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Mesenchymal stromal cells and the innate immune response

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ABSTRACT

Mesenchymal stromal cells (MSC) have been exploited for their immunomodulatory properties in the treatment of a number of immune-based disorders, including Graft *versus* Host Disease (GvHD) and type 1 diabetes. The mechanisms for inducing therapeutic effect still remain largely unknown however, with research focused on understanding how MSCs interact with individual immune cell subsets. Within this review we address what is known about the interactions of MSCs with cells of the innate immune system, how they respond to their microenvironment and how this relates to therapeutic effects we see both within *in vivo* animal models and in clinical trials.

1. Introduction

In contrast to the adaptive immune response, the innate system is non-specific and fast acting, with responses encoded within the germline DNA. Triggering is often by recognition of microbial pathogen associated molecular patterns (PAMPS) such as lipopolysaccharide (LPS), double stranded viral RNA (dsRNA) or prokaryotic DNA motifs such as CpG. PAMPS directly interact with toll-like ligand receptors (TLRs) expressed on both parenchyma and leukocytes, triggering a cascade of chemokines and cytokines involved in initiation of the inflammatory response and leukocyte migration [1]. The expression of TLRs on the cell surface of mesenchymal stromal cells (MSCs) suggests their inherent role in modulating this early immune response [2].

Mechanical tissue barriers, such as the skin and mucous membranes, provide the first line of defense in innate immunity. Many non-myeloid cells contribute to this defense strategy, including fibroblasts and epithelial cells, producing immunomodulatory and antimicrobial factors, in addition to their physical barrier function. Given the location of MSCs within epithelial niches close to the vessel walls and their phenotypic plasticity, it has been hypothesized that MSCs may orchestrate epithelial immune responses by surveilling the local milieu [3].

MSCs have been reported to exhibit immunomodulatory effects on both humoral and cellular components of the innate immune system. This review has focused on key cellular interactions and the potential role of complement in homing of MSCs to the site of injury.

1.1. MSCs and the complement system

The complement system is a central component of innate immunity and has been implicated in the rejection of transplanted allografts [4] and more recently linked to the rapid clearance of systemically circulating MSCs after infusion [5]. Triggering of the complement cascade can occur *via* three separate pathways (classical, lectin or alternative). Activation of the complement cascade provides chemotactic factors and enhancement of the inflammatory response, resulting in the recruitment and activation of leukocytes, enhanced phagocytosis and the formation of membrane attack complexes (MAC), which directly injure the target cells. Irrespective of the triggering pathway, the central step in complement activation is the cleavage of C3 into C3a and C3b [6]. C3b subsequently undergoes further cleavage into the surface-bound fragments iC3b and C3dg. These fragments, once cell bound, become ligands for various immune cells expressing the complement receptors CR1-4 [7].

The complement components C3 and C5 are cleaved by specific convertases to the anaphylatoxins C3a and C5a, both of which

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are found extensively within inflamed and injured tissues. Bone marrow MSCs (BMMSCs) express the receptors for these anaphylatoxins (C3aR and C5aR), suggesting that these breakdown products are chemotactic agents for the MSCs, promoting their migration towards the site of inflammation. Binding of C3a and C5a to their receptors on the cell surface of MSCs enhances MSC resistance to oxidative stress and prolongs the activation of intracellular signaling pathways involved in MSC proliferation and protection from apoptosis [8]. Binding of C3 to MSCs has also been correlated with a higher degree of suppression of peripheral blood mononuclear cell proliferation [9]. Cell surface expression of the complement inhibitors CD46, CD55 and most predominantly CD59, allows MSCs to partially inhibit activation of the complement system [10] and protect the MSCs from the lytic activity of complement components [8]. However, this mechanism of defense can be ineffective in a complement-activated environment and MSCs are vulnerable to MAC formation [5].

MSCs additionally secrete the complement inhibitor, factor H [10]. Factor H inhibits complement activation by limiting the activity of C3 and C5 convertases as well as acting as a co-factor for factor I in the inactivation of C3b and C4b. MSC production of factor H is constitutive, but further up-regulated by exposure to pro-inflammatory cytokines such as interferon-γ (IFNγ) and tumor necrosis factor α (TNFα), but not interleukin (IL)-6 [10].

MSCs themselves are able to trigger the complement cascade *via* all three complement pathways by secreting both C3 and C5 when exposed to ABO compatible blood and serum [5,8]. It is noted that the alternative pathway appears to play a major role in MSC induced complement activation [5]. As discussed previously, the rapid clearance of MSCs after systemic infusion, suggests triggering of the instant-blood-mediated-inflammatory-response (IBMIR) [9]. This may promote the activation and interaction of MSCs with immune cells within the blood, potentially initiating a cascade of intrinsic immunosuppressive functions within both the MSCs and immune cells i.e. regulatory T-cell (Treg) and M2 macrophage induction to generate a sophisticated immunosuppressive environment.

1.2. MSCs respond to their cellular microenvironment

The cellular microenvironment and inflammatory milieu play key roles in determining MSC phenotype and their effects on the immune system [11]. MSCs demonstrate marked plasticity, exerting both pro- and anti-inflammatory phenotypes dependent on these environmental stimuli [2,12]. Through balancing this activating or suppressive phenotype MSCs may be central to regulating immune control and tissue repair and regeneration.

MSCs with a predominantly pro-inflammatory signature are associated with early stage infection and inflammation. Induced by exposure to TLR2 (peptidoglycan) [13] or TLR4 (LPS) [2] activation, they migrate to the site of injury and promote immune induction [2]. This process is associated with the secretion of chemokine (C-X-C motif) ligand (CXCL)9, CXCL10, macrophage inflammatory protein (MIP)-1α, MIP-1β and Rantes, factors enhancing lymphocyte recruitment. It has been recently suggested that *in vitro* MSC expansion media can affect this phenotype, with platelet lysate supplemented media favoring a pro-inflammatory MSC phenotype and secretion of granulocyte macrophage–colony stimulating factor (GM-CSF), which may enhance immune cell recruitment and maintenance of macrophages in an M1 phenotype (Fig. 1) [14].

