Chapter 5
Mesenchymal Stem Cells as Endogenous Regulators of Inflammation

Hafsa Munir, Lewis S. C. Ward, and Helen M. McGettrick

Abstract This chapter discusses the regulatory role of endogenous mesenchymal stem cells (MSC) during an inflammatory response. MSC are a heterogeneous population of multipotent cells that normally contribute towards tissue maintenance and repair but have garnered significant scientific interest for their potent immunomodulatory potential. It is through these physicochemical interactions that MSC are able to exert an anti-inflammatory response on neighbouring stromal and haematopoietic cells. However, the impact of the chronic inflammatory environment on MSC function remains to be determined. Understanding the relationship of MSC between resolution of inflammation and autoimmunity will both offer new insights in the use of MSC as a therapeutic, and also their involvement in the pathogenesis of inflammatory disorders.

Keywords Mesenchymal stem cells · Endothelial cells · Neutrophils · Lymphocytes

5.1 Introduction

Mesenchymal stem cells (MSC) are non-haematopoietic, multipotent tissue-resident precursor cells with immunomodulatory capabilities [1]. They exist in small numbers in a variety of tissues including the bone marrow (BM), Wharton's jelly (WJ), adipose tissue (AD), dental pulp, brain, and spleen [2]. Even within different tissues, MSC are thought to exhibit heterogeneous phenotypes based on cellular size,

Hafsa Munir and Lewis S. C. Ward have contributed equally to this work

H. Munir
MRC Cancer Unit/Hutchison, University of Cambridge, Cambridge, UK

L. S. C. Ward
Discovery Sciences, AstraZeneca, Cambridge, UK

H. M. McGettrick (✉)
Rheumatology Research Group, Institute of Inflammation and Ageing, University of Birmingham, Birmingham, UK

B. M.J Owens, M. A. Lakins (eds.), *Stromal Immunology*, Advances in Experimental Medicine and Biology 1060, https://doi.org/10.1007/978-3-319-78127-3_5

surface marker expression, differentiation capacity, and function [3–6]. Thus, not all MSC are the same. Indeed, growing evidence suggests that the MSC niche is unique in distinct tissues and that variation in tissue microenvironments may lead to tissue-specific differences in MSC functions [7–10]. As well as their reparative roles, MSC possess immunomodulatory capabilities and therefore have the potential to regulate inflammation and its resolution. MSC-mediated immunomodulation occurs through two mechanisms: release of soluble factors and cell-cell contact-dependent interactions (Table 5.1). Here, we review the origins of tissue-resident MSC, their interaction with the tissue microenvironment, and how this may influence inflammatory responses. A brief synopsis on MSC as a therapeutic strategy for the treatment of graft-versus-host disease is also discussed.

Table 5.1 Immunomodulatory effects of MSC on haematopoietic and stromal cells

Affected cell	Effect	Mediator(s)	Species	Passage	References
Stem cells					
HSC	↓ BM egress	CXCL12	Mouse	–	[11–13]
	↑ Proliferation and maintain HSC in an undifferentiated state	β-catenin	Mouse	–	[14, 15]
Leukocytes					
Neutrophils	↑ Phagocytosis	Soluble factors	Human	3–5	[16]
	↓ Respiratory burst and apoptosis	Soluble factors	Human	3–5	[16, 17]
NK cells	↓ IFN‖ secretion and cytotoxicity	PGE_2, HLA-G5	Human	1–6	[18–20]
Monocytes	↓ IL-12 secretion	PGE_2	Human	≥2–4	[21, 22]
	↑ BM egress	CCL2	Mouse	–	[23]
	↓ Differentiation into DC	IL-6, M-CSF, PGE_2	Human	≤15	[21, 22]
	↑ Polarisation to M2 macrophage	IDO, PGE_2	Human/ mouse	3–7	[24–26]
T-cells	↓ Proliferation	TGFβ, HGF, PD-1-PD-L1/2, NO, PGE_2	Human/ mouse	1–6	[21, 27–37]
	↓ IFNγ secretion	Cell contact, IL-10	Human	≤6	[38, 39]
	↑ Expansion of T_{reg}	HLA-G	Human	1	[30]
B-cells	↓ Antibody production	Soluble factors	Human	–	[40]
	↓CXCR4, CXCR5, CCR7 expression inhibiting trafficking	Soluble factors	Human	–	[40]
	↓ Proliferation	Cell contact	Human/ mouse	–	[32, 40]
DC	↓ TNFα secretion	IL-10	Human	≤6	[38]
	↓ Antigen-presenting functions	–	Human/ mouse	–	[22, 39, 41]
	↓ CCR7 expression ↓ trafficking	Soluble factors	Human	–	[42]

(continued)

Table 5.1 (continued)

Affected cell	Effect	Mediator(s)	Species	Passage	References
Stromal cells					
Endothelial cells	↑ Proliferation and migration	CCL2,CXCL12VEGF, PDGF	Human/ rodent	3–5	[43–45]
	↑ Angiogenesis	ROS	Rat	–	[46]
	↓ Vascular permeability	S-1-P	Human	3–7	[47–50]
	↓ Leukocyte recruitment[a]	IL-6, TGFβ	Human	3	[51–53]

All behaviours were analysed with BMMSC
[a]Also analysed for WJ MSC
IDO indoleamine 2,3-dioxygenase, *PD1* programmed cell death 1, *PGE2* prostaglandin E2, *ROS* reactive oxygen species, *S-1-P* sphingosine-1-phosphate

5.2 Origin of MSC

Our best definition of an MSC is defined in the International Society for Cell Therapy 2006 guidelines (Fig. 5.1) [54]. Additional surface proteins (e.g. CD146 and CD271) are thought to identify highly potent (suppressive) MSC subpopulations as assessed by T-cell proliferation assays [55]. Despite this, no specific MSC marker – based on either surface expression or function – has been identified. Moreover, "MSC" markers are also found on non-MSC stromal populations (e.g. fibroblasts) indicating that this criterion is too generic for defining a specific population in tissue. Also of concern is that the morphology, differentiation capacity, and expression of "MSC" markers are modified to varying degrees by in vitro culture conditions [56]. Identification of a unique, functionally relevant marker is urgently required to truly elucidate the endogenous role of tissue-resident MSC in modulating inflammation and the effects of MSC therapy in vivo. Understanding the origin of MSC may identify early lineage-specific markers that are exclusively expressed on MSC and can be used to distinguish these cells from other stromal cells.

Little is known about the developmental origin of MSC, with recent evidence suggesting at least two distinct lineages: neural crest and mesoderm. MSC can differentiate into cells of the neural lineages, and subsets of murine BM-derived MSC have been reported to express neural crest stem cell-specific genes [57], leading several groups to postulate this as their origin [57, 58]. Additionally, murine neural crest-derived cells can migrate through the bloodstream to populate numerous tissues, including the bone marrow, where they exhibit a differentiation capacity indicative of stem cells [58]. In contrast, lineage tracing studies showed that cells from the primary vascular plexus give rise to perivascular cells that exhibit MSC-like properties [59–61]. Whilst the origin of MSC is still being debated, it is clear that the cells described in these studies exhibit the same phenotypic features of MSC in vitro. Identifying the origin of MSC and their organ distribution (i.e. differences between MSC populations) may explain functional variations observed in MSC isolated from different anatomical sites.

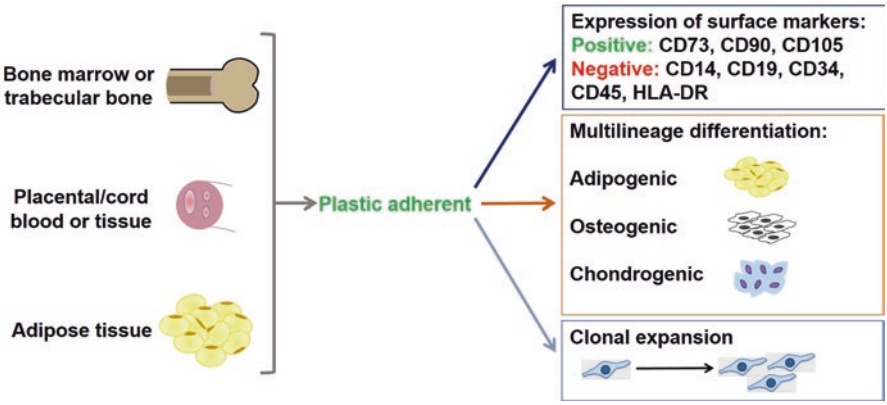

Fig. 5.1 Definition for mesenchymal stem cells. MSC can be isolated from a variety of sources (bone marrow, placenta/umbilical cord, and adipose tissue) primarily based on plastic adherence. Due to the heterogeneity of these cells, further characterisation is required. The International Society for Cell Therapy described the minimum criteria necessary to define MSC [54]. The cells must express the stromal markers, CD73, CD90, and CD105, and lack expression of haematopoietic and endothelial markers, CD14, CD19, CD34, CD45, and HLA-DR. They must also be able to differentiate into other mesodermal lineages (adipogenic, osteogenic, and chondrogenic). Lastly, MSC must be able to undergo clonal expansion during in vitro culture

5.3 MSC in the Bone Marrow Niche

BMMSC can contribute to the haematopoietic stem cell (HSC) niche by regulating haematopoiesis [11, 14, 15] and trafficking of BM-derived cells into the circulation [11–13]. Depletion of MSC or MSC-like progenitors caused an increase in HSC mobilisation [11] and augmented the expression of early myeloid selector genes by HSC, reducing their overall number in the bone marrow [15]. This indicates that the presence of MSC in the HSC niche is essential for inducing their proliferation and maintaining HSC in an undifferentiated state [15]. Indeed, stimulation of β-catenin in MSC has been shown to promote HSC self-renewal in vivo suggesting that this signalling pathway is involved [14]. MSC can also "hold" HSC in the perivascular niche through CXCL12-CXCR4-dependent interactions, preventing them from exiting the bone marrow into the bloodstream, akin to the mechanism reported for mature leukocytes [11, 12]. Importantly, the expression of CXCL12 by MSC can be regulated by CD169+ macrophages within the BM niche [13]. Depleting these BM macrophages reduced CXCL12 expression on MSC and in turn enhanced HSC egress [13]. Thus, MSC play an integral role in maintaining HSC within the BM niche through soluble mediators but also complex multicellular cross-talk with HSC and mature leukocytes.

