMSC were as efficient as human ADMSC at ameliorating the clinical signs of arthritis ($P &lt; 0.001$). CIA–chicken type II collagen with added heat-killed Mycobacterium Tuberculosis H37Ra</td>
</tr>
<tr>
<td>Bouffi et al. [44], No effect</td>
<td>CIA</td>
<td>(1) 1 or 2 doses of $1 \times 10^6$</td>
<td>IV</td>
<td>Assessed before and after onset—days 18, 24, 28, 32, and combinations of those</td>
<td>MSC effectively inhibit collagen-induced inflammation during a narrow therapeutic window ($P &lt; 0.05$). Day 18 and 24, injection after disease onset did not prevent arthritis signs. CIA–bovine type II collagen in CFA</td>
</tr>
<tr>
<td>Chen et al. [45], deterioration</td>
<td>CIA</td>
<td>(1) $1-2 \times 10^6$</td>
<td>IV</td>
<td>Day 0 or 21</td>
<td>Fli-1 + MSC did not confer therapeutic benefits. Clinical symptom scores and histological evaluation suggested a significant worsening of arthritis in mice treated with MSC at day 21 ($P &lt; 0.01$).</td>
</tr>
</tbody>
</table>

Hynes K. et al. Sem in Arth and Rheum 2016
30 studies included in this review demonstrate that different MSC-like populations exhibit variable therapeutic potential for the treatment of arthritis.

Out of the 30 studies, 19 reported a significant improvement in the clinical scores and 11 either identified a significant deterioration effect in arthritis scores or an absence of effect after MSC treatment.

What is the explanation for these discrepancies?
Plasticity of MSC in immune modulation


A

Acute Inflammation

Tissue Damage

Blood vessel

Chemokines

Lymphocyte recruitment

Licensed MSCs

IDO, NO

Tissue repair

Monocyte

M1 Macrophage

IFN-γ, TNF-α

MSCs

B

Chronic Inflammation

Tissue Damage

Blood vessel

Chemokines

Lymphocyte recruitment

MSCs

IDO, NO

Tissue damage

Monocyte

M2 Macrophage

IFN-γ, TNF-α

MSCs
MSC polarization

Pro-inflammatory MSC

Anti-inflammatory MSC

Effector T cells

TGF-β

Treg cells

PGE2, IL6, NO, IDO, chemokines

TNF, IFN-γ, IL-17, IL-1

Effector T cells
Combination of IFNγ and TNFa induces a synergist polarization of MSC

- Multiplex ELISA

Jin et al. Scientific Reports. 2016
LVD switches MSC toward an inflammatory phenotype and impairs their reparative potential

- Left ventricular dysfunction (LVD) and heart failure are associated with low grade, local and systemic inflammation

- The environment of the failing and infarcted myocardium drives resident and transplanted MSCs toward a pro-inflammatory phenotype, and restricts their reparative effects

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Naftali-Shani N. et al. Circulation 2017
Large scale analysis on 13 different tissues revealed that short-term persistence of hASC after injection is not related to the inflammatory status of mice.

Although therapeutic, hASC are not protected from elimination when present under moderate or severe inflammatory environment.

While inflammation is required for MSC immunosuppressive functions, it does not enhance their capacity to survive in vivo.
MSC from different sources have disparate immunosuppressive properties in vitro and in vivo

**In vitro**

- % Proliferation
- Count

**In vivo**

- Score

Luz-Crawford et al. ARD 2016
Luz-Crawford et al. Stem Cells 2015
These discrepancies between studies might be due to:

- MSC cell sources
- Route of MSC administration
- Transplant type (allogenic, syngenic, xenogenic)
- Treatment timing
- MSC dose
- The number of doses administrated
- Plasticity of MSC

While one or more of these factors have been identified to influence the therapeutic effect of MSC, there are no clear associations with efficacy
Questions:
- How to ensure that MSC-based therapies can meet the needs of the patient?
- How to select therapeutic agents/MSC?