In their unlicensed state, MSC exhibit immune homeostatic functions. This is enhanced by exposure of the cells to pro-inflammatory cytokines such as IFNγ and TNFα and/or TLR3 ligands such as dsRNA, as evidenced by increased production of immunosuppressive cytokines such as indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2; Fig. 2) [2,12]. This inflammatory licensing is likely to be akin to that after MSC infusion into patients with systemic inflammation, such as sepsis. *In vivo* murine models of sepsis and sepsis-associated kidney injury support this theory, reporting a decrease in inflammation and improved tubular recovery with infusion of MSCs [2,15,16].

1.3. Neutrophils

Neutrophils are the most prevalent innate immune cell, responding to microbial challenge by accumulating at the wound site within minutes of injury. These non-proliferative, phagocytic cells respond to microbial challenge by releasing bactericidal molecules, reactive oxygen species and producing neutrophil extracellular traps, webs of chromatin derived from the neutrophil nucleus containing proteases [17].

It has been demonstrated within mice that tissue resident MSCs are central to the recruitment of neutrophils, exhibiting a pro-inflammatory phenotype and secreting chemotactic cytokines such as IL-6, IL-8, GM-CSF and macrophage inhibitory factor (MIF; Fig. 1) [8]. IL-8 is a major chemoattractant for neutrophils, regulating CD11b expression and thereby mediating leukocyte extravasation [18]. These findings are further evidenced by results from a murine sepsis model where infusion of MSCs was shown to aid bacterial clearance through enhancing neutrophil phagocytic activity [19].

Neutrophils are notoriously short-lived immune cells and their survival is central to the elimination of infection and in facilitating tissue repair [20]. BMMSCs, unlicensed and to a greater extend TLR triggered, act to promote survival of both resting and activated neutrophils through the secretion of IL-6, IFNβ and GM-CSF. This is evidenced by increased expression of the anti-apoptotic factor MCL1 and down-regulation of the pro-apoptotic molecule BAX within neutrophils on exposure to MSCs. IL-6 promotion of neutrophil survival has been reported to be *via* the signal transducer and activator of transcription (STAT)-3 pathway [21,22]. Neutrophils are also maintained "healthy" by BMMSC enhancement of neutrophil burst activity, which in combination with increased survival enables maintenance of a neutrophil store for rapid release on detection of pathogen [8].

1.4. Mast cells

Mast cells (MCs) are the key innate responder cells in allergic inflammation. They reside in tissues close to the external environment, their location meaning that interactions between BMMSCs and MCs are highly probable during MSC homing. MCs originate from hematopoietic stem cells (HSC), circulating within the peripheral blood as CD34+ precursors where they migrate into the tissue and mature into effector cells. Shortly after allergen exposure the BM releases CD34+ precursors, which are recruited to the site of allergen [23]. Both these precursor and mature MCs play a central role in inducing allergic inflammation by producing high levels of Th2 cytokines. Although primarily known for their role in allergy, MCs have been shown to play a key role in defense and autoimmunity and therefore are also an important part of many Th2 mediated inflammatory diseases.

MCs mediate allergic anaphylaxis through the release of histamine during degranulation. Histamine has been demonstrated to stimulate the secretion of a number of cytokines including IL-1α and IL-6 in different cell types. BMMSCs have been reported to express the necessary receptors H1, H2 and H4 to interact with histamine [24]. Histamine and BMMSCs primarily interact *via* the H1 receptor, inducing IL-6 production within the MSCs in a dose and time-dependent manner. In addition PGD2, a MC lipid mediator, further stimulates IL-6 secretion by BMMSCs. These enhanced levels of IL-6 secretion aid in the prevention of pro-apoptotic activity on neutrophils whilst increasing superoxide production within the phagocytic cells [24].