Evidence suggests that MSC may also regulate the trafficking of monocytes and B cells from the bone marrow [13, 23]. During systemic infection, BMMSC up-regulated CCL2 in response to toll-like receptor (TLR) activation, promoting the

egress of $CCR2^+$ monocytes into the bloodstream [23]. This mobilisation of monocytes also promotes HSC egress away from the stem cell niche [13, 23] encouraging their maturation into leukocytes. This tightly regulated process requires cross-talk between MSC, monocytes, and HSC to coordinate an appropriate immune response. BMMSC also down-regulated expression of CXCR4 by B cells, which may promote their exit from the bone marrow [40]. Whether MSC influence maturation of other leukocyte populations remains to be determined (reviewed by [62]). The main function of BM-resident MSC is to endogenously regulate the proliferation and maturation of HSC and may therefore indirectly influence leukocyte generation. Additionally, MSC may also regulate leukocyte egress in response to infection and/or inflammatory cues. This indicates a novel and potentially tissue-specific role of BM-resident MSC.

5.4 MSC Regulation of Immune Cells

5.4.1 Effects on Innate Immunity

Within the tissue, resident MSC are thought to modulate the movement, effector functions, and survival of recruited neutrophils. Several studies have reported enhanced neutrophil chemotaxis across blank filters towards conditioned media from resting MSC, lipopolysaccharide (LPS)-primed MSC, or MSC isolated from diseased tissue (e.g. gastric cancer) [16, 63, 64]. However, direct coculture of MSC with neutrophils for 1 h, in contrast, had no effect on the ability of neutrophils to migrate along a gradient of C5a, IL-8, or fMLP [17]. In conflicting studies, BMMSC have been shown to dampen the fMLP-induced respiratory burst of neutrophils [17], whilst supernatants from BMMSC enhanced oxidative release in LPS-primed neutrophils [16]. Indeed, these supernatants were also demonstrated to augment neutrophil phagocytosis [16]. Furthermore, coculture with BMMSC or WJMSC or supernatants from parotid gland MSC reduced neutrophil apoptosis in vitro at 18–24 h [16, 17, 65]. Certain contexts require cell-cell contact in conjunction with soluble mediators to elicit the effects of MSC; however the reasons for this remain unknown. One possibility is that these rely on similar mechanisms to those observed with ICAM1-mediated suppression in lymphocytes [18, 19], but further investigations are required.

MSC have also been reported to dampen innate immune responses by suppressing the effector functions of natural killer (NK) cells and skewing the differentiation of monocytes towards a more anti-inflammatory M2 phenotype [20]. Human BMMSC suppressed IFNγ secretion by IL-2 [21, 38] or IL-15 [66] activated NK cells. In the case of the latter study, this was partially mediated through prostaglandin E_2 [PGE_2] and to a lesser extent TGFβ [66]. Cytotoxic effector functions of activated NK cells are also suppressed by BMMSC in vitro [21, 66] via indolamine-2,3-dioxygenase [IDO] and PGE_2 acting synergistically [21]. Similarly, contact with BMMSC also promoted monocyte polarisation to IL-10 producing M2

macrophages, once again in a soluble mediator (IDO and PGE_2)-dependent manner [24–26]. Indeed, IL-10 produced from M2 macrophages reduced neutrophil infiltration and lethality of sepsis in vivo following infusion of BMMSC [67]. In contrast, human BMMSC can suppress allogeneic $CD14^+$ monocyte differentiation into dendritic cells in vitro (driven by GM-CSF, IL-4, and LPS) when cells were cultured in close proximity, but not direct contact, on opposite sides of a porous filter [22]. MSC appear to have the ability to "turn off" inflammatory responses promoting resolution. Indeed preconditioning U937 cells (monocytic cell line) with BMMSC for 16 h reduced their adhesion to inflamed pulmonary endothelial cells in vitro [68]. Thus, tissue-resident MSC may act as endogenous sensors of inflammation, influencing the activity of recruited leukocytes. Moreover, they may also coordinate the switch from innate to adaptive immunity during protective inflammation.

5.4.2 Effects on Adaptive Immunity

MSC modulation of T-cell behaviour has been extensively studied (reviewed by [27]). MSC from a variety of tissues promote the survival of T-cells whilst maintaining them in a quiescent state by suppressing proliferation [28–30] and the production of pro-inflammatory cytokines (e.g. IFNγ) [38]. Indeed, these represent the standard assays used to test the potency of MSC. As with other cell types, MSC mediate their effects through soluble factors (e.g. TGF-β, IDO, and PGE2) and cell contact (e.g. programmed cell death 1 [PD-1]) (reviewed by [69]). These factors can synergistically induce maximal suppression of T-cell proliferation when MSC are in direct contact with the T-cells [31]. Cell-cell contact between MSC and T-cells leads to bidirectional cross-talk affecting both cell types. For example, ICAM-1 is upregulated by human ADMSC following interaction with T-cells and is necessary for the suppression of proliferation, where blocking ICAM-1 on ADMSC releases T-cells from IDO-induced inhibition [70]. BMMSC can also enhance the expansion of the T_{reg} population in peripheral blood mononuclear cells in a HLA-G-dependent manner, which may be further enhanced by IL-10 [30]. Moreover, human ADMSC have been shown to redirect B-cell plasmablast formation into a regulatory B-cell subset (B_{reg}), although the mechanism remains unknown [71, 72]. Consequently, MSC could potentially amplify their effects on T-cells indirectly, by promoting the proliferation of local T_{reg} and B_{reg} populations.

How MSC regulate other cells of the adaptive immune system is poorly understood. Human BMMSC have been reported to preserve naive B-cells in a resting state suppressing their proliferation and antibody production [19, 40]. Similar observations have been made in mice where BMMSC inhibited the expansion of follicular and marginal zone B-cells in vitro [73]. Coculture in contact with MSC reduced the expression of chemokine receptors on B-cells (CXCR5 and CCR7) and dendritic cells (CCR7; [42]) required for trafficking through lymphoid organs [40]. Additionally MSC are capable of promoting tolerance in vitro: coculture on opposite sides of a porous filter impaired NF-κB signalling in dendritic cells resulting in reduced CD80/CD86 and HLA expression and impaired stimulation of T-cell clonal

expansion [22, 39, 41, 74]. In contrast data from phase I to phase II clinical trials in patients undergoing liver transplants has observed no tolerogenic effect of BMMSC infusion [75]. In most cases MSC-derived agents are sufficient to drive their effects on adaptive immune cells. However in a few cases, direct cell contact appeared necessary to produce a maximal response possibly involving the PD-1 pathway [32, 73].

5.5 MSC Interactions with Platelets

MSC are also capable of interacting with circulating platelets. Whilst we know much less about these interactions, they are likely to be critically important in the context of MSC cell-based therapy and vascular damage where perivascular MSC become exposed to blood [59, 60]. Human MSC bind circulating platelets in a β_1-integrin-dependent manner [76], where such interactions enhanced MSC adhesion to arterial endothelium in vitro [77] and facilitated BMMSC recruitment to lung vasculature in a rat model of pulmonary arterial hypertension [78]. Similarly platelet-MSC interactions also impact the ability of the MSC therapy to bind to extracellular matrix proteins such as collagen and fibronectin [76]. Furthermore, depleting platelets have been shown to impair MSC homing, a murine model of LPS-induced dermal inflammation [79]. Collectively these studies indicate that platelet-MSC interactions may aid their "homing" to damaged sites following therapeutic administration. However, caution is required as recent evidence indicates that such interactions have the potential to induce platelet activation and cause thrombus formation. The glycoprotein podoplanin, which is expressed by human WJMSC, can bind to CLEC-2 on platelets and induce platelet activation and their subsequent aggregation [76]. When administered systemically, podoplanin-expressing WJMSC cause a significant reduction in platelet numbers in the blood, with the platelets forming higher-order aggregates of activated cells [76]. Thus, platelet-MSC interactions have the potential to be beneficial in facilitating MSC homing to inflammatory sites but also detrimental associated with increased the risk of thrombotic events. Further investigations are required to resolve the functional impact of MSC on platelets and vice versa.

5.6 MSC Regulation of Vascular Endothelial Cells and Tissue-Resident Stroma

MSC reside in the perivascular niche in close proximity with endothelial cells (EC) lining the vasculature (blood and lymphatic) and other tissue-resident (stromal) cells [59, 60]. Comparatively speaking we understand very little about the interactions of MSC with these populations and their functional consequences. Indeed the effects of MSC on the behaviour of endothelial cells have been analysed in three contexts (see below), whilst their interactions with stromal cells have solely focused on the reparative properties of both cell types.

5.6.1 Regulation of Angiogenesis

Under resting conditions, human and rodent BMMSC have been reported to release factors (e.g. VEGFα and PDGF-BB) known to enhance the proliferation and migration of endothelial cells [43–45]. The production of these agents indicates that MSC have the potential to promote angiogenesis. In a murine model of wound repair, BMMSC (injected intradermally) and BMMSC-derived conditioned media (injected subcutaneously at the site of injury) increased endothelial cell and macrophage numbers at the site of the wound [44, 80]. These studies suggest that MSC promote wound healing by inducing angiogenesis. In vitro, proliferation and migration of both human and murine endothelial cells was induced in the presence of conditioned media from BMMSC but not dermal fibroblasts [44]. For further information on the effects MSC have on in vitro tube-forming assays, see review [81]. Of note, the main stimulators of angiogenesis, like shear stress and oxygen tension, were not modelled in these studies. Furthermore, co-injection of MSC with B16 melanoma cells increased tumour size and vessel area in vivo, indicating that they are pro-angiogenic [82]. In contrast, MSC suppressed angiogenesis in a Matrigel model through production of reactive oxygen species when in direct contact with rat lung microvascular EC [46]. Whether these factors are the key drivers of MSC-induced angiogenesis has not been explored. Numerous putative angiogenic proteins have recently been identified in exosomes derived from MSC cultured under serum-starved hypoxic conditions [83]. MSC-derived factors may well communicate with endothelial cells to control angiogenesis during development and wound repair. Endogenous MSC regulation of angiogenesis in adult pathologies remains unclear.

