Received: 9 February 2021 | Accepted: 12 March 2021

DOI: 10.1002/stem.3380

CONCISE REVIEW

STEM CELLS

Macrophages at the nexus of mesenchymal stromal cell potency: The emerging role of chemokine cooperativity

Jacques Galipeau[1,2,3]

[1]Department of Medicine, School of Medicine and Public Health, University of Wisconsin in Madison, Madison, Wisconsin

[2]University of Wisconsin Carbone Comprehensive Cancer, Madison, Wisconsin

[3]University of Wisconsin Program for Advanced Cell Therapy, Madison, Wisconsin

Correspondence
Jacques Galipeau, MD, Department of Medicine, School of Medicine and Public Health, University of Wisconsin in Madison, Madison, WI.
Email: jgalipeau@wisc.edu

Funding information
National Institute of Diabetes and Digestive and Kidney Diseases, Grant/Award Number: R01DK109508

Abstract

Pharmacological depletion of macrophages in vivo with liposomal clodronate renders mice unresponsive to adoptive transfer of mesenchymal stromal cells (MSCs) for affecting outcomes of acute inflammatory pathology. This experimental observation identifies host macrophages as necessary in mediating the salutary anti-inflammatory properties of MSCs as a cellular pharmaceutical. This theory is supported by the observation that transfusion of MSCs leads to the prompt phagocytosis of nearly half of lung entrapped MSCs by lung resident macrophages, triggering an interleukin (IL)-10 suppressive efferocytotic response. In addition, non-phagocytosed MSCs with COX2 competency shape the immune milieu by inducing tissue macrophages to express IL-10. Additional experimental evidence identifies MSC-borne IL-6, IDO and TSG-6 as directly involved in macrophage polarization. Along similar lines of functional convergence, implantation of $CCL2^+$ MSCs in the extravascular space where interaction with lung resident perivascular macrophages is not operative, also leads to IL-10 polarization of $CCR2^+$ macrophages within acute injured tissue far removed from MSC depot. Intriguingly, MSC-derived CCL2 on its own is not sufficient to polarize macrophages and requires heterodimerization with MSC-borne CXCL12 to trigger macrophage IL-10 polarization via CCR2, but not CXCR4. Such chemokine cooperativity opens a new venue for analysis of MSC potency especially considering the rich chemokine secretome of MSC exposed to inflammatory stimulus. As an aggregate, these data highlight a necessary MSC and host macrophage functional dyad that may inform potency attribute analysis of MSCs—including the chemokine interactome—that may be directly linked to in vivo clinical anti-inflammatory and regenerative response.

KEYWORDS
CCL2, CCR2, chemokine, efferocytosis, IL-10, macrophage, mesenchymal stem/stromal cell, monocyte

1 | INTRODUCTION

Pragmatic human clinical trials have led to the marketing approval in Europe of allogeneic adipose-derived mesenchymal stromal cells (hereafter MSC[1]) as a living cellular pharmaceutical improving the clinical outcome of Crohn's disease associated entero-cutaneous perianal fistulas.[2] Similarly promising regulatory outcomes in USA for use of marrow-derived MSC in pediatric steroid resistant graft vs host disease (GVHD) were highly anticipated but unfulfilled.[3] Notwithstanding, placebo-controlled randomized phase III trials are ongoing worldwide exploiting the hypothesized paracrine mechanism of action of MSC for treatment of acute inflammatory tissue injury syndromes.[4] Biological insights on MSC mechanism of action in human clinical studies is typically limited to in vitro correlative studies attempting to plot a direct relationship between MSC cellular attributes and human subject clinical and biological predictive markers of objective response.[5] This missing link between MSC functional attributes (eg, "potency") and clinical outcomes remains the Achilles heel of marketing approval in international regulatory jurisdictions.[6,7] A remedy to this shortcoming is to eek insights from a comparative biology perspective examining preclinical models of disease especially the study of MSC attributes that are shared between human and animal systems as well as the host response.[8] The scope of this concise review is to provide such an analysis interrogating murine systems that provide insight in the mechanism of action of MSC and the emerging role of MSC-borne chemokines in host macrophage response for clinical effect in acute inflammatory tissue injury syndromes.

Significance statement

The present study examines the relationship between culture-adapted mesenchymal stromal cells (MSCs) and their effect on macrophage polarization as a cornerstone of pharmaceutical effect in resolving inflammatory and tissue injury syndromes in vivo. The contribution of MSC-borne chemokines, CCL2 in particular, and the recently recognized biological role of CC/CXC chemokine heterodimers found in MSC secretome as potent agents of immune modulation provide novel insights in identifying MSC cellular attributes predictive of clinical response.

