Maximizing the Therapeutic Potential of Mesenchymal Stromal Cells

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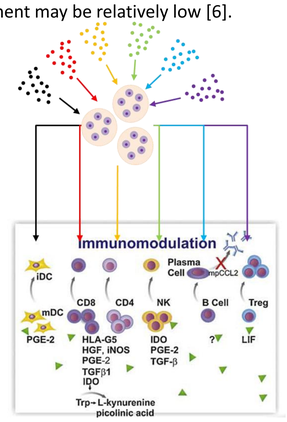
Background

Mesenchymal stem/stromal cells (MSCs) are adult stem cells that are attractive as a cellular therapeutic due to their non-immunogenicity and many therapeutic effects achieved primarily via paracrine mechanisms [1]. Of particular interest is their ability to modulate immune/inflammatory responses. Despite promising clinical trial results regarding the safety of administering single-cell suspensions of MSCs to human patients, achieving significant therapeutic efficacy has been more elusive [2,3]. Inefficient homing of these cells to target tissues and their rapid clearance necessitates the use of very large and often repeated doses [4]. One aspect of MSC biology that may be important to improving MSC therapies is that their therapeutic effects are not spontaneous, but need to be induced by external activating factors [5]. The degree to which this may be happening *in vivo* is not clear, especially when only a small fraction of cells make it to the target tissue or when the amount of stimulatory factors in the microenvironment may be relatively low [6].

We hypothesize that targeted exogenous activation and immobilization via alginate encapsulation of MSCs can improve the efficacy of MSC therapy by maximizing distinct immunomodulatory phenotypes (**Schematic 1**) and reducing the number of MSC needed to achieve significant therapeutic benefit.

As a whole, this translational work on MSC biology represents multiple thrusts of the Stem Cell IGERT on Integrated Science and Engineering of Stem Cells: molecular engineering of stem cells and environmental manipulation of stem cell behaviors (**Schematic 3**).

The purpose of the selected work presented here is to explore the activation of MSCs by a panel of exogenous inflammatory factors, using MSC-mediated modulation of innate immune cell phenotype as a metric.



Schematic 1

Experimental Setup

A panel of six inflammatory molecules were chosen to activate MSCs based on their common presence in inflammatory environments after injury or their previous use in literature in the context of MSCs [5]. To investigate the effects of combinations as well as single factors alone, a fractional factorial design (FFD) was generated using Statistical Analysis System (SAS) software, in which the 64 possible conditions were projected onto a subset of 32 conditions. (**Schematic 2**).

Schematic 2:

Factor (X_i)	Receptor	Concentration Level 0		Level 1	
X_1 LPS	TLR-4	0	µg/ml	1	µg/ml
X_2 Poly(I:C)	TLR-3	0	µg/ml	1	µg/ml
X_3 IL-6	IL6R/CD126	0	ng/ml	1	ng/ml
X_4 IL-1β	IL1R	0	ng/ml	1	ng/ml
X_5 IFN-β	IFNAR	0	ng/ml	1	ng/ml
X_6 IFN-γ	IFNGR1/2	0	ng/ml	1	ng/ml

2^6 possible activation cocktail combinations reduced by half to 2^5 using FFD

2^{6-1}(VII)
2 levels
6 factors
½ fraction
Resolution 6

Combination	MSC Activating Factors X_1 LPS	X_2 Poly(I:C)	X_3 IL-6	X_4 IL-1β	X_5 IFN-β	X_6 IFN-γ
1	0	0	0	0	0	0
2	1	0	0	0	0	1
3	0	1	0	0	0	1
4	1	1	0	0	0	0
5	0	0	1	0	0	1
6	1	0	1	0	0	0
7	0	1	1	0	0	0
8	1	1	1	0	0	1
9	0	0	0	1	0	1
10	1	0	0	1	0	0
11	0	1	0	1	0	0
12	1	1	0	1	0	1
13	0	0	1	1	0	0
14	1	0	1	1	0	1
15	0	1	1	1	0	1
16	1	1	1	1	0	0
17	0	0	0	0	1	1
18	1	0	0	0	1	0
19	0	1	0	0	1	0
20	1	1	0	0	1	1
21	0	0	1	0	1	0
22	1	0	1	0	1	1
23	0	1	1	0	1	1
24	1	1	1	0	1	0
25	0	0	0	1	1	0
26	1	0	0	1	1	1
27	0	1	0	1	1	1
28	1	1	0	1	1	0
29	0	0	1	1	1	1
30	1	0	1	1	1	0
31	0	1	1	1	1	0
32	1	1	1	1	1	1

48 hr Activated-MSC conditioned media (CM) spiked with 1 µg/ml LPS and used to culture macrophages (Macs)

Cocktails added to MSCs

Macs → Metrics: TNF-α (Y_2), IL-10 (Y_3)

MSCs → Metric: PGE_2(Y_1)

Analysis

NOTE:
+, 1, or +1 = "present in MSC-activating cocktail"
-, 0, or -1 = "not present in MSC-activating cocktail"

Human bone marrow-derived MSCs were plated as monolayers in a 96-well plate and subsequently cultured in fully supplemented medium containing the combinations of activating factors . The conditioned media from these activated MSCs were collected, spiked with LPS, and transferred to macrophages isolated from human peripheral blood. After culture, the media were collected and analyzed for macrophage-secreted TNF-α and IL-10. The resulting data was entered into SAS for statistical analysis of the single-factor and two-factor effects.