5.6.2 Regulation of Blood Vascular Permeability

Evidence suggests that perivascular MSC can communicate with endothelial cells to regulate vascular permeability and maintain vessel integrity in resting and acute inflammatory conditions [47–50, 84]. Coculture with MSC increased the stability of junctional molecules (e.g. VE-cadherin and β-catenin) by inhibiting their turnover at the plasma membrane of endothelial cells, reducing endothelial permeability to FITC-dextran [50]. This effect was reproduced when endothelial cells were treated with conditioned media from the coculture, implicating soluble mediators as the main drivers [50]. In LPS-driven infection, infusion of BMMSC reduced pulmonary microvessel permeability and increased endothelial barrier function in vivo, reducing murine lung vascular permeability [49]. Similar observations were made using both mouse and rat models of haemorrhagic shock [47, 84]. Nevertheless, therapeutic administration of MSC may have beneficial effects for individuals with severe vascular damage.

5.6.3 Regulation of Leukocyte Recruitment

In terms of regulating inflammatory responses, perivascular MSC communicate directly with neighbouring endothelium to indirectly regulate leukocyte recruitment during inflammation [47, 51, 68]. However, very few studies have examined this, and none have questioned whether MSC from different tissues have the same capacity to regulate this process (i.e. tissue-specific effects).

Therapeutic administration of murine BMMSC increased the number of circulating neutrophils whilst simultaneously decreasing circulating monocytes in a murine model of sepsis, suggesting MSC can actively influence leukocyte recruitment [67]. Moreover, pretreating pulmonary endothelial cells with conditioned media from human endothelial-BMMSC cocultures reduced their ability to support monocytic leukaemia cell line (U937) adhesion in response to TNFα in vitro, by tightening endothelial adherens junctions (VE-cadherin and β-catenin) and reducing adhesion molecule expression, ICAM-1 and VCAM-1 [47]. Thus, MSC can reduce leukocyte adhesion when they interact directly with target cells. However, these studies analysed adhesion under static conditions, which do not mimic physiological recruitment of leukocytes from flowing blood. Moreover, they focus on soluble mediator-induced effects on naive endothelium, rather than the direct bidirectional cross-talk between MSC and endothelial cells.

To address this, we developed an in vitro multicellular flow-based adhesion assay that mimicked intravenous BMMSC and WJMSC infusion and subsequent integration into the endothelial monolayer [51, 52]. We reported that MSC communicate with neighbouring vascular endothelial cells to limit leukocyte recruitment induced by inflammatory cytokines [51, 53]. Specifically, BMMSC potently down-regulated the recruitment of both neutrophils and lymphocytes by inflamed endothelium [51, 53]. Whilst WJMSC and TBMSC elicited similar effects, these MSC populations showed greater suppressive effects compared to BMMSC, which could be attributed to tissue-specific differences [51, 53]. A two-way conversation between MSC and endothelial cells was essential for these effects, with activation of TGFβ and release of IL-6 being critical factors [51, 53]. Coculture with MSC also inhibited the secretion of chemokines (CXCL8 and CXCL10) responsible for stabilising leukocyte adhesion and driving onward migration [51].

Alternatively, MSC and endothelial cells were cocultured together on opposite sides of a porous insert. This construct more accurately models the cross-talk that occurs within the tissue but can also be used to examine the effects of site-specific infusion of MSC [52, 53]. Like the therapeutic model, we observed that BMMSC and WJMSC suppressed neutrophil recruitment. Once again, coculture conditioned media mimicked the effects of coculture, indicating a soluble mediator-dependent mechanism. Indeed, IL-6 and TGFβ were identified as the main mediators. Interestingly, production of the soluble mediator by WJMSC, but not BMMSC, was dependent upon close proximity between the MSC and EC [53]. This suggests that BMMSC can communicate with endothelial cells in a contact-independent manner [53]. We have shown that MSC communicate directly with neighbouring

endothelium to modulate the inflammatory response. Whilst MSC from different anatomical sites have the same functional effects, they appear to utilise different mechanisms which may ultimately affect their regulatory capacity. These functional differences may be due to differences in developmental origin of different MSC populations, a phenomenon previously observed in different smooth muscle cell populations [85]. This has important implications for therapy, as it suggests that MSC from different sources may only suppress recruitment when administered in close proximity to the endothelium.

These observations are not restricted to tissue-resident MSC. We and others have shown that healthy stromal cells from a variety of tissues (e.g. fibroblasts, podocytes, and secretory smooth muscle cells) exhibit immunosuppressive capabilities, limiting leukocyte recruitment induced by inflammatory cytokines [[51, 86–88]; also see Chap. 3]. Moreover, stromal populations, including endothelial cells and fibroblasts, display distinct spatial identities [89] that govern their behaviour. This allows them to establish tissue-specific "address codes" that actively regulate the recruitment of leukocytes to inflamed sites (reviewed by [90]). Whether MSC exhibit such tissue-specific differences requires further investigation. Collectively these studies suggest that healthy mesenchymal tissue-resident cells use the same mechanism to act as endogenous regulators of the inflammatory infiltrate, with IL-6 and TGFβ acting as master regulators [51, 53]. Given these agents are present in endothelial-MSC conditioned media, infusion of culture supernatant or MSC-derived agents may be more efficacious than infusion of cells. Ultimately this would eliminate the need for MSC infusions where the long-term effects (safety and efficacy) of therapy are unknown.

5.6.4 Regulation of Tissue Repair: Interactions with Stromal Cells

Limited evidence suggests MSC may interact with other tissue-resident mesenchymal stromal cells to facilitate their reparative functions during tissue repair and bone remodelling [91–95]. BMMSC have been reported to migrate towards damaged bone in response to TGFβ1 released by osteoclastic bone at resorptive sites, where they differentiate into osteoblasts promoting bone remodelling [91]. Moreover, rheumatoid synovial fibroblasts secrete placental growth factor, promoting BMMSC chemotaxis [96]. In rodent models of tissue damage (surgically or chemically induced), injection of BMMSC or BMMSC conditioned media reduced tissue fibrosis in the affected organ (kidney, heart, liver, and skin; [92–95]). One interpretation is that MSC migrate into the damaged tissue to communicate with resident fibroblasts and influence their production and/or deposition of extracellular matrix components, reducing fibrosis. Indeed, Yates et al. have recently demonstrated that MSC and fibroblasts can synergistically reduce extracellular matrix production and thus scarring when transplanted into a CXCR3-deficient mouse model [97]. New lines of research are necessary to determine whether MSC manipulate stroma responses to regulate the tissue microenvironment during inflammation.

5.7 Regulation by the Physical Microenvironment

MSC respond to nanoscale features altering their growth and differentiation potentials according to the patterns of nanotopography they experience [98]. For example, soft (0.5 kPa) hydrogels promoted MSC differentiation towards neural cells, whilst stiff (40 kPa) gels drive osteogenesis in the absence of additional growth factors [99]. Moreover, MSC pluripotency can be maintained using a highly ordered distribution of nanopits on the culture surface [100]. Introducing a relatively small amount of disorder to such features was sufficient to stimulate osteogenesis [101]. Sensing topographical features smaller than adhesion molecules (~10 nm) indicates that MSC observe fine details (physical and chemical) within their environment and are able to mount potent responses in an effort to maintain tissue homeostasis. Such insights could enable the ex vivo expansion of MSC for therapeutic use on specially designed surfaces that can topographically maintain, e.g. “stemness”.

5.8 MSC Response to Acute Inflammation

The inflammatory microenvironment is complex with a context-specific medley of agents that can shape the behaviour of leukocytes, endothelial cells, and stromal cells. Do tissue-resident MSC also respond to their local environment and does this impact their effector functions?

One avenue that has been explored is the effects of exogenous cytokines on the phenotype of MSC (Table 5.2) and the functional consequences of these changes (Table 5.3). Pretreating MSC (BM, WJ, AD) with IFNγ in combination with TNFα for 18 h altered their phenotype: differentially modifying TLR expression (see Table 5.2) and increasing the release of cytokines (e.g. IL-6) and chemokines (e.g. CXCL8, CCL5) when compared to untreated MSC [104]. Murine BMMSC treated with IFNγ in combination with either TNFα, IL-1α, or IL-1β for 24 h up-regulated expression of adhesion molecules (e.g. ICAM1, VCAM1) and chemokine (e.g. CXCL9) compared to untreated MSC [18, 106]. Of note, single cytokine treatments had little effect on these parameters [18, 106]. In contrast, IFN‖, but not TNF‖, stimulation for 72 h induced IDO expression by BMMSC and WJMSC relative to resting MSC [102]. Many of these changes mirror the response of other stromal cell types to inflammation ([114, 115]; see Chap. 3) and support cell-cell interactions necessary for migration to the damaged tissue. In certain contexts, cytokines can further enhance the immunomodulatory effects of MSC when compared to naive MSC [102, 116, 117]. Indeed, pretreating MSC (BM or placental) with IFNγ for 48 h suppressed T-cell proliferation to a greater extent than untreated MSC [113]. Cord-derived MSC had a greater suppressive effect than BMMSC when primed with IFNγ as assessed by T-cell proliferation assays and mixed lymphocyte reactions in vitro [102]. Furthermore, IL-2 secretion by T cells was significantly reduced when BMMSC, but not WJMSC, were primed with TNFα for 72 h prior to coculture in the presence of PHA [102]. However, enhancing MSC functions can have detri-