2 | HOST MACROPHAGES ARE NECESSARY FOR MSC PHARMACEUTICAL EFFECT—IN VIVO VERITAS

Blood monocytes and their tissue bound congeners play an important role in host defense against infectious pathogens as well as in tissue homeostasis and injury response.[9] The canonical view of macrophage response to injury is reflected in a biphasic sequence of chemokine mobilized $CCR2^+$ $CD14^+$ monocytes to site of injury that deploy a pro-inflammatory innate host defense reaction followed by tissue repair and remodeling.[10,11] Functional classification of monocytes following their leukine secretome pegs resting monocytes as M0, pro-inflammatory as M1 and reparative as interleukin (IL)-10^+ M2[12] with the understanding that a spectrum of possible phenotypes span these categorizations. Canonical IRF/STAT signaling pathways are activated by interferons and TLR signaling to skew macrophage function toward the M1 phenotype (via STAT1) or by IL-4 and IL-13 to skew toward the M2 phenotype (via STAT6).[13] In the setting of tissue injury, the salutary effects of an abbreviated M1 phase and expedient M2 function would foreshadow lessened injured tissue loss and improved restoration outcomes.[14] Herein lies a biologically plausible mechanistic link between MSC transfusion and host macrophage biology as intimated by in vivo monocyte depletion studies. It is well established that intravenous administration of liposomal clodronate will lead to prompt apoptosis of blood monocytes as well as tissue-resident phagocytic macrophages in mice while sparing non-phagocytic blood and tissue borne lymphomyeloid cells.[15] Using this methodological approach to deplete monocyte/macrophages in vivo, the Mezey group at NIH demonstrated in 2009 the necessary role of host phagocytic myeloid cells in mediating the anti-inflammatory effects of intravenously transfused MSCs.[16] These data using clodronate depletion of host macrophages to validate their essential role in mediating MSC protection to murine acute tissue syndromes were replicated independently in preclinical models of acute kidney injury,[17] ARDS,[18] allergic airway disease,[19] experimental autoimmune uveitis,[20] and DSS-induced acute colitis.[21] An important insight is that clodronate treated mice retain a normal lymphoid compartment of T-, B-lymphocytes, and NK cells suggesting that—in the absence of macrophages—a direct MSC to lymphoid cell interaction in vivo is not sufficient for their clinical anti-inflammatory effects as predicated by surrogate in vitro assays in common use to assess MSC potency.[5] Therefore, the adoptive transfer of culture adapted MSCs in preclinical models of tissue injury and inflammation apparently provides an impetus for accelerated macrophage M2 IL-10^+ functional polarization that is required for improved clinical outcomes. Focusing on reductionist in vitro assay systems interrogating the interaction of MSC with human blood monocytes shows that co-culture of marrow-derived MSCs with purified human blood $CD14^+$ monocytes polarizes monocytes to a M2-like $CD206^+$, IL-10 high, IL-12 Low, IL-6 High, and TNF-α low cytokine expression profile which can be replicated in part using cell-free MSC supernatant.[22] The polarizing of blood monocytes toward IL-10 M2 functionality is in part due to indoleamine dioxygenase (IDO) MSC activity and that these M2 polarized macrophages inhibit proliferation of bystander T-cells.[23] Complementary in vitro approaches examining human blood monocyte subsets defined by CD14 and CD16 surface expression[24] demonstrated that MSC secretome skews a substantial subset of $CD14^{+++}CD16^-$ "classic" monocytes to an "intermediate" $CD14^{++}CD16^+$ phenotype[25] which represent a functionally separate subpopulation with high IL-10 competency and anti-inflammatory properties.[26] These data are congruent with the

murine in vivo defining of MSC licensing host tissue macrophages to produce IL-10 upon which the anti-inflammatory response rests.

3 | MSC AND MACROPHAGE DYAD—PARTNER AND PREY

Cell tracking studies of intravenously transfused MSCs in mice have shown that cells are retained in lung microvasculature, promptly extravasate within hours, and co-mingle with lung resident macrophages with nearly half the input subjected to phagocytosis.[16] The macrophage phagocytosis of whole MSCs (eg, efferocytosis) in itself leads to an IL-10 and TGFβ driven anti-inflammatory response,[27] a phenomenon also observed in uptake of mitochondria-laden MSC subcellular vesicles,[28] p-bodies,[29] or dead MSCs.[30] Efferocytosis of blood-borne stromal elements has also been invoked as a pivotal mechanism for feto-maternal tolerance driven by maternal macrophage recycling of placenta shed fetal allogeneic stromal elements[31] which supports the theorem that MSC transfusion may trigger tolerogenic pathways evolved from mammalian reproductive biology. The intravenous bolus transfusion of live or killed MSCs elicits a lung efferocytotic response with phagocytic engulfment and secondary migration of MSC-laden macrophages to liver within 72 hours[32] from which they promptly dissipate. Killed MSCs are an ineffective substitute for their live counterpart in affecting clinical outcomes in murine models of tissue injury[33] speaking to the physiological relevance of live MSC and macrophage intercellular functional dyad as necessary component of MSC mechanism of action, independently of efferocytosis.[34,35] Defining the MSC-derived elements that directly alter macrophage physiology as a necessary step in a cascade of cellular events leading to desirable anti-inflammatory pharmaceutical effect would inform the mapping of MSC cellular attributes that would be predictive of in vivo potency.

4 | INSIGHTS FROM ENDOGENOUS MSC PROGENITORS' ROLE IN MARROW MACROPHAGE MOBILIZATION

The study of marrow-resident mesodermal progenitors in vivo and their role in regulating the hematopoietic niche can serve as an important source of hypothesis generating insights in mapping MSC functional attributes linked to macrophage biology.[36] Murine in vivo marrow mesenchymal fate mapping studies have demonstrated that leptin receptor (LepR) marks CXCL12 and stem cell factor (SCF)-expressing niche cells with robust in vitro CFU-F competency,[37-42] a feature dependent upon functional expression of both PDGFRα and PDGFRβ.[43,44] Analogous analysis of human marrow-derived CD271$^+$ MSCs maps CFU-F ontogeny to LepR$^+$/PDGFRαβ$^+$ progenitors which highlights the functional convergence of PDGFRαβ from a comparative biology perspective between mouse and human CFU-F properties.[45-47] Reductionist in vivo study of mesodermal progenitor function in mice has demonstrated that marrow-resident LepR$^+$ MSC mesodermal precursors will dynamically respond to TLR4 agonists such as LPS by upregulating expression of CCL2 chemokine that triggers egress of CCR2$^+$ marrow-resident myeloid cells and monocytes to the periphery.[48,49] Considering that TLR4 agonists also include alarmins such HMGB1, we can hypothesize that endogenous mesodermal progenitors can be activated by tissue damage as well[50-52] suggesting an important role in mobilizing marrow myeloid cells in response to injury. The key functional insight here provided is that the regulated expression of CCL2 by marrow stroma is directly linked to monocyte physiology in the setting of infection or tissue injury.