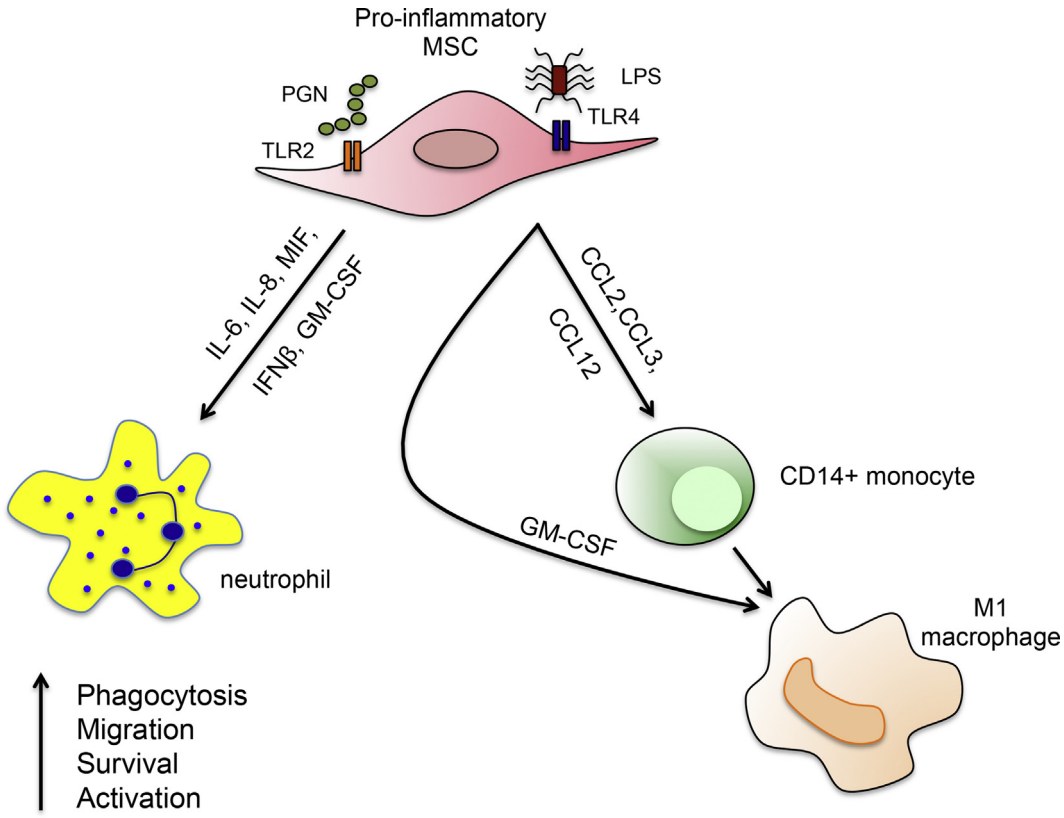

Fig. 1. Targeted actions of the pro-inflammatory MSC. MSCs challenged with Gram positive (peptidoglycan; PGN) and Gram negative (lipopolysaccharide; LPS) bacteria skew to a pro-inflammatory phenotype. MSC secretion of IL-6, IL-8, IFNβ, MIF and GM-CSF increases neutrophil migration to the site of infection/injury, enhancing their activation and phagocytosis whilst promoting their survival. Pro-inflammatory MSC secretion of CCL2, CCL3 and CCL12 recruits monocytes to the site of injury where they differentiate into M1 pro-inflammatory macrophages. The release of GM-CSF by the MSCs can maintain the macrophages in the M1 phenotype enhancing bacterial clearance and early stage wound healing responses.

MSCs can suppress allergic responses and chronic inflammation in experimental models of asthma [25] and allergic rhinitis [26]. IgE mediated MC degranulation, resulting in the release of pro-inflammatory mediators, promotes the recruitment of neutrophils and dendritic cells (DCs) by enhancing T-cell activation and regulating the cytokine microenvironment. MSCs can effectively suppress MC activation both *in vitro* and *in vivo*, in addition to reducing FcεRI-mediated degranulation, TNFα production and stem cell factor induced migration [27–29]. *In vitro* this inhibitory effect can be reversed by inhibiting the production of PGE2 and transforming growth factor β1 (TGFβ1; Fig. 2) [28,29]. The effect of the MSC secretome on MC function has further been demonstrated in a model of experimental allergic conjunctivitis (EAC) [30]. Within this model, MSC conditioned media effectively reduced IgE production, histamine release and activation of MCs during the effector phase of EAC; working specifically through the NF-κB pathway *via* cyclooxygenase (COX) 2 [30]. When in direct contact with MCs, BMMSCs are able to suppress degranulation, pro-inflammatory cytokine production, chemotaxis and chemokinesis. These effects are dependent on cross-talk between the cells, the up-regulation of COX2 within BMMSCs and the binding of secreted PGE2 to the EP4 receptor on MCs [27].

1.5. Natural killer cells

Natural killer (NK) cells have a surveillance role in eliminating both virally infected and stressed cells. These cells are of particular interest in understanding mechanisms of rejection and are central to the regulation of cytotoxicity in response to human leukocyte antigen (HLA) molecules. NK cells comprise the primary lymphocyte population after HSC transplantation (HSCT) and mediate a significant Graft *versus* Leukemia effect [31,32]. With an increasing trend in the use of MSCs in the treatment of Graft *versus* Host Disease (GvHD), it is crucial to understand the interactions and effects of MSCs and NK cells.

NK function is regulated by expression of both activating and inhibitory receptors, with activation controlled by balance of pro- and inhibitory signals [33]. NK cells are activated by exposure to IL-2 or IL-15, resulting in the secretion of IFNγ and TNFα, which both play pivotal roles in the subsequent adaptive immune response. NK cells target cells which have down-regulated HLA I expression, a characteristic of tumor and virally infected cells, as recognized by the inhibitory receptors NKG2A and Killer Ig-like Receptors (KIRs), and resulting in cytolysis by perforins, granzyme and Fas ligand [8].

BMMSCs directly interfere with the proliferation, cytokine production and in some cases cytotoxicity of NK cells. MSC-NK interactions are complex and largely dependent on the microenvironment and activation status of the NK cells. BMMSCs suppress IL-2 and IL-15 induced proliferation and IFNγ production, but not the cytotoxicity of freshly isolated NK cells [34,35]. In contrast, when confronted with previously activated NK cells, MSCs can interfere with NK mediated cytotoxicity, cytokine production, the expression of activating receptors on the cells surface of the NK cells (including NKp30, NKp44 and NKG2D) and granzyme B release. This is primarily mediated by cell–cell contact and the secretion of IDO, PGE2, TGFβ1, HLA-G5 and activin-A (Fig. 2) [8,35,36].

HLA mismatched MSCs are not immune to NK mediated cytotoxicity. Human BMMSCs express a number of ligands capable of activating NK cell receptors, such as ULBP-3 as well as nectin-2, CD54 and CD155. These MSCs are susceptible to lysis by IL-2 and/or