Table 5.2 Response of MSC to inflammatory environments

	Effect on MSC	MSC source	Species	Passage	References
Cytokine treatment					
IFNγ	↑ PD-L1, HGF and PGE_2 expression and IDO activity	BM/AD	Human/ mouse	2–10	[19, 102, 103]
	↓ TGFβ1 secretion	BM	Mouse	3–10	[103]
TNFα	↓ TGFβ1 and HGF secretion	BM/WJ	Human/ mouse	3–10	[102, 103]
	↑ TGFβ1 mRNA	WJ	Human	5–10	[94]
	↑ HGF, PGE_2 secretion	BM/WJ	Human/ mouse	3–8	[102, 103]
Poly(I:C)	↑ IDO, PGE_2, SMAD7 mRNA	BM	Human	≤4	
	↓ TGFβ1, IL-6, IL-8, CCL10, secretion	BM			[33]
	↑ Fibronectin deposition	–			
	↓ Differentiation capacity	–			
LPS	↑ Jagged-1/2, SMAD3 mRNA	BM	Human	≤4	[33, 94]
	↓ TGFβ1 and HGF expression	BM/AD			
	↑ Osteogenesis and collagen deposition	–			[33, 104]
	↓ Adipogenesis	–			
	↑ IL-1Ra, IL-6, IL-8, and IL-4 secretion	AD			
TGFβ1	↑ Migration	BM	Mouse	–	[91]
IFNγ+TNFα	↑ ICAM-1, VCAM-1, HIF-1α, VEGF, iNOS, PD-L1 expression	BM	Mouse	3–20	[18, 103, 105]
	↑ IL-6, IL-8, CXCL9, CXCL10 secretion	BM	Human/ mouse	–	[34, 106]
IL-1β+IFNγ +TNFα+IFNα	↑ IL-1β mRNA and IL-6 and IL-8 secretion	BM/WJ/ AD	Human	<2	[104]
	↑ TLR2, TLR3, ↓ TLR6 mRNA	BM/WJ/ AD			
	↑ TLR1 mRNA	WJ			
	↓ TLR5 mRNA	WJ/AD			
	↑ IFN-γ and ↓ HGF secretion	BM			
Disease					
RA	↓ MSC proliferation	BM	Human	1–6	[107, 108]
	Impaired ability to support haematopoiesis				
	↓ Cyclin-D; ↑ cyclin-D inhibitor				
SLE	↓ MSC proliferation	BM/WJ	Human/ mouse	>3	[109–112]
	↓ Differentiation into osteoblasts				

AD adipose, *BM* bone marrow, *DC* dendritic cell, *WJ* Wharton's jelly

Table 5.3 Effects of inflammatory cytokines on the immunomodulatory properties of MSC

	Effect	Mediator(s)	MSC source	Species	Passage	References
IFNγ	↓ Proliferation of T- and B-cells	IDO, PD-1	Placenta/ BM/AD	Human/ mouse	>2	[19, 73, 113]
	↓ B-cell differentiation into plasma cells	PD-1	BM	Mouse	20–25	[73]
	↓ Secretion of IFN-γ and TNFα by T-cell	–	BM	Human	≤10	[102]
	↓ Expansion of B_{reg}	IDO	AD	Human	2–5	[19]
TNFα	↓ DC maturation	–	BM	Mouse	3–10	[42]
	↓ CCR7 expression on DC					
	↓DC migration to CCL19					
	↓ Secretion of IFNγ and TNFα by T-cells	–	BM	Human	≤10	[113]
	↓ Splenocyte proliferation	PGE_2	BM	Mouse	3–10	[103]
IL-10	↓ T-cell proliferation	HLA-G5	BM	Human	1	[30]
	↓ NK cytotoxicity					
	↑ Expansion of T_{reg}					
IL-1β+IFNγ +TNFα+IFNα	↓ T-cell proliferation	–	BM/WJ/AD	Human	<2	[104]

AD adipose, *BM* bone marrow, *DC* dendritic cell, *IDO* indoleamine 2,3-dioxygenase, *PD1* programmed cell death 1, *PGE2* prostaglandin E2, *WJ* Wharton's jelly

mental effects. For example, IFNγ-stimulated MSC are better able to suppress B-cell proliferation but have a reduced capacity to induce B_{reg} [19]. Co-injection of primed murine BMMSC (12 h TNFα and IFNγ) with a C26 colonic cancer cell line caused a significant increase in tumour growth when compared to untreated MSC [105]. That said, priming itself is not essential for the suppressive actions of MSC [17, 20, 21, 24–26, 38, 66]. But it does suggest that the MSC can respond to their local microenvironment, which in turn could affect their behaviour (reviewed by [118]). Whether priming of MSC in vitro is representative of the in vivo situation requires further research.

Engagement of TLR-3 in vitro was initially reported to enhance the effects of BMMSC, inducing the release of anti-inflammatory factors (e.g. IDO) [33]. In contrast, TLR-4 activation of BMMSC abrogated their ability to suppress T-lymphocyte proliferation and induced the release of pro-inflammatory cytokines (e.g. TNFα) and deposition collagen [33]. In vivo, systemic administration of TLR3-primed MSC ameliorated symptoms of lung injury and diabetic neuropathy, whilst TLR4-

primed MSC exacerbated disease compared to infusion of naive MSC [33]. Although MSC were defined as MSC2 and MSC1, respectively, it should be noted that these terms refer to the phenotype acquired following TLR activation, rather than the origin of the cells. Subsequent in vitro studies presented conflicting findings: IDO or PGE_2 secretion and T-cell proliferation have been reported to be enhanced, reduced, or unchanged by TLR 3- and TLR 4-stimulated BMMSC [34, 104, 119]. Different experimental conditions (treatment concentrations and duration) and the number and source of MSC are the likely explanation for these contradictory outcomes. Furthermore, MSC infusion has previously been shown to reduce lung oedema and inflammatory infiltrates in murine models of sepsis where high levels of LPS (TLR4 ligand) are present [49, 120]. As MSC dampened inflammation, rather than augment it, it is likely that the effects of TLR priming observed in vitro may not reflect MSC responses in vivo.

The behaviour of MSC is highly plastic, with the local inflammatory milieu (cytokines, danger signals, and bacterial components) having the potential to shape the immune regulatory effects of tissue-resident MSC [33]. Further work is essential to fully understand MSC biology during inflammatory responses and the impact of chronic inflammation. Such plasticity could have implications for MSC as a cell therapy – can we guarantee that the cells administered will maintain their immunosuppressive effects in a chronically inflamed site?

5.9 The Dangers of Chronic Inflammatory Environments on MSC Behaviour

Mesenchymal stromal cells *(see other chapters)*, including MSC, endogenously moderate inflammation, so why does it persist? Also, does chronic inflammation adversely and/or permanently affect MSC function? Ex vivo studies report that human BMMSC isolated from patients with RA have impaired ability to support haematopoiesis [107]. Furthermore, BMMSC from systemic lupus erythematosus (SLE) and RA patients have reduced proliferative capacity and reduced telomere length, indicative of a senescent phenotype when compared to healthy controls [108–110]. Likewise, reduced proliferation and osteogenesis were observed in BMMSC from patients with SLE and a murine preclinical model of SLE [111, 112]. In contrast, no such changes were observed in BMMSC isolated from patients with multiple sclerosis (MS; [121, 122]) or systemic sclerosis (SS; [35]). Importantly, MSC from patients with SLE, RA, and SS appear to maintain their immunomodulatory effector functions – as measured by T-cell proliferation assays [35, 108, 110]. Culturing healthy BMMSC in the presence of 20% synovial fluid from patients with osteoarthritis, but not post-mortem donors with no signs of joint inflammation, increased the gene expression of IL-6 and IDO [123]. Moreover, proteomic analysis of RA BMMSC revealed changes in molecules responsible for regulating cell cycle from G1 to S-phase when compared to healthy age and gender-matched controls, namely, an increase in cyclin-D inhibitors and decrease in cyclin-D [108]. The

chronic inflammatory milieu appears to be capable of driving the proliferation and premature senescence of BMMSC, possibly contributing to further pathogenesis. Unfortunately, all of these studies analysed BMMSC, leaving the effect of the chronic inflammatory milieu on local tissue-resident MSC to be elucidated.

Ectopic fat deposits and/or alterations in local adipose tissue are associated with a number of disorders including Duchenne muscular dystrophy [124], myocardial infarction [125], type II diabetes [126], and RA [127–129]. Similarly aberrant bone formation or calcification has been described in fibrodysplasia ossificans progressiva [130], the vasculature of chronic kidney disease [131], and the adipose tissue in intra-abdominal surgery [132]. These deposits could be the result of inappropriate differentiation of tissue-resident MSC induced by inflammatory mediators in the affected tissue. Thus, under certain conditions, MSC could change their phenotype, no longer acting as brakes on the inflammatory response and possibly taking on a stimulatory state. This might occur during "classic" differentiation, e.g. into adipocytes, or conversion into a non-specific state in chronically insulted tissue. Indeed, MSC-derived adipocytes have lost the ability to suppress neutrophil capture to inflamed endothelium, as seen with undifferentiated MSC [133]. In a 3D multicellular migration assay, both MSC-derived adipocytes and osteoblasts were no longer able to suppress neutrophil adhesion to and migration through an inflamed endothelial monolayer, suggesting that transdifferentiation of MSC abrogates their immunomodulatory capacity [134]. In contrast, native stromal cells, adipocytes derived from them, and mature adipocytes from adipose tissue were all immuno-protective [133]. Thus disruption of normal tissue stroma homeostasis, as occurs in chronic inflammatory diseases, might drive "abnormal" adipogenesis which adversely influences the behaviour of MSC and contributes to pathogenic recruitment of leukocytes [133]. These novel findings parallel those we made when comparing stromal cells from healthy and diseased tissues, where stromal cells from chronically inflamed sites lost immunosuppressive properties and modified endothelial cells to inappropriately recruit leukocytes ([87, 88, 135–137]; see Chap. 3). Moreover, these effects were mediated by altering the bioactivity of IL-6 or TGFβ, making them act in a "pro-inflammatory" manner [138]. Whether a diseased environment (chronic inflammation or tumour) drives a similar pathogenic response in MSC remains to be addressed.