Macrophage Response to Activated MSC

Macrophages are important in innate immunity and play a big role after injuries and in many diseases with inflammatory aspects. They are capable of adopting a spectrum of phenotypes, including the pro-inflammatory (M1) and anti-inflammatory (M2), characterized in part by elevated TNF-α secretion and increased IL-10 secretion, respectively [7]. MSC have been demonstrated *in vitro* and *in vivo* to promote the M2 phenotype [8,9]. Therefore, macrophage-secreted TNF-α and IL-10 were used as metrics for this conversion mediated by activated MSC conditioned media (CM).

The individual effects of each activating factor were determined by comparing the TNF-α and IL-10 levels in all cases when the factor was present in the cocktail to the levels when it was not present (**Equation 1**). Statistics were then performed to determine if any individual factors caused significant MSC-mediated changes in macrophage TNF-α and/or IL-10 secretion.

$$\text{Main Factor Effects: } \begin{pmatrix}\text{avg. } Y_i \text{ when}\\ X_i \text{ is present}\end{pmatrix} \text{ vs. } \begin{pmatrix}\text{avg. } Y_i \text{ when}\\ X_i \text{ is not present}\end{pmatrix}$$

Equation 1

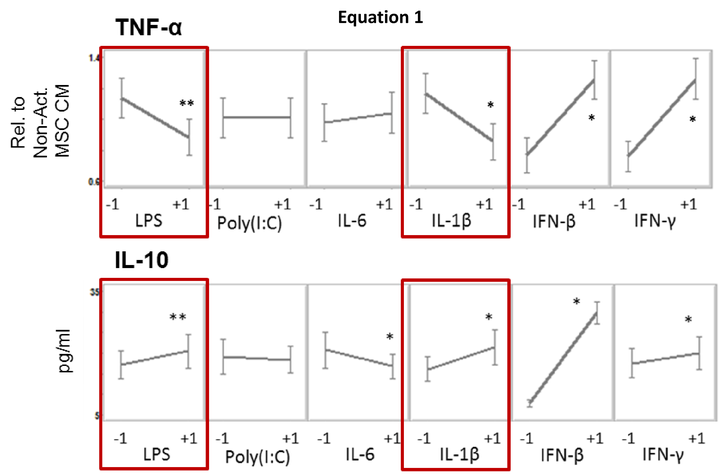


Figure 1 (*p<.0001, **p<.001 compared to "-1")

As seen in **Figure 1**, the treatment of MSC with multiple factors for 48 hours led to significant changes. LPS and IL-1β resulted in a simultaneous MSC-mediated reduction in TNF-α and increase in IL-10, indicating a switch from pro-inflammatory to anti-inflammatory macrophage behavior.

Treatment of MSCs with IFN-β or IFN-γ, however, led to a strong increase in the secretion of both of these cytokines, seemingly indicating an increase in both pro-inflammatory and anti-inflammatory macrophage behavior.

$$\text{Two Factor Interaction: } \begin{pmatrix}\text{avg. } Y_i \text{ when}\\ X_i -, X_j -\end{pmatrix} \text{ vs. } \begin{pmatrix}\text{avg. } Y_i \text{ when}\\ X_i +, X_j -\end{pmatrix} \text{vs. } \begin{pmatrix}\text{avg. } Y_i \text{ when}\\ X_i -, X_j +\end{pmatrix} \text{ vs. } \begin{pmatrix}\text{avg. } Y_i \text{ when}\\ X_i +, X_j +\end{pmatrix}$$

Equation 2

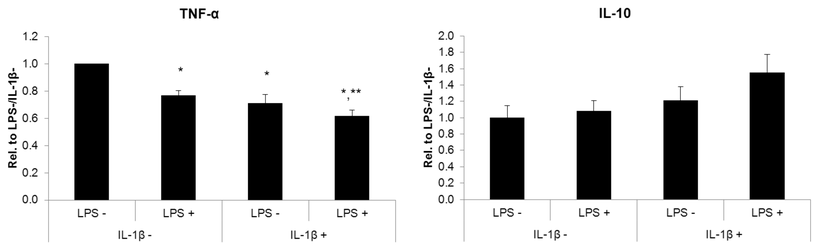


Figure 3 (*p<.01 compared to LPS-/IL-1β- , **p<.05 compared to LPS +/IL-1β -)

Two-factor interactions were explored to determine if combinations of two factors led to synergistic MSC-mediated effects on macrophages (**Equation 2**). Because they both produced a similar effect on MSC-mediated macrophage phenotypic switch from a pro- to an anti-inflammatory state, the two-factor interaction of LPS and IL-1β are shown in **Figure 3**. For the mitigation of macrophage TNF-α secretion, the presence of LPS together with IL-1β in the activating cocktail further enhanced MSC-mediated reduction of TNF-α, compared to LPS alone. Similarly for the MSC-mediated up-regulation of macrophage IL-10, IL-1β in combination with LPS trended higher than IL-1β or LPS alone.