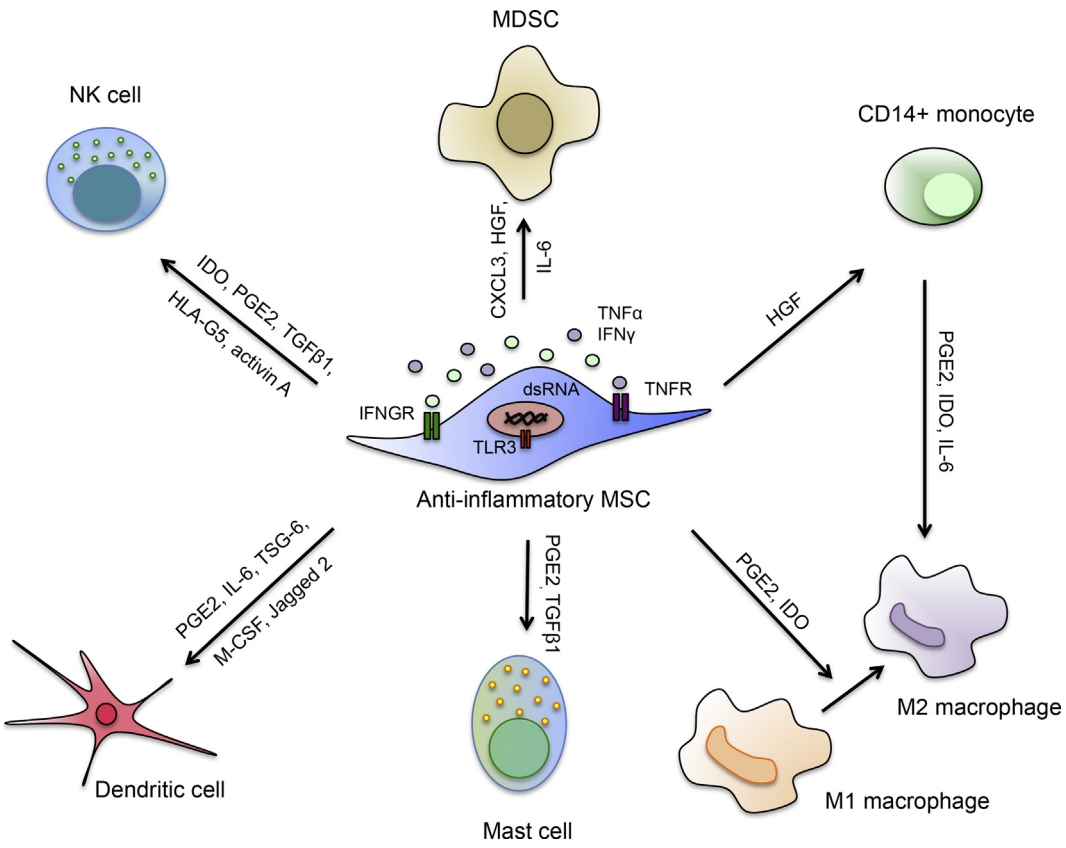

Fig. 2. The multi-faceted anti-inflammatory actions of MSCs. In response to pro-inflammatory cytokines or TLR3 stimuli, MSCs will develop an anti-inflammatory profile. Through the secretion of soluble factors these licensed cells can act on numerous innate immune cells affecting both effector function and phenotype. Individual effects are discussed further within the main text.

IL-15 activated or IL-12 and/or IL-18 activated NK cells, but not resting NK cells [8,34,37,38]. Licensed MSCs exposed to IFNγ are protected from NK mediated cell killing, potentially due to their up-regulated cell surface expression of HLA I (an inhibitory signal to the NK cells) and down-regulation of ULBP-3 (an activating signal), which alongside an increased production of both IDO and PGE2 offers multiple mechanisms for dampening NK responsiveness to the MSCs [8].

1.6. Dendritic cells

DCs provide a link between the innate and adaptive immune systems, presenting antigen to T-cells and regulating their activation, in addition to directly interacting with B [39] and NK cells [40]. DCs can be immune-inducing and tolerogenic, dependent on their maturation state and the specific DC subset. Immature DCs (iDCs), exhibit a tolerogenic phenotype, expressing low levels of HLA II and no co-stimulatory molecules. Like their mature (mDC) counterpart, iDCs possess the ability to recognize, process and present antigen to T-cells. In the absence of co-stimulatory molecules however, T-cell anergy or apoptosis is induced as apposed to activation.

MSCs can directly inhibit both the maturation of monocytes and CD34+ precursor cells into DCs and the direct activation of DCs *via* the secretion of PGE2, IL-6, tumor necrosis factor-inducible gene 6 (TSG-6), M-CSF and Jagged-2 mediated signaling (Fig. 2) [29,41–44]. The expression of cell surface receptors associated with DC maturation is reduced in response to MSC exposure, including HLA II, CD80 and CD86 [45]. A further shift in the DC secretome from pro-inflammatory cytokines such as TNFα and IL-12, to anti-inflammatory IL-10 suggests the induction of a tolerogenic phenotype, with downstream adaptive skewing to a Th2 and Treg response [46]. IL-6 mediated inhibition of DC maturation and tolerogenic skewing has been linked to the up-regulation of SOCS1, a central regulator of the immune system and preventer of systemic autoimmunity [47,48].

Taken together, iDCs through their exposure to MSCs are unable to effectively induce the activation of T-cells, with a reduced capacity to present antigen and stimulate T-cell proliferation and naïve T-cell differentiation, suggesting an indirect mechanism of MSC-mediated immunosuppression [45,46]. MSCs can also act directly on mDCs, reverting them to a renewed immature phenotype associated with a down-regulation of their cell surface expression of antigen-presenting and co-stimulatory molecules, IL-12 secretion and an inability to stimulate lymphocyte proliferation *in vitro* [49].

1.7. Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) comprise a heterogeneous population of myeloid precursors of macrophages, granulocytes and DCs. Exploiting their immunosuppressive nature, MDSCs have been implicated in autoimmune diseases including type 1 diabetes [50] as well as in maintaining chronic inflammation [51].