5.10 MSC in Therapeutics to Treat Inflammatory Disorders

The ability of MSC to modify immune responses has been the basis for clinical trials in a range of conditions [139]. Of these graft vs. host disease (GvHD) has been the most extensively studied, with early studies showing good therapeutic potential. To date there are 12 recently completed and 25 trials ongoing in this area; in all cases the outcomes have yet to be announced [139]. Systemic infusion of matched or mismatched BMMSC into patients with or at risk of GvHD improved clinical scores [140–142], with a few patients reporting complete remission at the 12-month

follow-up [141, 143–145]. BMMSC therapy improved survival at 12–24 months in ~50–60% of patients with steroid-refractory GvHD in phase II trials [142, 146]. Due to the nature of these studies, none included a placebo control arm necessary to assess the true clinical benefit of MSC infusion. Early trials report therapeutic efficacy of MSC. However, randomised multicentre phase III trials of steroid-refractory GvHD showed no significant difference between treatment ("off-the-shelf" allogeneic MSC) and placebo groups [147]. The lack of efficacy may be due to differences in disease severity (degree of steroid resistance) between patients. Individuals with moderate disease severity may have a better response to MSC infusion compared to those with more severe disease, which could affect the outcome of trials. Recent follow-up studies have shown an increased incidence of haematological malignancies [148] or risk of pneumonia [149] in GvHD patients treated with MSC. That said, there was no evidence of tumour formations following intravenous infusion of MSC in patients with neuromyelitis optica spectrum disorder at 2 years follow-up [150]. The long-term risks and potential side effects of MSC therapy will need further investigation.

Based on promising data from preclinical models, trials are also examining the efficacy of MSC in autoimmune diseases, with a significant number involving patients with Crohn's disease, SLE, and RA (reviewed by [151]). However, a concern with these studies is the cyclical nature of patient's symptoms, making it difficult to determine whether improvements in the condition are due to the MSC or the natural disease cycle. As mentioned for GvHD, many of these studies also lack the appropriate placebo controls. Nevertheless, preclinical and clinical studies have shown potential clinical benefits of MSC treatment [53, 141, 143–145].

5.10.1 Limitations of Current Clinical Trials

Conflicting outcomes in clinical trials may arise from differences in trial design and lack of understanding of MSC biology. Variations in the clinical outcome of these trials may also be due to ex vivo expansion (passaging) of MSC which has a negative effect on their proliferation, differentiation, and immunosuppressive effects [152]. Due to the scarcity of MSC in tissues, large-scale culture ex vivo expansion is necessary to generate sufficient cell numbers for therapeutic administration, which may limit their clinical benefits. MSC are a heterogeneous population of cells with similar phenotypic features as other stromal populations such as fibroblasts. As such, MSC will need to be more stringently defined before becoming an "off-the-shelf" therapeutic strategy for treatment of inflammatory disorders.

Key concerns regarding the optimum route of administration, dose of MSC, the best source of cells, and the fate of the cells after infusion also need to be addressed (reviewed by [151]). Systemically infused MSC have a low homing efficiency (<1%) and become mechanically trapped in the lungs (reviewed by [153]), suggesting that the beneficial effects of MSC treatment are mostly likely due to soluble mediators [56]. However, a recent study reported that intravenously infused fluorescently labelled BMMSC initially lodged in the lungs but importantly were no longer

detected at 24 h [153]. They subsequently have suggested that previous studies showing MSC redistribution in other tissues were detecting cell debris or phagocytosed MSC that are still labelled and postulated that any long-term immunosuppressive effects observed after MSC infusion are mediated by other cell types and not the MSC themselves [153]. For example, infused MSC can be phagocytosed by monocytes, inducing the monocytes to acquire the non-classical anti-inflammatory phenotype through up-regulation of CD16 and therefore transferring their MSC immunomodulatory effects onto the monocytes [154]. Alternatively, human BMMSC-derived apoptotic bodies have been suggested to initiate MSC-induced immunosuppressive in a murine model of GvHD [155].

The long-term effects of MSC treatment (5–10 years follow-up) have not been carried out. Any long-term risks of MSC treatment are currently unknown, and issues such as MSC response to other therapeutic interventions, potential tumorigenicity, and tissue distribution upon administration will need to be addressed to eliminate possible risks of MSC treatment. Manipulating the functions of endogenous MSC for therapeutic use may therefore be an attractive alternative to current treatment modalities for inflammatory conditions. However, without fully ascertaining the mode of actions of endogenous MSC, it will be difficult to elucidate their true therapeutic potential.

5.11 Conclusions

Tissue-resident MSC are endogenous regulators of inflammation. They have an inherent capacity to sense even the subtlest of changes in their microenvironment and respond accordingly. MSC maintain tissue homeostasis: replacing damaged cells through their differentiation into the target stromal cell and also supporting the haematopoietic niche. During inflammation, MSC inhibit the archetypical inflammatory behaviours of their target cell whilst simultaneously promoting anti-inflammatory, pro-resolution agents and/or the generation of regulatory cells. We, ourselves, have shown that MSC communicate with blood vascular endothelial cells to regulate the inflammatory infiltrate. MSC predominately mediate their effects through the release of soluble factors, but in certain context, direct cell-cell interactions are thought to be required to enhance these further. Whilst MSC cell therapy is currently being explored for clinical benefit, many of the clinical trials are in the earliest phases with the outcomes yet to be announced or inconclusive. Such studies are confounded by differences in their design, source, and dose of MSC and the absence of placebo controls, making it difficult to ascertain the true clinical benefit of MSC treatment. Further research is needed to understand how MSC communicate with cells, other than leukocytes, within tissues and whether these interactions change during an inflammatory response. Moreover, it is critical we understand the impact chronic inflammation has on the function of MSC. Can we guarantee that therapeutic MSC will maintain their immunosuppressive effects in a chronically inflamed site? Whether MSC-derived media or effector molecules (either from

MSC or cocultures with other cell types) would be a safer and more efficacious alternative intervention remains to be seen.

Conflicts of Interest The authors declare that they have no conflicts of interest.

Funding HM and LSCW were supported by BBSRC and MRC PhD studentships, respectively. HMM was supported by an Arthritis Research UK Career Development Fellowship (19899) and Systems Science for Health, University of Birmingham (5212).

References

1. Pal R, Hanwate M, Jan M, Totey S. Phenotypic and functional comparison of optimum culture conditions for upscaling of bone marrow-derived mesenchymal stem cells. J Tissue Eng Regen Med. 2009;3:163–74.
2. Sotiropoulou PA, Perez SA, Salagianni M, Baxevanis CN, Papamichail M. Characterization of the optimal culture conditions for clinical scale production of human mesenchymal stem cells. Stem Cells. 2006;24(2):462–71.
3. Christodoulou I, Kolisis FN, Papaevangeliou D, Zoumpourlis V. Comparative evaluation of human mesenchymal stem cells of fetal (Wharton's jelly) and adult (adipose tissue) origin during prolonged in vitro expansion: considerations for Cytotherapy. Stem Cells Int. 2013;2013:246134.
4. Baksh D, Yao R, Tuan RS. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells. 2007;25:1384–92.
5. Jin H, Bae Y, Kim M, Kwon S-J, Jeon H, Choi S, et al. Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. Int J Mol Sci. 2013;14:17986–8001.
6. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells. 2006;24:1294–301.
7. Magatti M, De Munari S, Vertua E, Gibelli L, Wengler GS, Parolini O. Human amnion mesenchyme harbors cells with allogeneic T-cell suppression and stimulation capabilities. Stem Cells. 2008;26:182–92.
8. Wolbank S, Peterbauer A, Fahrner M, Hennerbichler S, van Griensven M, Stadler G, et al. Dose-dependent immunomodulatory effect of human stem cells from amniotic membrane: a comparison with human mesenchymal stem cells from adipose tissue. Tissue Eng. 2007;13:1173–83.
9. Chen P-M, Yen M-L, Liu K-J, Sytwu H-K, Yen B-L. Immunomodulatory properties of human adult and fetal multipotent mesenchymal stem cells. J Biomed Sci. 2011;18(1):49.
10. Chang C-J, Yen M-L, Chen Y-C, Chien C-C, Huang H-I, Bai C-H, et al. Placenta-derived multipotent cells exhibit immunosuppressive properties that are enhanced in the presence of interferon-gamma. Stem Cells. 2006;24:2466–77.
11. Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010;466(7308):829–34.
12. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006;25(6):977–88.