MSC Secreted Factors

To begin to elucidate the mechanism by which these activating factors induced MSC-mediated modulation of macrophages towards the M2 phenotype, MSC CM was analyzed for prostaglandin E_2 (PGE_2), a secreted molecule demonstrated to be a very potent promoter of M2 macrophages [10].

MSC secretion of PGE_2 was greatly affected in response to the current panel of activating molecules, with LPS, Poly(I:C), and IL-1β inducing the strongest increases in PGE_2 (**Figure 4**).

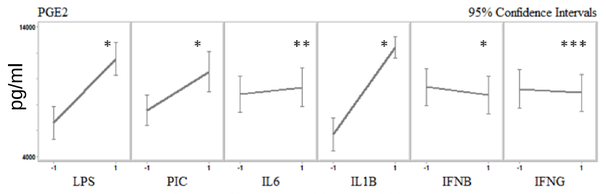
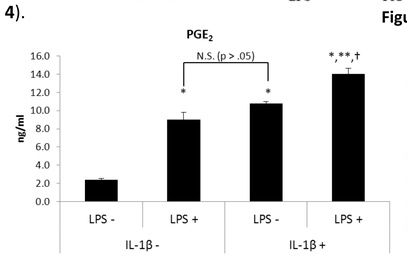


Figure 4 (*p<.0001, **p<.01, ***p<.05)



Because LPS and IL-1β had the most consistent effect on MSC-mediated macrophage phenotypic switch and MSC-secreted PGE_2, the two-factor interaction of these factors was again explored. **Figure 5** shows that these factors individually induced an elevation in PGE_2 to a similar degree, but synergistically increased PGE_2 even further.

Figure 5 (*p<.0001 compared to LPS -/IL-1β -; **p<.01 compared to LPS +/IL-1β -; †p<.0001 compared to LPS -/IL-1β +)

Summary, Discussion, and Future Work

FFD was used to investigate the effect of activating monolayer MSC with a panel of factors on MSC-mediated modulation of macrophage phenotype.

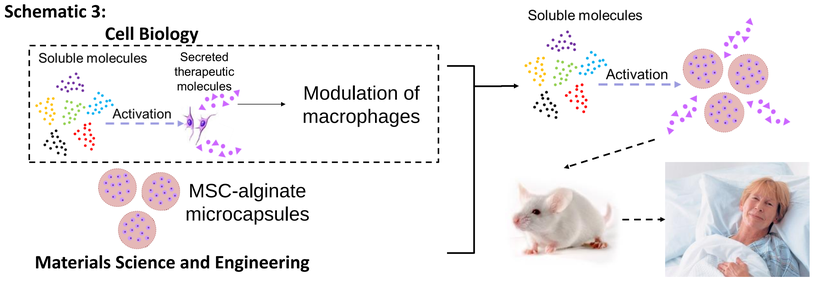
LPS and IL-1β similarly activated MSC in a way that resulted in a MSC-mediated macrophage phenotypic switch from M1 to M2. IFN-β and IFN-γ also had a significant effect, but seemingly promoted MSC-mediated pro- and anti-inflammatory macrophage characteristics simultaneously.

- These respective pairs of molecules share similar intracellular signaling pathways. These overlapping pathways can be further investigated to elucidate the mechanism(s) by which activated MSCs modulate macrophage inflammatory phenotype. These dose response of MSCs to these factors will also be characterized by titrating the cytokines over a range of concentrations.

LPS and IL-1β had strong individual and synergistic effects on MSC PGE_2 secretion. This correlated with synergistic effects between LPS and IL-1β seen for MSC-mediated promotion of macrophage IL-10 and reduction of TNF-α.

- As seen in **Figure 4**, Poly(I:C) and IL-6 appeared to also induce MSC PGE_2 secretion, but did not lead to MSC-mediated modulation of macrophage TNF-α and IL-10 secretion. This may indicate that other MSC-secreted factors may be important in differentially regulating MSC-mediated effects on macrophages. Therefore other MSC-secreted factors will be analyzed to further understand the mechanism(s) by which activated MSCs modulate macrophages.

Schematic 3:



The results of this work (dotted line, **Schematic 3**) will be validated and optimized for alginate-encapsulated MSCs. These activated, immobilized MSCs will then be tested in a murine model of disease/injury involving macrophages, such as spinal cord injury. These interdisciplinary efforts may one day lead to the development of improved MSC therapy that can achieve significant efficacy in human patients.

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