Within mice, MDSCs have been demonstrated to suppress cytotoxic T-cell effector function through the up-regulation of Arginase 1 and nitric oxide synthase 2 [52]. Limited work has been undertaken to investigate the link between MSCs and MDSCs, although MSC derived soluble factors play a significant role in their interaction. MSCs secrete CXCL3, a member of the growth-related oncogenes (GRO-γ), directly inhibiting the differentiation and function of MDDCs, promoting their skewing to a MDSC phenotype both *in vitro* and *in vivo* (Fig. 2) [53]. This is accompanied by an

up-regulation of the MDSC related genes, COX2, IDO, programmed death ligand (PD-L) 1 and 2 and matrix metalloproteinase 9 (MMP9) in human MDDCs. MSC secreted hepatocyte growth factor (HGF) can further support the expansion of these cells by directly binding the HGF receptor, c-met and increasing STAT3 phosphorylation status within the MDSCs (Fig. 2) [54]. IL-6, a cytokine constitutively secreted by MSCs has also been demonstrated to support this expansion process [55].

1.8. Monocytes

Monocytes represent approximately 10% of circulating leukocytes within humans [56]. Derived from myeloid precursors, this population of cells represents the precursors for macrophages and DCs. Human monocytes are heterogeneous and can be divided into at least 3 subsets based on their expression of CD14 and CD16; the classical monocytes (CD14++CD16−) and the minor subsets of CD16+ monocytes (CD14++CD16+ and the pro-inflammatory CD14+CD16+)[57].

Murine and human BMMSCs promote the movement of monocytes out of the BM space after detecting microbial pathogens and enhance the recruitment of monocytes and macrophages into inflamed tissues to promote wound repair through the secretion of the chemokine (C-C motif) ligands CCL2, CCL3 and CCL12 (Fig. 1)[58]. In addition to their effects on monocyte progeny, MSCs directly act on CD14+ monocytes through the secretion of HGF and potentially factors downstream of the COX2 pathway [45,59]. HGF is known to modulate T-cell function, however in order to exert these effects monocytes expressing c-met are required. Splenic, but not BM or peripheral blood derived CD14+ monocytes respond to MSC secreted HGF by inducing an immunomodulatory phenotype, and are able to rapidly expand within the circulation before becoming an adherent macrophage (Fig. 2) [59].

1.9. Macrophages

Macrophages are characterized into two phenotypes, the M1 pro-inflammatory macrophage with antimicrobial activity and the M2 anti-inflammatory macrophage. These categories relate primarily to their differential secretion of cytokines and expression of cell surface markers. The interactions between macrophages and BMMSCs have been demonstrated within the BM, working in symbiosis for stem cell homeostatic maintenance and potential microbial challenge.

Monocytes entering the inflammatory environment will respond to local chemical signals, differentiating into either M1 macrophages secreting IFNγ and TNFα to support and enhance inflammation, or M2 macrophages promoting transition to the reparative stage of wound healing by secreting anti-inflammatory factors such as IL-10 and TGFβ1.

MSC educated macrophages demonstrate increased migratory and proliferative capacity [60]. Recent studies have reported the ability of MSCs to modulate the phenotype of macrophages by inducing a shift from M1 to M2, thereby accelerating the wound healing process [12,61]. Co-cultures of human BMMSCs with macrophages induce expression of CD206 and CD163 and a down-regulation of the co-stimulatory molecule CD86, suggesting an M2 phenotype. This is accompanied by high-level secretion of IL-10 and IL-6, low levels of IL-12 and TNFα and a functionally higher phagocytic activity [8]. The secretion of IL-10 by both monocytes and M2 polarized macrophages can prevent neutrophils migrating into the inflamed tissue and thereby reduce oxidative damage, indirectly aiding bacterial clearance due to a resulting higher number of neutrophils within the blood [19]. This illustrates the role of MSCs in orchestrating the inflammatory response, enhancing neutrophil migration into the inflamed environment during the early phases to promote an innate immune response and switching to a inhibitory, inflammation dampening role later to prevent prolonged damage to the tissue.

The role of MSCs in post-injury inflammation has also been documented within the brain [62]. Microglia (brain resident macrophages) were reported to polarize to an M2 phenotype in mice with brain injuries injected intracerebroventricularly with human MSCs. This effect was accompanied by a reduction in phagocytosis, early recovery of neurological functions and reparative changes within the lesion site, suggesting a role in both growth stimulation and tissue repair [62].

MSCs have also been reported to directly induce an M2 phenotype from monocytes. This directed maturation is partially regulated *via* direct cell contact but also by BMMSC secretion of PGE2, IL-6 and IDO (Fig. 2) [63–65]. Secretion of PGE2 by MSCs simultaneously stimulates the proliferation of epithelial cells and therefore it has been hypothesized that the MSC role as an immunomodulator and healing promoting cell may be linked [8]. Changes in concentrations of these soluble signaling molecules may aid in the regulation and balancing of both the shift from M1 to M2 and the transition of pro- to anti-inflammatory MSCs.

A strong link between polarization of macrophages by MSCs and modulation of T-cell behavior has been demonstrated. Pro-inflammatory cytokine release by activated T-cells, including IFNγ and TNFα increases the expression of COX2 and IDO in MSCs, further enhancing macrophage polarization [12]. M2 polarization of macrophages has also been associated with the induction of Tregs and therefore linking to the adaptive immune response [63]. Interestingly, it has been demonstrated that removal of monocytes from PBMC:MSC co-cultures reduces suppressive effects [63,66]. The relevance of these findings linking MSCs to macrophage polarity has been supported by *in vivo* investigations. In a mouse model of sepsis, infusion of murine BMMSCs only decreased lethality in the presence of active macrophages, with depletion of macrophages or the presence of IL-10 neutralizing antibodies preventing this response [8].

Summary

MSCs have been trialed extensively for their immunomodulatory and trophic properties within the clinic, earning these cells the term “injury drugstore” [67]. With increasing evidence for the role of MSCs in direct modulation of the innate immune system, MSC therapy is increasingly being viewed as a novel and promising treatment for diseases such as bacterial pneumonia [68], sepsis [65] and acute lung injury [69]. It is clear, that although the specific mechanisms of action by which MSCs exert their immunomodulatory effects *in vivo* remain largely unknown, these therapeutic cells are highly regulated by their microenvironment and paracrine signals. Within this review we have discussed the mechanisms of action by which MSCs exert key effects on different innate immune cell subsets, demonstrating the importance of both cellular interactions and the MSC secretome. Further understanding of these interactions will be crucial in improving and developing new clinical protocols for MSC based cell therapy in the future.