13. Chow A, Lucas D, Hidalgo A, Méndez-Ferrer S, Hashimoto D, Scheiermann C, et al. Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. J Exp Med. 2011;208(2):261–71.
14. Ahn JY, Park G, Shim JS, Lee JW, Oh IH. Intramarrow injection of beta-catenin-activated, but not naive mesenchymal stromal cells stimulates self-renewal of hematopoietic stem cells in bone marrow. Exp Mol Med. 2010;42(2):122–31.
15. Omatsu Y, Sugiyama T, Kohara H, Kondoh G, Fujii N, Kohno K, et al. The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. Immunity. 2010;33(3):387–99.
16. Brandau S, Jakob M, Hemeda H, Bruderek K, Janeschik S, Bootz F, et al. Tissue-resident mesenchymal stem cells attract peripheral blood neutrophils and enhance their inflammatory activity in response to microbial challenge. J Leukoc Biol. 2010;88(5):1005–15.
17. Raffaghello L, Bianchi G, Bertolotto M, Montecucco F, Busca A, Dallegri F, et al. Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for neutrophil preservation in the bone marrow niche. Stem Cells. 2008;26:151–62.
18. Ren G, Zhao X, Zhang L, Zhang J, L'Huillier A, Ling W, et al. Inflammatory cytokine-induced intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in mesenchymal stem cells are critical for immunosuppression. J Immunol. 2010;184(5):2321–8.
19. Luk F, Carreras-Planella L, Korevaar SS, de Witte SFH, Borràs FE, Betjes MGH, et al. Inflammatory conditions dictate the effect of mesenchymal stem or stromal cells on B cell function. Front Immunol. 2017;8:1042.
20. Le Blanc K, Mougiakakos D. Multipotent mesenchymal stromal cells and the innate immune system. Nat Rev Immunol. 2012;12:383–96.
21. Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L. Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood. 2008;111(3):1327–33.
22. Jiang X-X, Zhang Y, Liu B, Zhang S-X, Wu Y, Yu X-D, et al. Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood. 2005;105(10):4120–6.
23. Shi C, Jia T, Mendez-Ferrer S, Hohl TM, Serbina NV, Lipuma L, et al. Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. Immunity. 2011;34(4):590–601.
24. Kim J, Hematti P. Mesenchymal stem cell-educated macrophages: a novel type of alternatively activated macrophages. Exp Hematol. 2009;37:1445–53.
25. Maggini J, Mirkin G, Bognanni I, Holmberg J, Piazzón IM, Nepomnaschy I, et al. Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. PLoS One. 2010;5(2):e9252.
26. François 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;20:187–95.
27. Ma S, Xie N, Li W, Yuan B, Shi Y, Wang Y. Immunobiology of mesenchymal stem cells. Cell Death Differ. 2014;21(2):216–25.
28. Benvenuto F, Ferrari S, Gerdoni E, Gualandi F, Frassoni F, Pistoia V, et al. Human mesenchymal stem cells promote survival of T cells in a quiescent state. Stem Cells. 2007;25:1753–60.
29. Glennie S, Soeiro I, Dyson PJ, Lam EW-F, Dazzi F. Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood. 2005;105:2821–7.
30. Selmani Z, Naji A, Zidi I, Favier B, Gaiffe E, Obert L, et al. Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4+CD25highFOXP3+ regulatory T cells. Stem Cells. 2008;26(1):212–22.
31. Gu YZ, Xue Q, Chen YJ, Yu GH, de Qing M, Shen Y, et al. Different roles of PD-L1 and FasL in immunomodulation mediated by human placenta-derived mesenchymal stem cells. Hum Immunol. 2013;74(3):267–76.

32. Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, et al. Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol. 2005;35(5):1482–90.
33. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM. A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an immunosuppressive MSC2 phenotype. PLoS One. 2010;5(4):e10088.
34. Liotta F, Angeli R, Cosmi L, Filì L, Manuelli C, Frosali F, et al. Toll-like receptors 3 and 4 are expressed by human bone marrow-derived mesenchymal stem cells and can inhibit their T-cell modulatory activity by impairing Notch signaling. Stem Cells. 2008;26:279–89.
35. Larghero J, Farge D, Braccini A, Lecourt S, Scherberich A, Fois E, et al. Phenotypical and functional characteristics of in vitro expanded bone marrow mesenchymal stem cells from patients with systemic sclerosis. Ann Rheum Dis. 2008;67(4):443–9.
36. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood. 2002;99(10):3838–43.
37. Sato K, Ozaki K, Oh I, Meguro A, Hatanaka K, Nagai T, et al. Nitric oxide plays a critical role in suppression of T-cell proliferation by mesenchymal stem cells. Blood. 2007;109(1):228–34.
38. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105:1815–22.
39. Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, et al. Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood. 2005;105(5):2214–9.
40. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, et al. Human mesenchymal stem cells modulate B-cell functions. Blood. 2006;107(1):367–72.
41. Ramasamy R, Fazekasova H, Lam EW-F, Soeiro I, Lombardi G, Dazzi F. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. Transplantation. 2007;83(1):71–6.
42. English K, Barry FP, Mahon BP. Murine mesenchymal stem cells suppress dendritic cell migration, maturation and antigen presentation. Immunol Lett. 2008;115(1):50–8.
43. Rahbarghazi R, Nassiri SM, Khazraiinia P, Kajbafzadeh A, Ahmadi SH, Mohammadi E, et al. Juxtacrine and paracrine interactions of rat marrow derived mesenchymal stem cells, muscle derived satellite cells and neonatal cardiomyocytes with endothelial cells in angiogenesis dynamics. Stem Cells Dev. 2013;22(6):855–65.
44. Chen L, Tredget EE, Wu PYG, Wu Y, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One. 2008;3(4):e1886.
45. Dhar K, Dhar G, Majumder M, Haque I, Mehta S, Van Veldhuizen PJ, et al. Tumor cell-derived PDGF-B potentiates mouse mesenchymal stem cells-pericytes transition and recruitment through an interaction with NRP-1. Mol Cancer. 2010;9:209.
46. Otsu K, Das S, Houser SD, Quadri SK, Bhattacharya S, Bhattacharya J. Concentration-dependent inhibition of angiogenesis by mesenchymal stem cells. Hematop Stem Cells. 2009;113(18):4197–205.
47. Pati S, Gerber MH, Menge TD, Wataha KA, Zhao Y, Baumgartner JA, et al. Bone marrow derived mesenchymal stem cells inhibit inflammation and preserve vascular endothelial integrity in the lungs after hemorrhagic shock. PLoS One. 2011;6(9):e25171.
48. Zhao YD, Ohkawara H, Vogel SM, Malik AB, Zhao Y-Y. Bone marrow-derived progenitor cells prevent thrombin-induced increase in lung vascular permeability. Am J Physiol Lung Cell Mol Physiol. 2010;298(1):L36–44.
49. Zhao YD, Ohkawara H, Rehman J, Wary KK, Vogel SM, Minshall RD, et al. Bone marrow progenitor cells induce endothelial adherens junction integrity by sphingosine-1-phosphate-mediated Rac1 and Cdc42 signaling. Circ Res. 2009;105(7):696–704.

50. Pati S, Khakoo AY, Zhao J, Jimenez F, Gerber MH, Harting M, et al. Human mesenchymal stem cells inhibit vascular permeability by modulating vascular endothelial cadherin/b--catenin signaling. Stem Cells Dev. 2010;20(1):89–101.
51. Luu NT, McGettrick HM, Buckley CD, Newsome P, Ed Rainger G, Frampton J, et al. Crosstalk between mesenchymal stem cells and endothelial cells leads to down-regulation of cytokine-iNduced leukocyte recruitment. Stem Cells. 2013;31:2690–702.
52. Munir H, Rainger GE, Nash GBMH, McGettrick H. Analyzing the effects of stromal cells on the recruitment of leukocytes from flow. J Vis Exp. 2015;95:e52480.
53. Munir H, Luu N-T, Clarke LSC, Nash GB, McGettrick HM. Comparative ability of mesenchymal stromal cells from different tissues to limit neutrophil recruitment to inflamed endothelium. PLoS One. 2016;11(5):e0155161.
54. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy. 2006;8:315–7.
55. Tormin A, Li O, Brune JC, Walsh S, Schütz B, Ehinger M, et al. CD146 expression on primary nonhematopoietic bone marrow stem cells is correlated with in situ localization. Blood. 2011;117:5067–77.
56. Eggenhofer E, Luk F, Dahlke MH, Hoogduijn MJ. The life and fate of mesenchymal stem cells. Front Immunol. 2014;5:148.
57. Morikawa S, Mabuchi Y, Niibe K, Suzuki S, Nagoshi N, Sunabori T, et al. Development of mesenchymal stem cells partially originate from the neural crest. Biochem Biophys Res Commun. 2009;379(4):1114–9.
58. Nagoshi N, Shibata S, Kubota Y, Nakamura M, Nagai Y, Satoh E, et al. Ontogeny and multipotency of neural crest-derived stem cells in mouse bone marrow, dorsal root ganglia, and whisker pad. Cell Stem Cell. 2008;2(4):392–403.
59. Crisan M, Yap S, Casteilla L, Chen C-W, Corselli M, Park TS, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell. 2008;3(3):301–13.
60. Feng J, Mantesso A, De Bari C, Nishiyama A, Sharpe PT. Dual origin of mesenchymal stem cells contributing to organ growth and repair. Proc Natl Acad Sci U S A. 2011;108(16):6503–8.
61. Vodyanik MA, Yu J, Zhang X, Tian S, Stewart R, Thomson JA, et al. A mesoderm-derived precursor for mesenchymal stem and endothelial cells. Cell Stem Cell. 2010;7(6):718–29.
62. Li N, Hua J. Interactions between mesenchymal stem cells and the immune system. Cell Mol Life Sci. 2017;74(13):2345–60.
63. Brandau S, Jakob M, Bruderek K, Bootz F, Giebel B, Radtke S, et al. Mesenchymal stem cells augment the anti-bacterial activity of neutrophil granulocytes. Jacobs R, editor. PLoS One. 2014;9(9):e106903.
64. Zhu Q, Zhang X, Zhang L, Li W, Wu H, Yuan X, et al. The IL-6-STAT3 axis mediates a reciprocal crosstalk between cancer-derived mesenchymal stem cells and neutrophils to synergistically prompt gastric cancer progression. Cell Death Dis. 2014;5:e1295.
65. Khan I, Zhang L, Mohammed M, Archer FE, Abukharmah J, Yuan Z, et al. Effects of Wharton's jelly-derived mesenchymal stem cells on neonatal neutrophils. J Inflamm Res. 2014;8:1–8.
66. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail M. Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells. 2006;24:74–85.
67. Németh K, Leelahavanichkul A, Yuen PST, Mayer B, Parmelee A, Doi K, 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;15:42–9.
68. Letourneau PA, Menge TD, Wataha KA, Wade CE, Cox SC Jr, Holcomb JB, et al. Human bone marrow derived mesenchymal stem cells regulate leukocyte-endothelial interactions and activation of transcription factor. J Tissue Sci Eng. 2011;3:1–7.
69. Bernardo ME, Fibbe WE. Mesenchymal stromal cells: sensors and switchers of inflammation. Cell Stem Cell. 2013;13:392–402.