5 | CULTURE ADAPTED MSCS ARE FUNCTIONALLY ANALOGOUS TO TLR4 ACTIVATED MARROW MESODERMAL PROGENITORS

It is estimated that mice and humans contain $(1.1\text{-}2.1) \times 10^{10}$ nucleated bone marrow cells per kg body weight of which 0.1% are CD140a$^+$ (PDGFRα) stromal progenitors which translates to $(1.1\text{-}2.1) \times 10^7$ CD140a$^+$ marrow stromal progenitors/kg.[53] From this population of CD140a$^+$ stromal progenitors would arise the CFU-F clonogenic cells necessary for culture adaptation of marrow stromal progenitors for manufacture of MSC for pharmaceutical use. Typical clinical dosing of culture adapted MSC in human clinical trials is $(0.1\text{-}1.0) \times 10^7$ MSC/kg (when given intravenously as a bolus)[4] which numerically equates nearly the entire body content of analogous CD140a$^+$ marrow stromal progenitors. The presumed pharmaceutical effect of MSC administration may be attributed to the bulk effect of surnumerary stromal cellular elements transfused, however there are substantial qualitative properties of culture adapted MSCs which distinguish them from homeostatic endogenous marrow mesodermal progenitors from which they arise which may play a substantial role in their potency.[54] Indeed, single cell RNAseq analysis of mouse endogenous LepR$^+$ marrow stromal progenitors deploy a steady state transcriptome that is entirely distinct from mitogen stimulated culture adapted and actively replicating MSC. Focusing on chemokine secretome, endogenous marrow progenitors are mitotically quiescent and express CXCL12 constitutively and robustly, but little to no CCL2.[41,48] Whereas, mitotically active culture adapted MSC, not only express CXCL12 but spontaneously gain CCL2 competency[55,56] mirroring the transcriptome of TLR4-activated endogenous progenitors. The insight here provided is the direct functional relationship between TLR4-activated CCL2-expressing mesodermal progenitors and the mobilization of CCR2$^+$ myelomonocytic cells in vivo that provides a plausible mechanistic link to the necessary immune modulating role of CCL2 arising from use of culture-adapted MSC.[19,21,57,58] The importance of CCL2 expression as part of a potency functional attribute of culture adapted MSCs is reflected in the loss of anti-inflammatory function of CCL2-null MSCs in vivo and the reciprocal unresponsiveness of CCR2-null hosts to CCL2-sufficient MSCs.[21]

6 | MSC FUNCTIONALITY INVOLVED IN MACROPHAGE POLARIZATION

In a mouse model of bacterial sepsis, COX2 expression and prostaglandin E2 (PGE_2) production of intravenously administered MSC was essential for rescue of mice via tissue macrophages expressing prostaglandin receptors EP1/EP2 and latter IL-10 polarization.[16] In vitro reductionist analysis of human MSC suppressor effect also depends, in part, on MSC-expressed TSG-6, IL-6 and indoleamine dioxgenase (IDO) on bystander monocytes.[14,59-61] Similar close proximity immune suppression functional attributes are assignable to MSC surface bound CD271 (PD-L1),[62] CD73 (aspariginase),[63] and CD54 (ICAM-1).[64] All of the above imply physical contact, or very close proximity, to tissue resident macrophage responder cells to allow for their IL-10 polarization or anti-inflammatory functionality. Indeed, this cell-cell communication functionality is implied in studies of intravenous delivery of MSCs interacting with lung resident myelomonocytic cells. The aforementioned MSC tracking studies suggest that MSC and macrophage direct contact as well as efferocytic engulfment in vivo are a necessary component of MSC mechanism of action. However, administration of MSC in extravascular space—subdermal, intramuscular, or intra-peritoneal—leads to systemic anti-inflammatory effects in murine tissue injury models speaking to contact independent MSC/macrophage interplay.[65] Indeed, intra-peritoneal delivery of MSCs is more clinically potent than the highest tolerable intravenous bolus in mice to prevent death from gut injury, all of which is dependent on host macrophages.[33] A biologically plausible mechanism would entail that MSC secretome can act on tissue bound macrophage at a substantial distance from MSC depot site through persistence of live functional MSC and systemic recirculation of their secretome and subcellular elements.

7 | THE MSC/MACROPHAGE CHEMOKINE AXIS

Human and mouse blood monocytes and tissue macrophages abundantly express the chemokine receptor CCR2 which is functionally required for responsiveness to CCL2 in leading to macrophage mobilization and IL-10 polarization in vitro and in vivo.[19] The apparent paradox of CCL2/CCR2 dichotomy is that it is contradictorily invoked in the macrophage M1-driven inflammatory response as well as in anti-inflammatory M2 polarization. Recent developments in the understanding of chemokine protein biochemistry may provide some insights. The ~50 known chemokines are subdivided into four subfamilies based on relative positioning of the first two of four highly conserved cysteine residues: CXC, CC, C, and CX_3C.[66] All chemokines oligomerize into homodimers, and some into tetramers. Chemokine biology informs us that chemokines far outnumber chemokine receptors speaking to the concept of a chemokine interactome where a chemokine may activate more than one receptor.[67,68] Chemokine biology is nonetheless more complex than was initially predicted, as several studies suggest that heterologous chemokines can dimerize and that their receptors are found as dimers and/or higher order oligomers at the cell surface.[69] It has been found that CXC and CC chemokines can form heterodimers within family members and that it has been suggested that heterodimerization between members of CXC and CC subfamilies can also occur. The functional result is that heterodimerization dramatically modulates the biological activities—synergy as well as antagonism—of these chemokines.[70] Functional synergism can be achieved through receptor heteromerization or receptor retention at the cell surface or on conformational changes affecting receptor signaling.[67] CCL2 deploys vigorous promiscuity in heterodimerizing with non-self chemokines. In silico protein/protein binding affinities foreshadow CCL2 coupling with CC chemokines: CCL5 and CCL8 as well as with CXC chemokines: CXCL4 and CXCL8.[71,72] Chemokine receptors themselves can heterodimerize on cell surface of responder cells speaking to a chemokine/receptor signaling network that may alter responsive cell functionality far beyond mere chemotaxis.[73]