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References

[1] K. Ozato, H. Tsujimura, T. Tamura, Toll-like receptor signaling and regulation of cytokine gene expression in the immune system, Biotechniques (2002) 66–68, 70, 72 passim.
[2] R.S. Waterman, S.L. Tomchuck, S.L. Henkle, A.M. Betancourt, A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype, PLoS ONE 5 (2010) e10088.
[3] L. Diaz-Flores, R. Gutierrez, J.F. Madrid, H. Varela, F. Valladares, E. Acosta, et al., Pericytes. Morphofunction, interactions and pathology in a quiescent and activated mesenchymal cell niche, Histol. Histopathol. 24 (2009) 909–969.
[4] P.D. Hughes, S.J. Cohney, Modifiers of complement activation for prevention of antibody-mediated injury to allografts, Curr. Opin. Organ Transpl. 16 (2011) 425–433.
[5] Y. Li, F. Lin, Mesenchymal stem cells are injured by complement after their contact with serum, Blood 120 (2012) 3436–3443.
[6] B.J. Janssen, E.G. Huizinga, H.C. Raaijmakers, A. Roos, M.R. Daha, K. Nilsson-Ekdahl, et al., Structures of complement component C3 provide insights into the function and evolution of immunity, Nature 437 (2005) 505–511.
[7] D. Ricklin, J.D. Lambris, Complement-targeted therapeutics, Nat. Biotechnol. 25 (2007) 1265–1275.
[8] K. Le Blanc, D. Mougiakakos, Multipotent mesenchymal stromal cells and the innate immune system, Nat. Rev. Immunol. 12 (2012) 383–396.
[9] G. Moll, R. Jitschin, L. von Bahr, I. Rasmusson-Duprez, B. Sundberg, L. Lonnies, et al., Mesenchymal stromal cells engage complement and complement receptor bearing innate effector cells to modulate immune responses, PLoS ONE 6 (2011) e21703.
[10] Z. Tu, Q. Li, H. Bu, F. Lin, Mesenchymal stem cells inhibit complement activation by secreting factor H, Stem Cells Dev. 19 (2010) 1803–1809.
[11] K. Le Blanc, C. Tammik, K. Rosendahl, E. Zetterberg, O.H.L.A. Ringden, expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells, Exp. Hematol. 31 (2003) 890–896.
[12] M.E. Bernardo, W.E. Fibbe, Mesenchymal stromal cells: sensors and switchers of inflammation, Cell Stem Cell 13 (2013) 392–402.
[13] S.L. Tomchuck, K.J. Zwezdaryk, S.B. Coffelt, R.S. Waterman, E.S. Danka, A.B. Scandurro, Toll-like receptors on human mesenchymal stem cells drive their migration and immunomodulating responses, Stem Cells 26 (2008) 99–107.
[14] V. Ulivi, R. Tasso, R. Cancedda, F. Descalzi, Mesenchymal stem cell paracrine activity is modulated by platelet lysate: induction of an inflammatory response and secretion of factors maintaining macrophages in a proinflammatory phenotype, Stem Cells Dev. 23 (2014) 1858–1869.
[15] Y.H. Chao, H.P. Wu, K.H. Wu, Y.G. Tsai, C.T. Peng, K.C. Lin, et al., An increase in CD3+CD4+CD25+ regulatory T cells after administration of umbilical cord-derived mesenchymal stem cells during sepsis, PLOS ONE 9 (2014) e110338.
[16] C.J. Luo, F.J. Zhang, L. Zhang, Y.Q. Geng, Q.G. Li, Q. Hong, et al., Mesenchymal stem cells ameliorate sepsis-associated acute kidney injury in mice, Shock 41 (2014) 123–129.
[17] S.J. Weiss, Tissue destruction by neutrophils, N. Eng. J. Med. 320 (1989) 365–376.
[18] J. Lundahl, S.H. Jacobson, J.M. Paulsson, IL-8 from local subcutaneous wounds regulates CD11b activation, Scand. J. Immunol. 75 (2012) 419–425.
[19] S.R. Hall, K. Tsoyi, B. Ith, R.F. Padera Jr., J.A. Lederer, Z. Wang, et al., Mesenchymal stromal cells improve survival during sepsis in the absence of heme oxygenase-1: the importance of neutrophils, Stem Cells 31 (2013) 397–407.
[20] H.R. Luo, F. Loison, Constitutive neutrophil apoptosis: mechanisms and regulation, Am. J. Hematol. 83 (2008) 288–295.
[21] L. Raffaghello, G. Bianchi, M. Bertolotto, F. Montecucco, A. Busca, F. Dallegri, et al., Human mesenchymal stem cells inhibit neutrophil apoptosis: a model for neutrophil preservation in the bone marrow niche, Stem Cells 26 (2008) 151–162.
[22] T. Hirano, K. Ishihara, M. Hibi, Roles of STAT3 in mediating the cell growth, differentiation and survival signals relayed through the IL-6 family of cytokine receptors, Oncogene 19 (2000) 2548–2556.
[23] R. Sehmi, K. Howie, D.R. Sutherland, W. Schragge, P.M. O'Byrne, J.A. Denburg, Increased levels of CD34+ hemopoietic progenitor cells in atopic subjects, Am. J. Respir. Cell Mol. Biol. 15 (1996) 645–655.
[24] K. Nemeth, T. Wilson, B. Rada, A. Parmelee, B. Mayer, E. Buzas, et al., Characterization and function of histamine receptors in human bone marrow stromal cells, Stem Cells 30 (2012) 222–231.
[25] K. Nemeth, A. Keane-Myers, J.M. Brown, D.D. Metcalfe, J.D. Gorham, V.G. Bundoc, et al., Bone marrow stromal cells use TGF-beta to suppress allergic responses in a mouse model of ragweed-induced asthma, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 5652–5657.
[26] K.S. Cho, H.K. Park, H.Y. Park, J.S. Jung, S.G. Jeon, Y.K. Kim, et al., IFATS collection: immunomodulatory effects of adipose tissue-derived stem cells in an allergic rhinitis mouse model, Stem Cells 27 (2009) 259–265.
[27] J.M. Brown, K. Nemeth, N.M. Kushnir-Sukhov, D.D. Metcalfe, E. Mezey, Bone marrow stromal cells inhibit mast cell function via a COX2-dependent mechanism, Clin. Exp. Allergy 41 (2011) 526–534.
[28] H. Kim, J. Yun, T. Shin, S. Lee, B. Lee, K. Yu, et al., Human umbilical cord blood mesenchymal stem cell-derived PGE and TGF-beta1 alleviate atopic dermatitis by reducing mast cell degranulation, Stem Cells (2014).
[29] W.R. Su, Q.Z. Zhang, S.H. Shi, A.L. Nguyen, A.D. Le, Human gingiva-derived mesenchymal stromal cells attenuate contact hypersensitivity via prostaglandin E2-dependent mechanisms, Stem Cells 29 (2011) 1849–1860.