Means:
Identification of a marker that could predict and direct on the long term both MSC immunosuppressive and therapeutic properties
Peroxisome proliferator-activated receptors (PPARs) are key regulators of physiological and immunological processes.

- PPARβ/δ is the most widely expressed PPAR family member, expressed in different organs such as brain, heart, lung, liver, kidney but also skeletal muscle and intestine.

- PPARβ/δ activation promotes the differentiation of MSC into osteoblasts.

- PPARβ/δ possesses anti-inflammatory activities including inhibition of cytokine production, NF-κB signaling and cell adhesion molecule expression.

*Scholtysek C et al. Nature Medicine 2013*
PPARβ/δ is a inhibitor of NF-κB signaling

- Inflammatory cytokine-induced ICAM1 and VCAM1 in MSC are critical for immunosuppression (Ren et al. JI 2010)
- Does PPARβ/δ govern the molecular mechanisms of immunosuppression on MSC?

Daynes and Jones, Nat Rev Immunol 2002
Inverse correlation between *Ppard* expression and MSC immunosuppressive potential

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Luz-Crawford et al. ARD 2016
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PPARβ/δ inhibition increased the suppressive properties of hBM-MSC

- Day -1: irreversible antagonist of PPARβ/δ
- Day 0: Activated PBMC
- Day 3: Analysis of PBMC proliferation by FACS
Inhibition or knockdown of PPARβ/δ in MSC primes their immunoregulatory functions
PPARβ/δ inhibition significantly increases ICAM, VCAM and NO by MSC.
PPARβ/δ inhibition significantly increases ICAM, VCAM and NO by MSC

- PPARβ/δ might play a critical role on the immunosuppressive properties of MSCs likely by enhancing the expression of adhesion molecules and the production of NO₂.
Role of PPARβ/δ in the interaction between MSC and T cells
Role of PPARβ/δ in the interaction between MSC and T cells

These data correlate with the significantly higher expression levels of VCAM and ICAM in PPARβ/δ−/− MSC as compared to PPARβ/δ+/+ MSC.
hMSC modulate T-cell responses via TNFα-mediated activation of NF-κB

Dorronsoro et al, Eur J Immunology, 2014
PPARβ/δ orchestrates NF-κβ activity in MSC
PPARβ/δ modulates iNOS expression through NF-κβ

- PPARβ/δ modulates iNOS expression through NF-κβ
- iNOS expression
- iNOS promotor
Mechanisms that regulate the secretion of soluble factors

- NF-κB
- PPAR
- Cox2
- iNOS
- NO
- VCAM-1
- ICAM-1
- TNFR-1
- TNF-α
- PGE2
- Cox2
- NO
- iNOS
PPARβ/δ deficiency increases the benefit of MSC in arthritis when injected before and after the onset of the disease.
MSC for the treatment of autoimmune diseases:

Clinical trials
2004

- First successful clinical application of MSCs to treat severe steroid- and cyclosporine-resistant GvHD in a 9-year-old boy (Le Blanc et al. Lancet, 2004)

2015

- 29 clinical trials using MSC in the treatment of GvHD
- Currently, there are 653 registered clinical trials in different clinical phases evaluating the potential of MSC-based cell therapy

To date, 63 autoimmune-disease clinical trials have been registered, out of which 15 are completed, 20 are open for recruitment (ClinicalTrials.gov).
MSC for the treatment of Crohn’s disease
- Phase II study: 49 patients with complex perianal fistulas (14 with Crohn)
  2 arms: injection of autologous ASC (20x10^6) + fibrin glue/fibrin glue alone

- Fistula healing in 70% of patients who received ASCs in addition to fibrin glue
  compared with 16% of patients who received fibrin glue alone

Garcia-Olmo et al, Dis Colon Rectum 2009
Local injection of BM-MSC and Crohn in humans