Culture adapted mouse and human marrow MSCs express a number of chemokines and the MSC chemokine transcriptome is substantially induced by inflammatory cues derived from activated lymphocytes[55,56,74] (Table 1). Aforementioned in silico and in vitro predictive heterologous chemokine studies affinity studies would foreshadow that MSC secretome contains CCL2 tandems involving CCL5, CCL8, CXCL8 or CXCL12 which may interact with $CCR2^+$ responder macrophages as well as other potential functional chemokine heteromers. Indeed, our group has identified that MSC-secreted CCL2 and CXCL12 cooperate as a heterodimer to upregulate IL-10 expression in a CCR2-dependent response by macrophages in vitro, an effect which could not be replicated by CCL2 on its own speaking to the biological relevance of the CCL2/CXCL12 heterodimer.[21] We also demonstrated that MSC CCL2 competency is absolutely required for $IL\text{-}10^+$ polarization of intestinal and peritoneal resident macrophages in vivo for reversal of colitis. We observed that tissue macrophage IL-10 polarization in vivo was widespread involving extra-intestinal tissues and secondarily led to bystander IL-10 expression in intestine-resident B and T cells. Though MSC-derived CCL2 is necessary for macrophage mobilization and IL-10 polarization in vitro and in vivo,[19] there are likely additional functionalities attributable to the chemokine secretome of MSCs and the theoretical interactome arising thereof (Figure 1). Indeed, through the release of CCL2, CCL3, and CCL12, human and mouse MSCs can recruit monocytes and macrophages into inflamed tissues and promote wound repair.[14] The three most common tissue sources of MSCs for human clinical trials are marrow (M), adipose (A) and Wharton jelly from umbilical cord (UC).[4] Interestingly, the steady state chemokine secretome from culture adapted MSC for these tissue sources is distinct as MSC(M) are $CCL2^+CXCL12^+$, MSC(A) are $CCL2^-CXCL12^+$ and MSC(UC) are $CCL2^+CXCL12^-$ which speaks to potential divergent functional attributes of MSCs as informed by their tissue origin.[76] However, the chemokine secretome of MSCs is profoundly affected by inflammatory cytokines produced by activated lymphoid cells such as interferon-γ which markedly upregulates the

TABLE 1 Chemokine expression in mouse and human culture adapted mesenchymal stromal cell (MSCs)

		Human MSC		Mouse MSC	
Name[a]	Other name(s)[a]	resting[b]	+hPBMC[c]	resting[b]	+mPBMC[d]
CC chemokines					
CCL1	I-309, TCA-3	+			
CCL2	MCP-1	++	++	+++	++++
CCL3	MIP-1α			+	
CCL4	MIP-1β	++			
CCL5	RANTES	+	++	+	++++
CCL6	C10, MRP-2				
CCL7	MARC, MCP-3	–	++		++
CCL8	MCP-2	–			++
CCL9/CCL10	MIP-1δ, CCF18	+			+
CCL11	Eotaxin	–		–	
CCL12	MCP-5			++	
CCL13	MCP-4, NCC-1	–			
CCL14	HCC-1, NCC-2				
CCL15	MIP-1δ, HCC-2				
CCL16	NCC-4				
CCL17	TARC, ABCD-2	–		+	++
CCL18	MIP-4, PARC	+			
CCL19	MIP-3β, Exodus-3			+	
CCL20	LARC, Exodus-1				
CCL21	SLC, Exodus-2			–	
CCL22	MDC	+			
CCL23	MIP-3	–			
CCL24	Eotaxin-2	–			
CCL25	TECK				
CCL26	Eotaxin-3	+			
CCL27	CTACK, Eskine			+	
CCL28	MEC				
CXC chemokines					
CXCL1	GRO1	–		+++	+++++
CXCL2	MIP-2α/GRO2			+++	+++++
CXCL3	MIP-2β/GRO3				
CXCL4	PF-4				
CXCL5	ENA78				++++
CXCL6	GCP-2				
CXCL7	NAP-2	++			
CXCL8	IL-8/NAP-1	++	++		
CXCL9	MIG	–	++		+++++
CXCL10	IP-10	+	++		+++++
CXCL11	I-TAC/IP-9		++		++
CXCL12	SDF-1	+	+	+	
CXCL13	BCA-1	–			++
CXCL14	BRAK/bolekine				
CXCL15	Lungkine, WECHE				
CXCL16	SRPSOX				
CXCL17	DMC, VCC-1				

(Continues)

TABLE 1 (Continued)

Name[a]	Other name(s)[a]	Human MSC resting[b]	Human MSC +hPBMC[c]	Mouse MSC resting[b]	Mouse MSC +mPBMC[d]
C chemokines					
XCL1	Lymphotactine α				
XCL2	Lymphotactine β				
CX3C chemokines					
CX3CL1	Fractalkine	++			++++

Note: Qualitative units of expression: (–) absent, (+) to (++++), present with increasing levels. Boxes left blank were not evaluated.
[a]Chemokine nomenclature informed by Laing and Secombes.[75]
[b]Expression by MSCs in culture, informed by Chen et al.[55]
[c]Expression human MSC co-cultured with activated human PBMCs, informed by Chinnadurai et al.[74]
[d]Expression mouse MSC co-cultured with activated mouse PBMCs, informed by Ren et al.[56]

expression of numerous chemokines (Table 1) with direct anti-inflammatory macrophage effects such as CX3CL1.[77]

8 | MSC EXOSOMES AS POTENTIAL CHEMOKINE CARRIERS

Chemokines adhere to extracellular matrix via their GAG-binding domain to form a haptic gradient for chemotaxis. However, MSC-derived chemokines—and possibly higher order chemokine quarternary assemblies—can circulate in plasma providing plausible link between chemokines and responder tissue macrophages in injured tissue, a feature not shared by MSC borne small molecules with short half-lives such as KYN or PGE_2. MSC-derived subcellular microvesicles, exosomes in particular, may also provide long-range communication bridge between MSC and tissue macrophages not in direct contact with administered MSCs. Considering that the MSC-dependent mechanism of action is anchored in its secretome,[78] a cogent argument can be made that subcellular elements of the secretome such as components derived from extracellular vesicles (EV) like exosomes are biologically relevant.[79,80] Indeed, characterization and translational development of MSC-derived exosomes[81,82] and related EVs are of great interest.[83,84] The therapeutic potency of MSC exosomes is usually rationalized on the presence of a biologically relevant protein or RNA in the MSC exosome.[79] Like miRNA in MSC exosomes, proteins in MSC exosomes have the potential to modulate many of the biological processes that are involved in disease pathogenesis or tissue repair and regeneration and it has been proposed that MSC exosomes in part work through the protein cargo more so than the RNA content.[85] Analysis of proteins specifically present in and on MSC-EV highlights the significant abundance of collagens I and VI presumably attached to the outer surface of these vesicles through their strong binding to EV associated integrins α1β1, α2β1, α10β1, and α11β1.[86] Additionally, generic EVs can bear a diverse cargo of cytokines either encapsulated or surface attached, including chemokines such as CCL2[87,88] which supports a notional role of EVs in regulating the immune response of $CCR2^+$ responder cells, potentially enabling long-distance cellular communication and obviating the need for direct cell-to-cell contact.[89]