[30] W. Su, Q. Wan, J. Huang, L. Han, X. Chen, G. Chen, et al., Culture medium from TNF-alpha-stimulated mesenchymal stem cells attenuates allergic conjunctivitis through multiple antiallergic mechanisms, J. Allergy Clin. Immunol. (2015).
[31] R. Jacobs, M. Stoll, G. Stratmann, R. Leo, H. Link, R.E. Schmidt, CD16− CD56+ natural killer cells after bone marrow transplantation, Blood 79 (1992) 3239–3244.
[32] L. Ruggeri, M. Capanni, E. Urbani, K. Perruccio, W.D. Shlomchik, A. Tosti, et al., Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants, Science 295 (2002) 2097–2100.
[33] A. Moretta, C. Bottino, M. Vitale, D. Pende, R. Biassoni, M.C. Mingari, et al., Receptors for HLA class-I molecules in human natural killer cells, Annu. Rev. Immunol. 14 (1996) 619–648.
[34] G.M. Spaggiari, A. Capobianco, S. Becchetti, M.C. Mingari, L. Moretta, Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation, Blood 107 (2006) 1484–1490.
[35] G.M. Spaggiari, A. Capobianco, H. Abdelrazik, F. Becchetti, M.C. Mingari, L. Moretta, Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2, Blood 111 (2008) 1327–1333.
[36] D. Chatterjee, N. Marquardt, D.M. Tufa, T. Hatlapatka, R. Hass, C. Kasper, et al., Human umbilical cord-derived mesenchymal stem cells utilize activin-A to suppress interferon-gamma production by natural killer cells, Front. Immunol. 5 (2014) 662.
[37] C. Noone, A. Kihm, K. English, S. O'Dea, B.P. Mahon, IFN-gamma stimulated human umbilical-tissue-derived cells potently suppress NK activation and resist NK-mediated cytotoxicity in vitro, Stem Cells Dev. 22 (2013) 3003–3014.
[38] C. Gotherstrom, A. Lundqvist, I.R. Duprez, R. Childs, L. Berg, K. le Blanc, Fetal and adult multipotent mesenchymal stromal cells are killed by different pathways, Cytotherapy 13 (2011) 269–278.
[39] B. Dubois, J.M. Bridon, J. Fayette, C. Barthelemy, J. Banchereau, C. Caux, et al., Dendritic cells directly modulate B cell growth and differentiation, J. Leukoc. Biol. 66 (1999) 224–230.
[40] F. Gerosa, B. Baldani-Guerra, C. Nisii, V. Marchesini, G. Carra, G. Trinchieri, Reciprocal activating interaction between natural killer cells and dendritic cells, J. Exp. Med. 195 (2002) 327–333.
[41] Q. Zhang, S. Shi, Y. Liu, J. Uyanne, Y. Shi, S. Shi, et al., Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis, J. Immunol. 183 (2009) 7787–7798.
[42] A.J. Nauta, A.B. Kruisselbrink, E. Lurvink, R. Willemze, W.E. Fibbe, Mesenchymal stem cells inhibit generation and function of both CD34+-derived and monocyte-derived dendritic cells, J. Immunol. 177 (2006) 2080–2087.
[43] X.X. Jiang, Y. Zhang, B. Liu, S.X. Zhang, Y. Wu, X.D. Yu, et al., Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells, Blood 105 (2005) 4120–4126.
[44] Y. Liu, Z. Yin, R. Zhang, K. Yan, L. Chen, F. Chen, et al., MSCs inhibit bone marrow-derived DC maturation and function through the release of TSG-6, Biochem. Biophys. Res. Commun. 450 (2014) 1409–1415.
[45] B. Du Rocher, A.L. Mencalha, B.E. Gomes, E. Abdelhay, Mesenchymal stromal cells impair the differentiation of CD14(++) CD16(−) CD64(+) classical monocytes into CD14(++) CD16(+) CD64(++) activate monocytes, Cytotherapy 14 (2012) 12–25.
[46] S. Beyth, Z. Borovsky, D. Mevorach, M. Liebergall, Z. Gazit, H. Aslan, et al., Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness, Blood 105 (2005) 2214–2219.
[47] T. Hanada, H. Yoshida, S. Kato, K. Tanaka, K. Masutani, J. Tsukada, et al., Suppressor of cytokine signaling-1 is essential for suppressing dendritic cell activation and systemic autoimmunity, Immunity 19 (2003) 437–450.
[48] Y. Deng, S. Yi, G. Wang, J. Cheng, Y. Zhang, W. Chen, et al., Umbilical cord-derived mesenchymal stem cells instruct dendritic cells to acquire tolerogenic phenotypes through the IL-6-mediated upregulation of SOCS1, Stem Cells Dev. 23 (2014) 2080–2092.
[49] B. Zhang, R. Liu, D. Shi, X. Liu, Y. Chen, X. Dou, et al., Mesenchymal stem cells induce mature dendritic cells into a novel Jagged-2-dependent regulatory dendritic cell population, Blood 113 (2009) 46–57.
[50] B. Yin, G. Ma, C.Y. Yen, Z. Zhou, G.X. Wang, C.M. Divino, et al., Myeloid-derived suppressor cells prevent type 1 diabetes in murine models, J. Immunol. 185 (2010) 5828–5834.
[51] S.K. Bunt, P. Sinha, V.K. Clements, J. Leips, S. Ostrand-Rosenberg, Inflammation induces myeloid-derived suppressor cells that facilitate tumor progression, J. Immunol. 176 (2006) 284–290.
[52] D.I. Gabrilovich, S. Nagaraj, Myeloid-derived suppressor cells as regulators of the immune system, Nat. Rev. Immunol. 9 (2009) 162–174.
[53] H.W. Chen, H.Y. Chen, L.T. Wang, F.H. Wang, L.W. Fang, H.Y. Lai, et al., Mesenchymal stem cells tune the development of monocyte-derived dendritic cells toward a myeloid-derived suppressive phenotype through growth-regulated oncogene chemokines, J. Immunol. 190 (2013) 5065–5077.
[54] B.L. Yen, M.L. Yen, P.J. Hsu, K.J. Liu, C.J. Wang, C.H. Bai, et al., Multipotent human mesenchymal stromal cells mediate expansion of myeloid-derived suppressor cells via hepatocyte growth factor/c-met and STAT3, Stem Cell Reports 1 (2013) 139–151.
[55] S.C. Hung, R.R. Pochampally, S.C. Chen, S.C. Hsu, D.J. Prockop, Angiogenic effects of human multipotent stromal cell conditioned medium activate the PI3K-Akt pathway in hypoxic endothelial cells to inhibit apoptosis, increase survival, and stimulate angiogenesis, Stem Cells 25 (2007) 2363–2370.