70. Rubtsov Y, Goryunov K, Romanov A, Suzdaltseva Y, Sharonov G, Tkachuk V. Molecular mechanisms of immunomodulation properties of mesenchymal stromal cells: a new insight into the role of ICAM-1. Stem Cells Int. 2017;2017:6516854.
71. Franquesa M, Mensah FK, Huizinga R, Strini T, Boon L, Lombardo E, et al. Human adipose tissue-derived mesenchymal stem cells abrogate plasmablast formation and induce regulatory B cells independently of T helper cells. Stem Cells. 2015;33(3):880–91.
72. Blair PA, Noreña LY, Flores-Borja F, Rawlings DJ, Isenberg DA, Ehrenstein MR, et al. CD19+CD24hiCD38hi B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. Immunity. 2010;32(1):129–40.
73. Schena F, Gambini C, Gregorio A, Mosconi M, Reverberi D, Gattorno M, et al. Interferon-γ–dependent inhibition of B cell activation by bone marrow–derived mesenchymal stem cells in a murine model of systemic lupus erythematosus. Arthritis Rheum. 2010;62(9):2776–86.
74. Wu J, Ji C, Cao F, Lui H, Xia B, Wang L. Bone marrow mesenchymal stem cells inhibit dendritic cells differentiation and maturation by microRNA-23b. Biosci Rep. 2017;37(2):BSR20160436.
75. Detry O, Vandermeulen M, Delbouille M-H, Somja J, Bletard N, Briquet A, et al. Infusion of mesenchymal stromal cells after deceased liver transplantation: a phase I–II, open-label, clinical study. J Hepatol. 2017;67(1):47–55.
76. Sheriff L, Alanazi A, Ward LSC, Ward C, Munir H, Rayes J, et al. Origin-specific adhesive interactions of mesenchymal stem cells with platelets influence their behaviour after infusion. Stem Cells. 2018; https://doi.org/10.1002/stem.2811. [Epub ahead of print]
77. Langer HF, Stellos K, Steingen C, Froihofer A, Schönberger T, Krämer B, et al. Platelet derived bFGF mediates vascular integrative mechanisms of mesenchymal stem cells in vitro. J Mol Cell Cardiol. 2009;47(2):315–25.
78. Jiang L, Song XH, Liu P, Zeng CL, Huang ZS, Zhu LJ, et al. Platelet-mediated mesenchymal stem cells homing to the lung reduces monocrotaline-induced rat pulmonary hypertension. Cell Transplant. 2012;21(7):1463–75.
79. Teo GSL, Yang Z, Carman CV, Karp JM, Lin CP. Intravital imaging of mesenchymal stem cell trafficking and association with platelets and neutrophils. Stem Cells. 2015;33(1):265–77.
80. Wu Y, Chen L, Scott PG, Tredget EE. Mesenchymal stem cells enhance wound healing through differentiation and angiogenesis. Stem Cells. 2007;25(10):2648–59.
81. Nassiri SM, Rahbarghazi R. Interactions of mesenchymal stem cells with endothelial cells. Stem Cells Dev. 2014;23(4):319–32.
82. Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, et al. Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med. 2011;17(7–8):579–87.
83. Anderson JD, Johansson HJ, Graham CS, Vesterlund M, Pham MT, Bramlett CS, et al. Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis via nuclear factor-kappaB signaling. Stem Cells. 2016;34(3):601–13.
84. Potter DR, Miyazawa BY, Gibb SL, Deng X, Togaratti PP, Croze RH, et al. Mesenchymal stem cell-derived extracellular vesicles attenuate pulmonary vascular permeability and lung injury induced by hemorrhagic shock and trauma. J Trauma Acute Care Surg. 2018;84(2):245–56.
85. Sinha S, Iyer D, Granata A. Embryonic origins of human vascular smooth muscle cells: implications for in vitro modeling and clinical application. Cell Mol Life Sci. 2014;71(12):2271–88.
86. Kuravi SJ, McGettrick HM, Satchell SC, Saleem MA, Harper L, Williams JM, et al. Podocytes regulate neutrophil recruitment by glomerular endothelial cells via IL-6-mediated crosstalk. J Immunol. 2014;193(1):234–43.
87. Lally F, Smith E, Filer A, Stone MA, Shaw JS, Nash GB, et al. A novel mechanism of neutrophil recruitment in a coculture model of the rheumatoid synovium. Arthritis Rheum. 2005;52(11):3460–9.
88. McGettrick HM, Smith E, Filer A, Kissane S, Salmon M, Buckley CD, et al. Fibroblasts from different sites may promote or inhibit recruitment of flowing lymphocytes by endothelial cells. Eur J Immunol. 2009;39:113–25.

89. Aird WC. Endothelial cell heterogeneity. Cold Spring Harb Perspect Med. 2012;2(1):a006429.
90. Parsonage G, Filer AD, Haworth O, Nash GB, Rainger GE, Salmon M, et al. A stromal address code defined by fibroblasts. Trends Immunol. 2005;26(3):150–6.
91. Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, et al. TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. Nat Med. 2009;15(7):757–65.
92. Abdel Aziz MT, Atta HM, Mahfouz S, Fouad HH, Roshdy NK, Ahmed HH, et al. Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver fibrosis. Clin Biochem. 2007;40(12):893–9.
93. Semedo P, Correa-Costa M, Cenedeze MA, Malheiros DMAC, Dos Reis MA, Shimizu MH, et al. Mesenchymal stem cells attenuate renal fibrosis through immune modulation and remodeling properties in a rat remnant kidney model. Stem Cells. 2009;27(12):3063–73.
94. Wu Y, Peng Y, Gao D, Feng C, Yuan X, Li H, et al. Mesenchymal stem cells suppress fibroblast proliferation and reduce skin fibrosis through a TGF-β3-dependent activation. Int J Low Extrem Wounds. 2015;14(1):50–62.
95. Li L, Zhang Y, Li Y, Yu B, Xu Y, Zhao S, et al. Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure. Transpl Int. 2008;21(12):1181–9.
96. Park SJ, Kim KJ, Kim WU, Cho CS. Interaction of mesenchymal stem cells with fibroblast-like synoviocytes via cadherin-11 promotes the angiogenesis by enhanced secretion of placental growth factor. J Immunol. 2014;192(7):3003–10.
97. Yates CC, Rodrigues M, Nuschke A, Johnson ZI, Whaley D, Stolz D, et al. Multipotent stromal cells/mesenchymal stem cells and fibroblasts combine to minimize skin hypertrophic scarring. Stem Cell Res Ther. 2017;8(1):193.
98. Dalby MJ, Gadegaard N, Oreffo ROC. Harnessing nanotopography and integrin-matrix interactions to influence stem cell fate. Nat Mater. 2014;13(6):558–69.
99. Lee J, Abdeen AA, Kilian KA. Rewiring mesenchymal stem cell lineage specification by switching the biophysical microenvironment. Sci Rep. 2014;4:5188.
100. McMurray RJ, Gadegaard N, Tsimbouri PM, Burgess KV, McNamara LE, Tare R, et al. Nanoscale surfaces for the long-term maintenance of mesenchymal stem cell phenotype and multipotency. Nat Mater. 2011;10(8):637–44.
101. Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, Herzyk P, et al. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nat Mater. 2007;6(12):997–1003.
102. Prasanna SJ, Gopalakrishnan D, Shankar SR, Vasandan AB. Pro-inflammatory cytokines, IFNgamma and TNFalpha, influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. PLoS One. 2010;5:e9016.
103. English K, Barry FP, Field-Corbett CP, Mahon BP. IFN-gamma and TNF-alpha differentially regulate immunomodulation by murine mesenchymal stem cells. Immunol Lett. 2007;110:91–100.
104. Raicevic G, Najar M, Stamatopoulos B, De Bruyn C, Meuleman N, Bron D, et al. The source of human mesenchymal stromal cells influences their TLR profile as well as their functional properties. Cell Immunol. 2011;270:207–16.
105. Liu Y, Han ZP, Zhang SS, Jing YY, Bu XX, Wang CY, et al. Effects of inflammatory factors on mesenchymal stem cells and their role in the promotion of tumor angiogenesis in colon cancer. J Biol Chem. 2011;286(28):25007–15.
106. Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, et al. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell. 2008;2(2):141–50.
107. Papadaki HA, Kritikos HD, Gemetzi C, Koutala H, JCW M, Boumpas DT, et al. Bone marrow progenitor cell reserve and function and stromal cell function are defective in rheumatoid arthritis: evidence for a tumor necrosis factor alpha-mediated effect. Blood. 2002;99(5):1610–9.