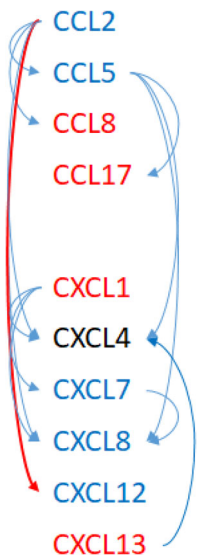

FIGURE 1 Hypothetical and verified chemokine heterocomplexes from mesenchymal stromal cell (MSC) secretome. Human and mouse chemokines identified in MSC secretome (blue) and following PBMC co-culture (red) which have been found in silico to predictably form heterocomplexes are linked by blue lines. CXCL4 is not expressed by MSCs but shown here with predicted partners found in MSC secretome. The CCL2/CXCL12 dyad was validated experimentally in mouse MSC system and shown linked in red

9 | HOST MONOCYTE STATUS AS PLAUSIBLE BIOMARKERS OF MSC RESPONSE

Notwithstanding the quality attributes of MSC defining their mechanism of action in preclinical inbred murine models of human pathology, the human condition entails genetic diversity of subjects that challenges predictable MSC responsiveness. It is therefore not

surprising, despite use of mass-produced "off-the-shelf" allogeneic MSCs bearing identical identity and functional features, that human subject clinical response will lack uniformity. Hence, the value in defining surrogate biomarkers of human subjects reflective of a biological predisposition that are predictive of MSC response prior to or following initiation of therapy. Mapping of such markers is of value for defining plausible in vivo correlative mechanism of action which further strengthens the path toward marketing approval by regulatory agencies.[7] Indeed, the FDA guidance on potency testing for Potency Tests for Cellular and Gene Therapy Products[90] aspirationally seeks for mapping of a relationship between potency and clinical effectiveness where "clinical data may be used to establish a correlation(s) between biological activity and a more practical potency measurement(s) that can be used for lot release, stability, and/or comparability studies." The MSC/monocyte functional dyad may provide correlates that meet the regulators test of coupling MSC quality attributes (such as IDO, COX2, TSG-6, or chemokine expression) and host monocyte/macrophage response as surrogate biomarkers of pharmaceutical activity. As a premise, the observation that clodronate depletion of macrophages in vivo abolishes MSC responsiveness foreshadows that surrogate biological markers of host monocyte number, phenotype or function may serve as a direct correlate of MSC functional attributes. In support of this hypothesis is the observation that intravenous MSC treatment results in a significant decrease in the number of circulating monocytes and an increase in the number of circulating neutrophils in septic mice. Because most tissue macrophages are derived from circulating monocytes, it was speculated that the decrease in monocyte numbers might be due to their tissue invasion, including lung.[16] This data foreshadows that blood monocyte number and phenotype may be directly modulated in human clinical trials by MSC administration. Exploratory companion studies in early phase human clinical trials do provide traction to the theorem. In a Phase I/II clinical study of allogeneic human MSCs inoculated into a hollow-fiber hemofilter, which promotes contact with patient blood via a semipermeable membrane in critically ill patients with Dialysis-Requiring Acute Kidney Injury (NCT03015623) observed modulation of IL-10 and TNFα plasma levels in addition to reduction in total blood monocytes.[91] In a clinical trial of MSC for patients with severe, refractory cGvHD treated with repeated infusions of MSCs (NCT01522716), 6 of 11 treated patients responded with reductions in disease severity and doses of immunosuppression as well as improved quality of life. All responders displayed a distinct immune phenotype characterized by higher levels of naïve T and B cells as well as increased levels of plasma CXCL9 and CXCL10 in responder patients.[92] Whereas monocyte numbers and cytokines plausibly derived from host can be arguably linked to MSC biology and predictors of response, evidence for alterations in monocyte composition in inflammatory compartments can be observed as seen in a clinical study of intra-articular injection of autologous marrow-derived MSC (NCT02351011) for osteoarthritis, where it was found that decrease in synovial IL-12p40 levels along with a decrease in pro-inflammatory monocyte/macrophage subsets after MSC injection.[93] The sum of these early clinical correlative biological data speaks to the feasibility for identification and prospective validation of monocyte/macrophage driven host predictive markers of response to MSC.

10 | CONCLUSION

The MSC and macrophage dyad theorem addresses one of the more puzzling aspects of potency of intravenously transfused MSCs. Namely, that transient MSC lung entrapment leads to a systemic anti-inflammatory effect in an array of rodent models of acute septic and sterile inflammatory tissue injury, and that the phenomenon is initiated and sustained by tissue-resident IL-10 polarized macrophages. The polarization of macrophages follows one of many complementary mechanisms, from efferocytosis to proximate licensing by contact (PD-L1, ICAM) or short distance acting small molecule effectors (KYN, PGE2) to more complex licensing leukines, including heterocomplexed chemokines, and exosomes which can act at a distance and systemically through recirculation. The effect of route of administration and in vivo persistence of transferred MSCs likely will also likely impact on the pharmacodynamics of macrophage polarization and anti-inflammatory response.[33] Thus, the insights gained from understanding of mechanism of action of MSCs related to their effect on host macrophages inform on optimizing their clinical deployment as well as providing leads in developing predictive biomarkers of clinical response in human subjects.

ACKNOWLEDGMENT

This work was supported by National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases award R01DK109508 to J.G.