- Phase I study: 10 Crohn patients with external fistulas
  + local injection of autologous BM-MSC
  (up to 4 injections of $20 \times 10^6$ cells at 4w intervals)

Disease remission: CDAI $\leq 150$
  at 3-12 month

Increased CD25$^+$Foxp3$^+$ cells in rectal mucosa and blood
IV injection of BM-MSC and Crohn in humans

- Phase II study: 16 Crohn patients refractory to anti-TNF with active luminal CD CDAI > 250
  + IV injection of allogeneic $2 \times 10^6$ BM-derived MSC /Kg/week for 4 weeks

Day 0

Month 4

- 12 patients with a clinical response
- Clinical remission achieved in 8 patients
- 7 patients with endoscopic improvement
MSC for the treatment of SLE
BM-MSC transplantation in active SLE

- A pilot clinical study: 15 patients with persistently active SLE were enrolled and underwent BM-MSCT. A total of 13 patients have been followed for more than 12 months.
  - No serious adverse events were reported
  - All patients clinically improved following treatment with MSC with a marked decrease in the SLEDAI score and 24 h proteinuria
  - At 12-month follow-up, SLEDAI scores and proteinuria decreased

Allogeneic BM-MSCT in patients with refractory lupus resulted in amelioration of disease activity, improvement in serological markers and stabilisation of renal function

Liang et al., ARD 2010
- Phase I/II: Multicenter clinical trial to assess the safety and efficacy of allogeneic UC MSC transplantation in 40 patients with active SLE + injection of allogeneic UC IV at D0 and D7.

  - UC-MSC injection was well tolerated, and no transplantation-related adverse events
  - 13 patients achieved MCR, 11 PCR and 3 and four 4 experienced disease relapse at 9 months and 12 months of follow up

  UC-MSCT results in satisfactory clinical response in SLE patients. However, the patients that experienced disease relapse after 6 months, suggest the necessity to repeat MSC after 6 months.

Wang et al., Arthr Res Ther 2014
MSC for the treatment of RA
Phase IB/IIA study: 53 patients with moderate to high disease activity received 3 IV ASC infusion at day 1, 8 and 15:

- First cohort: 1 million stem cells/kg administered at days 1, 8 and 15
- Second cohort: 2 million stem cells/kg administered at days 1, 8 and 15
- Third cohort: 4 million stem cells/kg administered at days 1, 8 and 15

Placebo Lactate Ringer’s solution

- IV infusion of ASC was well tolerated along 24 weeks
Clinical activity scores after ASC injection in RA patients

Safety of the treatment

Potential efficacy of cell therapy with allogeneic eASC in RA
Preliminary results with Phase I/II studies are encouraging: safety and some preliminary evidence of efficacy,

Evidence that MSCs could modulate the immune responses via Treg cell induction

Possibility that MSCs may contribute to tissue repair (differentiation, paracrine effect: reduced apoptosis,...)

Still questions:
  • what administration route?
  • what doses? One or several injections?
  • safety of ex vivo expanded MSCs?
  • autologous versus allogeneic source?
Acknowledgments

IRMB- INSERM U1183- Montpellier, FRANCE
Group « Stem Cells, Blastema and Regeneration »

Patricia LUZ-CRAWFORD
Rafael CONTRERAS
Marc MATHIEU
Gautier TEJEDOR
Audrey BARTHÉLAIX
Beryl LAPLACE-BUILHE
Mai NGUYEN CHI
Sébastien RIQUIER
Mireille GALLONI
Dora SPAEDE

Christian JORGENSEN
Danièle NOEL
Karine TOUPET

Collaborators
Maroun Khoury (University de Los Andes, Chile)
Fernando Figueroa (University de Los Andes, Chile)
Gerhard Kronke (Erlangern, Germany)
Natacha Ipseiz (Cardiff)
Eric Morand (Monash Medical Center, Australia)