108. Kastrinaki M-C, Sidiropoulos P, Roche S, Ringe J, Lehmann S, Kritikos H, et al. Functional, molecular and proteomic characterisation of bone marrow mesenchymal stem cells in rheumatoid arthritis. Ann Rheum Dis. 2008;67:741–9.
109. Sun L, Wang D, Liang J, Zhang H, Feng X, Wang H, et al. Umbilical cord mesenchymal stem cell transplantation in severe and refractory systemic lupus erythematosus. Arthritis Rheum. 2010;62:2467–75.
110. Nie Y, Lau C, Lie A, Chan G, Mok M. Defective phenotype of mesenchymal stem cells in patients with systemic lupus erythematosus. Lupus. 2010;19(7):850–9.
111. El-Badri NS, Hakki A, Ferrari A, Shamekh R, Good RA. Autoimmune disease: is it a disorder of the microenvironment? Immunol Res. 2008;41(1):79–86.
112. Tang Y, Xie H, Chen J, Geng L, Chen H, Li X, et al. Activated NF-kappaB in bone marrow mesenchymal stem cells from systemic lupus erythematosus patients inhibits osteogenic differentiation through downregulating Smad signaling. Stem Cells Dev. 2013;22(4):668–78.
113. Jones BJ, Brooke G, Atkinson K, McTaggart SJ. Immunosuppression by placental Indoleamine 2,3-dioxygenase: a role for mesenchymal stem cells. Placenta. 2007;28(11–12):1174–81.
114. Bahra P, Rainger GE, Wautier JL, Nguyet-Thin L, Nash GB. Each step during transendothelial migration of flowing neutrophils is regulated by the stimulatory concentration of tumour necrosis factor-alpha. Cell Adhes Commun. 1998;6:491–501.
115. Filer A, Parsonage G, Smith E, Osborne C, Thomas AMC, Curnow SJ, et al. Differential survival of leukocyte subsets mediated by synovial, bone marrow, and skin fibroblasts: site-specific versus activation-dependent survival of T cells and neutrophils. Arthritis Rheum. 2006;54(7):2096–108.
116. Deuse T, Stubbendorff M, Tang-Quan K, Phillips N, Kay MA, Eiermann T, et al. Immunogenicity and immunomodulatory properties of umbilical cord lining mesenchymal stem cells. Cell Transplant. 2011;20:655–67.
117. Yoo KH, Jang IK, Lee MW, Kim HE, Yang MS, Eom Y, et al. Comparison of immunomodulatory properties of mesenchymal stem cells derived from adult human tissues. Cell Immunol. 2009;259:150–6.
118. Najar M, Krayem M, Meuleman N, Bron D, Lagneaux L. Mesenchymal stromal cells and toll-like receptor priming: a critical review. Immune Netw. 2017;17(2):89–102.
119. Opitz CA, Litzenburger UM, Lutz C, Lanz TV, Tritschler I, Köppel A, et al. Toll-like receptor engagement enhances the immunosuppressive properties of human bone marrow-derived mesenchymal stem cells by inducing indoleamine-2,3-dioxygenase-1 via interferon-beta and protein kinase R. Stem Cells. 2009;27:909–19.
120. Tyndall A, Pistoia V. Mesenchymal stem cells combat sepsis. Nat Med. 2009;15:18–20.
121. Mallam E, Kemp K, Wilkins A, Rice C, Scolding N. Characterization of in vitro expanded bone marrow-derived mesenchymal stem cells from patients with multiple sclerosis. Mult Scler. 2010;16(8):909–18.
122. Papadaki HA, Tsagournisakis M, Mastorodemos V, Pontikoglou C, Damianaki A, Pyrovolaki K, et al. Normal bone marrow hematopoietic stem cell reserves and normal stromal cell function support the use of autologous stem cell transplantation in patients with multiple sclerosis. Bone Marrow Transplant. 2005;36(12):1053–63.
123. Leijs MJC, van Buul GM, Lubberts E, Bos PK, Verhaar JAN, Hoogduijn MJ, et al. Effect of arthritic synovial fluids on the expression of immunomodulatory factors by mesenchymal stem cells: an explorative in vitro study. Front Immunol. 2012;3:231.
124. Uezumi A, Fukada S, Yamamoto N, Takeda S, Tsuchida K. Mesenchymal progenitors distinct from satellite cells contribute to ectopic fat cell formation in skeletal muscle. Nat Cell Biol. 2010;12(2):143–52.
125. Goldfarb JW, Roth M, Han J. Myocardial fat deposition after left ventricular myocardial infarction: assessment by using MR water-fat separation imaging. Radiology. 2009;253(1):65–73.
126. Goodpaster BH, Wolf D. Skeletal muscle lipid accumulation in obesity, insulin resistance, and type 2 diabetes. Pediatr Diabetes. 2004;5(4):219–26.

127. Arend WP, Mehta G, Antonioli AH, Takahashi M, Takahashi K, Stahl GL, et al. Roles of adipocytes and fibroblasts in activation of the alternative pathway of complement in inflammatory arthritis in mice. J Immunol. 2013;190(12):6423–33.
128. Clements KM, Ball AD, Jones HB, Brinckmann S, Read SJ, Murray F. Cellular and histopathological changes in the infrapatellar fat pad in the monoiodoacetate model of osteoarthritis pain. Osteoarthr Cartil. 2009;17(6):805–12.
129. Schweitzer ME, Falk a, Pathria M, Brahme S, Hodler J, Resnick D. MR imaging of the knee: can changes in the intracapsular fat pads be used as a sign of synovial proliferation in the presence of an effusion? Am J Roentgenol. 1993;160(4):823–6.
130. Culbert AL, Chakkalakal SA, Theosmy EG, Brennan TA, Kaplan FS, Shore EM. Alk2 regulates early chondrogenic fate in fibrodysplasia ossificans progressiva heterotopic endochondral ossification. Stem Cells. 2014;32(5):1289–300.
131. Mizobuchi M, Towler D, Slatopolsky E. Vascular calcification: the killer of patients with chronic kidney disease. J Am Soc Nephrol. 2009;20(7):1453–64.
132. Zamolyi RQ, Souza P, Nascimento AG, Unni KK. Intraabdominal myositis ossificans: a report of 9 new cases. Int J Surg Pathol. 2006;14:37–41.
133. Munir H, Ward LSC, Sheriff L, Kemble S, Nayar S, Barone F, et al. Adipogenic differentiation of mesenchymal stem cells alters their immunomodulatory properties in a tissue-specific manner. Stem Cells. 2017;35(6):1636–46.
134. Munir H. *Mesenchymal stem cells as endogenous regulators of leukocyte recruitment; the effects of differentiation*. [PhD thesis on the Internet]. Birmingham: University of Birmingham; 2016 [cited 2018 Feb 28]. Available from: http://etheses.bham.ac.uk/.
135. McGettrick HM, Buckley CD, Filer A, Rainger GE, Nash GB. Stromal cells differentially regulate neutrophil and lymphocyte recruitment through the endothelium. Immunology. 2010;131(3):357–70.
136. Rainger GE, Nash GB. Cellular pathology of atherosclerosis: smooth muscle cells prime cocultured endothelial cells for enhanced leukocyte adhesion. Circ Res. 2001;88(6):615–22.
137. Filer A, Ward LSC, Kemble S, Davies CS, Munir H, Rogers R, et al. Identification of a transitional fibroblast function in very early rheumatoid arthritis. Ann Rheum Dis. 2017;76(12):2105–12.
138. McGettrick HM, Butler LM, Buckley CD, Ed Rainger G, Nash GB. Tissue stroma as a regulator of leukocyte recruitment in inflammation. J Leukoc Biol. 2012;91:385–400.
139. National Institutes of Health US. https://clinicaltrials.gov/
140. Weng JY, Du X, Geng SX, Peng YW, Wang Z, Lu ZS, et al. Mesenchymal stem cell as salvage treatment for refractory chronic GVHD. Bone Marrow Transplant. 2010;45(12):1732–40.
141. Ringden O, Uzunel M, Rasmusson I, Remberger M, Sundberg B, Lonnies H, et al. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation. 2006;81(10):1390–7.
142. Le Blanc K, Frassoni F, Ball L, Locatelli F, Roelofs H, Lewis I, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet. 2008;371(9624):1579–86.
143. Le Blanc K, Rasmusson I, Sundberg B, Gotherstrom C, Hassan M, Uzunel M, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet. 2004;363(9419):1439–41.
144. von Bonin M, Stolzel F, Goedecke A, Richter K, Wuschek N, Holig K, et al. Treatment of refractory acute GVHD with third-party MSC expanded in platelet lysate-containing medium. Bone Marrow Transplant. 2009;43(3):245–51.
145. Arima N, Nakamura F, Fukunaga A, Hirata H, Machida H, Kouno S, et al. Single intra-arterial injection of mesenchymal stromal cells for treatment of steroid-refractory acute graft-versus-host disease: a pilot study. Cytotherapy. 2010;12:265–8.
146. Kurtzberg J, Prockop S, Teira P, Bittencourt H, Lewis V, Chan KW, et al. Allogeneic human mesenchymal stem cell therapy (remestemcel-L, Prochymal) as a rescue agent for

severe refractory acute graft-versus-host disease in pediatric patients. Biol Blood Marrow Transplant. 2014;20(2):229–35.
147. Bernardo ME, Ball LM, Cometa AM, Roelofs H, Zecca M, Avanzini MA, et al. Co-infusion of ex vivo-expanded, parental MSCs prevents life-threatening acute GVHD, but does not reduce the risk of graft failure in pediatric patients undergoing allogeneic umbilical cord blood transplantation. Bone Marrow Transplant. 2011;46(2):200–7.
148. Ning H, Yang F, Jiang M, Hu L, Feng K, Zhang J, et al. The correlation between cotransplantation of mesenchymal stem cells and higher recurrence rate in hematologic malignancy patients: outcome of a pilot clinical study. Leukemia. 2008;22(3):593–9.
149. Forslow U, Blennow O, LeBlanc K, Ringden O, Gustafsson B, Mattsson J, et al. Treatment with mesenchymal stromal cells is a risk factor for pneumonia-related death after allogeneic hematopoietic stem cell transplantation. Eur J Haematol. 2012;89(3):220–7.
150. Fu Y, Yan Y, Qi Y, Yang L, Li T, Zhang N, et al. Impact of autologous mesenchymal stem cell infusion on neuromyelitis optica spectrum disorder: a pilot, 2-year observational study. CNS Neurosci Ther. 2016;22(8):677–85.
151. Munir H, McGettrick HM. Mesenchymal stem cells therapy for autoimmune disease: risks and rewards. Stem Cells Dev. 2015;24(18):2091–100.
152. von Bahr L, Sundberg B, Lönnies L, Sander B, Karbach H, Hägglund H, et al. Long-term complications, immunologic effects, and role of passage for outcome in mesenchymal stromal cell therapy. Biol Blood Marrow Transplant. 2012;18:557–64.
153. Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol. 2012;3:297.
154. de Witte SFH, Luk F, Sierra Parraga JM, Gargesha M, Merino A, Korevaar SS, et al. Immunomodulation by therapeutic mesenchymal stromal cells (MSC) is triggered through phagocytosis of MSC by monocytic cells. Stem Cells. 2018;36(4):602–15.
155. Galleu A, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS, et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci Transl Med. 2017;9(416):eaam7828.