CONFLICT OF INTEREST

The author declared no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

ORCID

Jacques Galipeau https://orcid.org/0000-0002-9374-1996

REFERENCES

1. Viswanathan S, Shi Y, Galipeau J, et al. Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT(R)) Mesenchymal Stromal Cell committee position statement on nomenclature. *Cytotherapy*. 2019;21:1019-1024.
2. Hoogduijn MJ, Lombardo E. Mesenchymal stromal cells anno 2019: Dawn of the therapeutic era? *Stem Cells Translational Medicine*. 2019;8:1126-1134.
3. Galipeau J. Mesenchymal stromal cells for graft-versus-host disease: a trilogy. *Biol Blood Marrow Transplant*. 2020;26:e89-e91.
4. Kabat M, Bobkov I, Kumar S, Grumet M. Trends in mesenchymal stem cell clinical trials 2004-2018: is efficacy optimal in a narrow dose range? *Stem Cells Translational Medicine*. 2020;9:17-27.

5. Krampera M, Galipeau J, Shi Y, Tarte K, Sensebe L, Therapy MSCC o t IS f C. Immunological characterization of multipotent mesenchymal stromal cells—the International Society for Cellular Therapy (ISCT) working proposal. *Cytotherapy*. 2013;15:1054-1061.
6. Martin I, Galipeau J, Kessler C, Le Blanc K, Dazzi F. Challenges for mesenchymal stromal cell therapies. *Sci Transl Med*. 2019;11:eaat2189.
7. Galipeau J, Krampera M. The challenge of defining mesenchymal stromal cell potency assays and their potential use as release criteria. *Cytotherapy*. 2015;17:125-127.
8. Galipeau J, Sensebe L. Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. *Cell Stem Cell*. 2018;22:824-833.
9. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *J Pathol*. 2013;229:176-185.
10. Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol*. 2018;233:6425-6440.
11. Brown BN, Sicari BM, Badylak SF. Rethinking regenerative medicine: a macrophage-centered approach. *Front Immunol*. 2014;5:510.
12. Italiani P, Boraschi D. From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. *Front Immunol*. 2014;5:514.
13. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. *J Clin Invest*. 2012;122:787-795.
14. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell*. 2013;13:392-402.
15. van Rooijen N, Hendrikx E. Liposomes for specific depletion of macrophages from organs and tissues. *Methods Mol Biol*. 2010;605:189-203.
16. Nemeth K, Leelahavanichkul A, Yuen PS, et al. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. *Nat Med*. 2009;15:42-49.
17. Geng Y, Zhang L, Fu B, et al. Mesenchymal stem cells ameliorate rhabdomyolysis-induced acute kidney injury via the activation of M2 macrophages. *Stem Cell Res Ther*. 2014;5:80.
18. Jackson MV, Morrison TJ, Doherty DF, et al. Mitochondrial transfer via tunneling nanotubes is an important mechanism by which mesenchymal stem cells enhance macrophage phagocytosis in the in vitro and in vivo models of ARDS. *Stem Cells*. 2016;34:2210-2223.
19. Takeda K, Webb TL, Ning F, et al. Mesenchymal stem cells recruit CCR2(+) monocytes to suppress allergic airway inflammation. *J Immunol*. 2018;200:1261-1269.
20. Ko JH, Lee HJ, Jeong HJ, et al. Mesenchymal stem/stromal cells precondition lung monocytes/macrophages to produce tolerance against allo- and autoimmunity in the eye. *Proc Natl Acad Sci USA*. 2016;113:158-163.
21. Giri J, Das R, Nylen E, Chinnadurai R, Galipeau J. CCL2 and CXCL12 derived from mesenchymal stromal cells cooperatively polarize IL-10 + tissue macrophages to mitigate gut injury. *Cell Rep*. 2020;30:1923-1934.e4.
22. Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. *Exp Hematol*. 2009;37:1445-1453.
23. Francois M, Romieu-Mourez R, Li M, Galipeau J. Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation. *Mol Ther*. 2012;20:187-195.
24. Ziegler-Heitbrock L, Ancuta P, Crowe S, et al. Nomenclature of monocytes and dendritic cells in blood. *Blood*. 2010;116:e74-e80.
25. Allen A, Vaninov N, Li M, et al. Mesenchymal stromal cell bioreactor for ex vivo reprogramming of human immune cells. *Sci Rep*. 2020;10:10142.
26. Skrzeczynska-Moncznik J, Bzowska M, Loseke S, Grage-Griebenow E, Zembala M, Pryjma J. Peripheral blood CD14high CD16+ monocytes are main producers of IL-10. *Scand J Immunol*. 2008;67:152-159.
27. Carty F, Mahon BP, English K. The influence of macrophages on mesenchymal stromal cell therapy: passive or aggressive agents? *Clin Exp Immunol*. 2017;188:1-11.
28. Morrison TJ, Jackson MV, Cunningham EK, et al. Mesenchymal stromal cells modulate macrophages in clinically relevant lung injury models by extracellular vesicle mitochondrial transfer. *Am J Respir Crit Care Med*. 2017;196:1275-1286.
29. Min H, Xu L, Parrott R, et al. Mesenchymal stromal cells reprogram monocytes and macrophages with processing bodies. *Stem Cells*. 2021;39:115-128.
30. Weiss ARR, Dahlke MH. Immunomodulation by mesenchymal stem cells (MSCs): mechanisms of action of living, apoptotic, and dead MSCs. *Front Immunol*. 2019;10:1191.
31. Askelund KJ, Chamley LW. Trophoblast deportation. Part I: review of the evidence demonstrating trophoblast shedding and deportation during human pregnancy. *Placenta*. 2011;32:716-723.
32. de Witte SFH, Luk F, Sierra Parraga JM, et al. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. *Stem Cells*. 2018;36:602-615.
33. Giri J, Galipeau J. Mesenchymal stromal cell therapeutic potency is dependent upon viability, route of delivery, and immune match. *Blood Adv*. 2020;4:1987-1997.
34. Weiss DJ, English K, Krasnodembskaya A, Isaza-Correa JM, Hawthorne IJ, Mahon BP. The necrobiology of mesenchymal stromal cells affects therapeutic efficacy. *Front Immunol*. 2019;10:1228.
35. Cheung TS, Dazzi F. Mesenchymal-myeloid interaction in the regulation of immunity. *Semin Immunol*. 2018;35:59-68.
36. Batsivari A, Haltalli MLR, Passaro D, Pospori C, Lo Celso C, Bonnet D. Dynamic responses of the haematopoietic stem cell niche to diverse stresses. *Nat Cell Biol*. 2020;22:7-17.
37. Farahani RM, Xaymardan M. Platelet-derived growth factor receptor alpha as a marker of mesenchymal stem cells in development and stem cell biology. *Stem Cells Int*. 2015;2015:362753.
38. Mabuchi Y, Matsuzaki Y. Prospective isolation of resident adult human mesenchymal stem cell population from multiple organs. *Int J Hematol*. 2016;103:138-144.
39. Wei Q, Frenette PS. Niches for hematopoietic stem cells and their progeny. *Immunity*. 2018;48:632-648.
40. Kfoury Y, Scadden DT. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell*. 2015;16:239-253.
41. Tikhonova AN, Dolgalev I, Hu H, et al. The bone marrow microenvironment at single-cell resolution. *Nature*. 2019;569:222-228.
42. Zhou BO, Yue R, Murphy MM, Peyer JG, Morrison SJ. Leptin-receptor-expressing mesenchymal stromal cells represent the main source of bone formed by adult bone marrow. *Cell Stem Cell*. 2014;15:154-168.
43. Matsuzaki Y, Mabuchi Y, Okano H. Leptin receptor makes its mark on MSCs. *Cell Stem Cell*. 2014;15:112-114.
44. Id Boufker H, Lagneaux L, Najar M, et al. The Src inhibitor dasatinib accelerates the differentiation of human bone marrow-derived mesenchymal stromal cells into osteoblasts. *BMC Cancer*. 2010;10:298.
45. Ghazanfari R, Li H, Zacharaki D, Lim HC, Scheding S. Human non-hematopoietic CD271pos/CD140alow/neg bone marrow stroma cells fulfill stringent stem cell criteria in serial transplantations. *Stem Cells Dev*. 2016;25:1652-1658.
46. Lo Furno D, Mannino G, Cardile V, Parenti R, Giuffrida R. Potential therapeutic applications of adipose-derived mesenchymal stem cells. *Stem Cells Dev*. 2016;25:1615-1628.
47. Pinho S, Lacombe J, Hanoun M, et al. PDGFRalpha and CD51 mark human nestin+ sphere-forming mesenchymal stem cells capable of