[56] A.J. Rees, Monocyte and macrophage biology: an overview, Semin. Nephrol. 30 (2010) 216–233.
[57] C. Auffray, M.H. Sieweke, F. Geissmann, Blood monocytes: development, heterogeneity, and relationship with dendritic cells, Annu. Rev. Immunol. 27 (2009) 669–692.
[58] L. Chen, E.E. Tredget, P.Y. Wu, Y. Wu, Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing, PLoS ONE 3 (2008) e1886.
[59] P.M. Chen, K.J. Liu, P.J. Hsu, C.F. Wei, C.H. Bai, L.J. Ho, et al., Induction of immunomodulatory monocytes by human mesenchymal stem cell-derived hepatocyte growth factor through ERK1/2, J. Leukoc. Biol. 96 (2014) 295–303.
[60] S. Gao, F. Mao, B. Zhang, L. Zhang, X. Zhang, M. Wang, et al., Mouse bone marrow-derived mesenchymal stem cells induce macrophage M2 polarization through the nuclear factor-kappaB and signal transducer and activator of transcription 3 pathways, Exp. Biol. Med. 239 (2014) 366–375.
[61] Q.Z. Zhang, W.R. Su, S.H. Shi, P. Wilder-Smith, A.P. Xiang, A. Wong, et al., Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing, Stem Cells 28 (2010) 1856–1868.
[62] E.R. Zanier, F. Pischiutta, L. Riganti, F. Marchesi, E. Turola, S. Fumagalli, et al., Bone marrow mesenchymal stromal cells drive protective M2 microglia polarization after brain trauma, Neurotherapeutics 11 (2014) 679–695.
[63] S.M. Melief, E. Schrama, M.H. Brugman, M.M. Tiemessen, M.J. Hoogduijn, W.E. Fibbe, et al., Multipotent stromal cells induce human regulatory T cells through a novel pathway involving skewing of monocytes toward anti-inflammatory macrophages, Stem Cells 31 (2013) 1980–1991.
[64] M. Francois, R. Romieu-Mourez, M. Li, J. Galipeau, Human MSC suppression correlates with cytokine induction of indoleamine 2,3-dioxygenase and bystander M2 macrophage differentiation, Mol. Therapy 20 (2012) 187–195.
[65] K. Nemeth, A. Leelahavanichkul, P.S. Yuen, B. Mayer, A. Parmelee, K. Doi, 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. 15 (2009) 42–49.
[66] A.J. Cutler, V. Limbani, J. Girdlestone, C.V. Navarrete, Umbilical cord-derived mesenchymal stromal cells modulate monocyte function to suppress T cell proliferation, J. Immunol. 185 (2010) 6617–6623.
[67] A.I. Caplan, Correa D. The, MSC: an injury drugstore, Cell Stem Cell 9 (2011) 11–15.
[68] N. Gupta, A. Krasnodembskaya, M. Kapetanaki, M. Mouded, X. Tan, V. Serikov, et al., Mesenchymal stem cells enhance survival and bacterial clearance in murine *Escherichia coli* pneumonia, Thorax 67 (2012) 533–539.
[69] N. Gupta, X. Su, B. Popov, J.W. Lee, V. Serikov, M.A. Matthay, Intrapulmonary delivery of bone marrow-derived mesenchymal stem cells improves survival and attenuates endotoxin-induced acute lung injury in mice, J. Immunol. 179 (2007) 1855–1863.