hematopoietic progenitor cell expansion. *J Exp Med*. 2013;210:1351-1367.
48. Shi C, Jia T, Mendez-Ferrer S, et al. Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. *Immunity*. 2011;34:590-601.
49. Jung H, Mithal DS, Park JE, Miller RJ. Localized CCR2 activation in the bone marrow niche mobilizes monocytes by desensitizing CXCR4. *PLoS One*. 2015;10:e0128387.
50. Chan JK, Roth J, Oppenheim JJ, et al. Alarmins: awaiting a clinical response. *J Clin Invest*. 2012;122:2711-2719.
51. Lee G, Espirito Santo AI, Zwingenberger S, et al. Fully reduced HMGB1 accelerates the regeneration of multiple tissues by transitioning stem cells to GAlert. *Proc Natl Acad Sci USA*. 2018;115:E4463-E4472.
52. Lin F, Xue D, Xie T, Pan Z. HMGB1 promotes cellular chemokine synthesis and potentiates mesenchymal stromal cell migration via Rap1 activation. *Mol Med Rep*. 2016;14:1283-1289.
53. Nombela-Arrieta C, Manz MG. Quantification and three-dimensional microanatomical organization of the bone marrow. *Blood Adv*. 2017;1:407-416.
54. Bara JJ, Richards RG, Alini M, Stoddart MJ. Concise review: bone marrow-derived mesenchymal stem cells change phenotype following in vitro culture: implications for basic research and the clinic. *Stem Cells*. 2014;32:1713-1723.
55. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. *PLoS One*. 2008;3:e1886.
56. Ren G, Zhang L, Zhao X, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. *Cell Stem Cell*. 2008;2:141-150.
57. Rafei M, Hsieh J, Fortier S, et al. Mesenchymal stromal cell-derived CCL2 suppresses plasma cell immunoglobulin production via STAT3 inactivation and PAX5 induction. *Blood*. 2008;112:4991-4998.
58. Lee HK, Kim HS, Kim JS, et al. CCL2 deficient mesenchymal stem cells fail to establish long-lasting contact with T cells and no longer ameliorate lupus symptoms. *Sci Rep*. 2017;7:41258.
59. English K. Mechanisms of mesenchymal stromal cell immunomodulation. *Immunol Cell Biol*. 2013;91:19-26.
60. Prockop DJ. Concise review: two negative feedback loops place mesenchymal stem/stromal cells at the center of early regulators of inflammation. *Stem Cells*. 2013;31:2042-2046.
61. Ortiz-Virumbrales M, Menta R, Perez LM, et al. Human adipose mesenchymal stem cells modulate myeloid cells toward an anti-inflammatory and reparative phenotype: role of IL-6 and PGE2. *Stem Cell Res Ther*. 2020;11:462.
62. Chinnadurai R, Copland IB, Patel SR, Galipeau J. IDO-independent suppression of T cell effector function by IFN-gamma-licensed human mesenchymal stromal cells. *J Immunol*. 2014;192:1491-1501.
63. Tan K, Zhu H, Zhang J, et al. CD73 expression on mesenchymal stem cells dictates the reparative properties via its anti-inflammatory activity. *Stem Cells Int*. 2019;2019:8717694.
64. Espagnolle N, Balguerie A, Arnaud E, Sensebe L, Varin A. CD54-mediated interaction with pro-inflammatory macrophages increases the immunosuppressive function of human mesenchymal stromal cells. *Stem Cell Rep*. 2017;8:961-976.
65. Caplan H, Olson SD, Kumar A, et al. Mesenchymal stromal cell therapeutic delivery: translational challenges to clinical application. *Front Immunol*. 2019;10:1645.
66. Hughes CE, Nibbs RJB. A guide to chemokines and their receptors. *FEBS J*. 2018;285:2944-2971.
67. von Hundelshausen P, Agten SM, Eckardt V, et al. Chemokine interactome mapping enables tailored intervention in acute and chronic inflammation. *Sci Transl Med*. 2017;9. https://doi.org/10.1126/scitranslmed.aah6650
68. Miller MC, Mayo KH. Chemokines from a structural perspective. *Int J Mol Sci*. 2017;18. https://www.mdpi.com/1422-0067/18/10/2088
69. Thelen M, Munoz LM, Rodriguez-Frade JM, Mellado M. Chemokine receptor oligomerization: functional considerations. *Curr Opin Pharmacol*. 2010;10:38-43.
70. Proudfoot AE, Uguccioni M. Modulation of chemokine responses: synergy and Cooperativity. *Front Immunol*. 2016;7:183.
71. Nesmelova IV, Sham Y, Gao J, Mayo KH. CXC and CC chemokines form mixed heterodimers: association free energies from molecular dynamics simulations and experimental correlations. *J Biol Chem*. 2008;283:24155-24166.
72. Crown SE, Yu Y, Sweeney MD, Leary JA, Handel TM. Heterodimerization of CCR2 chemokines and regulation by glycosaminoglycan binding. *J Biol Chem*. 2006;281:25438-25446.
73. Kraemer S, Alampour-Rajabi S, El Bounkari O, Bernhagen J. Hetero-oligomerization of chemokine receptors: diversity and relevance for function. *Curr Med Chem*. 2013;20:2524-2536.
74. Chinnadurai R, Rajan D, Qayed M, et al. Potency analysis of mesenchymal stromal cells using a combinatorial assay matrix approach. *Cell Rep*. 2018;22:2504-2517.
75. Laing KJ, Secombes CJ. Chemokines. *Dev Comp Immunol*. 2004;28:443-460.
76. Kehl D, Generali M, Mallone A, et al. Proteomic analysis of human mesenchymal stromal cell secretomes: a systematic comparison of the angiogenic potential. *NPJ Regen Med*. 2019;4:8.
77. Giunti D, Parodi B, Usai C, et al. Mesenchymal stem cells shape microglia effector functions through the release of CX3CL1. *Stem Cells*. 2012;30:2044-2053.
78. Spees JL, Lee RH, Gregory CA. Mechanisms of mesenchymal stem/stromal cell function. *Stem Cell Res Ther*. 2016;7:125.
79. Phinney DG, Pittenger MF. Concise review: MSC-derived exosomes for cell-free therapy. *Stem Cells*. 2017;35:851-858.
80. Yanez-Mo M, Siljander PR, Andreu Z, et al. Biological properties of extracellular vesicles and their physiological functions. *J Extracell Vesicles*. 2015;4:27066.
81. Elahi FM, Farwell DG, Nolta JA, Anderson JD. Preclinical translation of exosomes derived from mesenchymal stem/stromal cells. *Stem Cells*. 2020;38:15-21.
82. Dal Collo G, Adamo A, Gatti A, et al. Functional dosing of mesenchymal stromal cell-derived extracellular vesicles for the prevention of acute graft-versus-host-disease. *Stem Cells*. 2020;38:698-711.
83. Mendt M, Rezvani K, Shpall E. Mesenchymal stem cell-derived exosomes for clinical use. *Bone Marrow Transplant*. 2019;54:789-792.
84. Tieu A, Slobodian M, Fergusson DA, et al. Methods and efficacy of extracellular vesicles derived from mesenchymal stromal cells in animal models of disease: a preclinical systematic review protocol. *Syst Rev*. 2019;8:322.
85. Toh WS, Lai RC, Zhang B, Lim SK. MSC exosome works through a protein-based mechanism of action. *Biochem Soc Trans*. 2018;46:843-853.
86. van Balkom BWM, Gremmels H, Giebel B, Lim SK. Proteomic signature of mesenchymal stromal cell-derived small extracellular vesicles. *Proteomics*. 2019;19:e1800163.
87. Fitzgerald W, Freeman ML, Lederman MM, Vasilieva E, Romero R, Margolis L. A system of cytokines encapsulated in extracellular vesicles. *Sci Rep*. 2018;8:8973.
88. Chen T, Guo J, Yang M, Zhu X, Cao X. Chemokine-containing exosomes are released from heat-stressed tumor cells via lipid raft-dependent pathway and act as efficient tumor vaccine. *J Immunol*. 2011;186:2219-2228.
89. Beninson LA, Fleshner M. Exosomes: an emerging factor in stress-induced immunomodulation. *Semin Immunol*. 2014;26:394-401.
90. U.S. Food & Drug Administration. Guidance Document: Potency Tests for Cellular and Gene Therapy Products. Available at https://www.fda.gov/regulatory-information/search-fda-guidancedocuments/potency-tests-cellular-and-gene-therapy-products
91. Barcia R, Nguyen S, O'Rourke B, et al. Immune reprogramming in human subjects after extracorporeal mesenchymal stromal cell Therapy. *Cytotherapy*. 2020;22:S11.

92. Boberg E, von Bahr L, Afram G, et al. Treatment of chronic GvHD with mesenchymal stromal cells induces durable responses: a phase II study. *Stem Cells Translational Medicine*. 2020;9:1190-1202.
93. Chahal J, Gomez-Aristizabal A, Shestopaloff K, et al. Bone marrow mesenchymal stromal cell treatment in patients with osteoarthritis results in overall improvement in pain and symptoms and reduces synovial inflammation. *Stem Cells Translational Medicine*. 2019;8:746-757.

How to cite this article: Galipeau J. Macrophages at the nexus of mesenchymal stromal cell potency: The emerging role of chemokine cooperativity. *Stem Cells*. 2021;39:1145–1154. https://doi.org/10.1002/stem.3380