

Chapter 2

Mesenchymal Stromal Cell Mechanisms of Immunomodulation and Homing

J. Barminko, A. Gray, T. Maguire, R. Schloss, and M.L. Yarmush

Abstract The identification of therapeutic immunomodulatory mesenchymal stromal cells (MSC) with specific homing capabilities has simultaneously contributed to the potential development of powerful cellular immune therapies, with applications for a variety of inflammatory associated diseases. MSC have the ability to directly abrogate T cell, macrophage, dendritic cell (DC), neutrophil, and B cell pro-inflammatory functions. Specifically, T cell, macrophage, and DC MSC-mediated immunosuppression results in the adoption of phenotypes indicative of type II anti-inflammatory functional cells. These findings collectively suggest that MSC directly combat inflammation by controlling endogenous immune mechanisms. In this chapter, the molecular/cellular mechanisms governing these phenomena are discussed for each MSC-immune cell interaction. Furthermore, MSC homing mechanisms are discussed, highlighting our current understanding of the modes and limitations of MSC direct implantation modalities.

Keywords Inflammation • Mesenchymal stromal cell

2.1 Introduction

Mesenchymal stromal/stem cells (MSC) have become a promising therapy for various inflammatory disease applications. MSC are being explored as a treatment for myocardial infarction [1], graft-versus-host disease (GVHD), colitis [2], liver failure [3], kidney failure [4], Crohn's disease [5], central nervous system (CNS) trauma [6], and several autoimmune diseases [7–9]. Despite the fact that the precise MSC therapeutic mechanisms are unclear, to date there are many clinical trials

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ongoing to evaluate their safety and efficacy [10]. In many of these applications MSC differentiation was thought to be the primary mechanism of action. However, a considerable amount of data suggest improved outcomes after transplantation even in the absence of apparent MSC long-term engraftment [11]. The contemporary paradigm being popularized is that MSC promote therapeutic benefits via secretion of soluble factors and cues which control immune cell functions and provide trophic support. This idea is supported by in vitro coculture studies as well as in vivo transplantation studies. As such, numerous researchers have begun to regard MSC, not only as traditional differentiating stem cells, but also as cellular drug delivery vehicles [11, 12]. Therefore, it has become evident that by evaluating the molecular factors that these cells may contribute to the local inflammatory milieu, we will ultimately be able to assess the effectiveness of their therapeutic applications. While the evaluation of MSC as a potential therapy is moving forward, the mechanisms of action are still uncertain. Ostensibly, MSC provide immune support in these systems via different mechanisms, depending on the specific disease state. Therefore, depending on the application, different MSC-secreted products will be vital. To effectively characterize these, one must understand the specific cellular mediator(s) which accompany the respective pathologies. MSC effects could then be evaluated based on the MSC's ability to modulate particular immune cell(s) functions. As a result, therapies could be tailored to maximize these interactions. Just as important to the success of MSC as a therapy is the efficiency of MSC targeting as well as persistence at the site of injury. MSC abundance and persistence in vivo will likely be crucial for therapy evaluation and clinical translation. However, specific MSC mechanisms, homing potential and persistence in vivo are currently controversial. Here we discuss the current knowledge pertaining to MSC mechanisms of immunomodulation and review the effects of MSC on several immune system networks. In addition, the chapter will conclude with a discussion on MSC mechanisms of homing.

2.2 MSC Modulation of T Cells

Thymus-derived (T) cells recognize antigens and are critical for acquired immunity. These cells originate in the bone marrow and mature within the thymus into one of several subtypes with diverse functions as either direct effector cells or immunomodulating cells. These functions include maintenance of self-tolerance, lysis of infected cells, activation of other lymphocytes, and interaction with cells of the innate immune system. Some of the T cell subsets that have been investigated in the context of MSC-mediated immunomodulation are described in Table 2.1.

The first evidence that MSC can regulate immunosuppression in vivo came from models of GVHD [13]. These studies demonstrated that MSC could reduce allograft rejection, which is partly mediated by T lymphocytes [14, 15]. Shortly after, MSC T cell immunosuppression was demonstrated in vitro [16]. Subsequently, MSC became a candidate therapy for several autoimmune-related syndromes, where

Table 2.1 T cell subsets

T cell subtype		Major functions	Identifying proteins	Important secreted proteins	Effect of excessive or disrupted activity	References
Helper T cells	T _h 1	Cell-mediated immunity. Enhancement of inflammation by promoting cytotoxic T cell proliferation and macrophage eradication of intracellular microbes	CD4	TNF- α , TNF- β , IFN- γ	Autoimmunity	[146, 147]
	T _h 2	Antibody-mediated (humoral) immunity; induction of B cell production of IgE2 and promotion of eradication of parasites; antagonize effects of T _h 1	CD4	IL-4, IL-5, IL-13	Allergies, hypersensitivity	[146, 147]
	T _h 17	Induction of neutrophil mobilization in response to injury and infection to eradicate pathogens	CD4	IL-17, IL-23	Autoimmunity	[43, 148–150]
Cytotoxic T cells (CTL)		Destruction of cancerous or infected cells expressing MHC class I and foreign antigens	CD8	Perforin, granzymes	Autoimmunity	[56, 151, 152]
Regulatory T cells	T _{reg} * including T _h 1 and T _h 3	Maintenance of self-tolerance and immune homeostasis via immunosuppression and inhibition of helper T cells	CD4, CD25, Foxp3	IL-10, TGF- β	Autoimmunity	[153, 154]

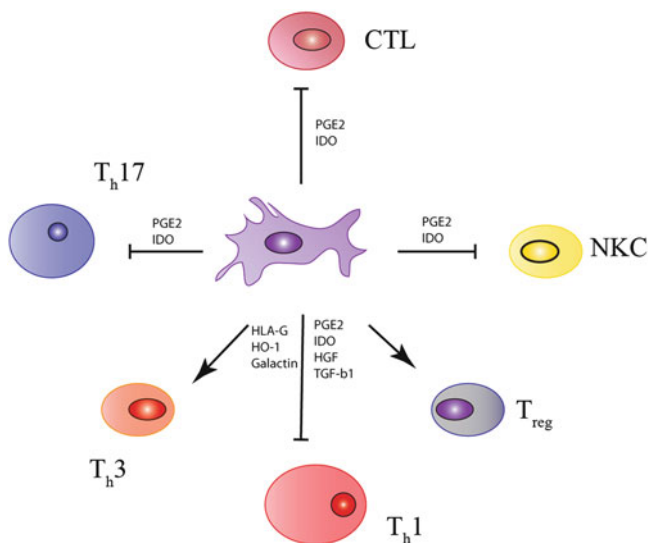


Fig. 2.1 The control of T lymphocyte differentiation and effector function by MSC. MSC modulate several aspects of T cell behaviors, primarily preventing the differentiation and expansion of pro-inflammatory mediators, T_h1 and T_h17 . Simultaneously, MSC promote T cell phenotypes which possess immunomodulatory behaviors, T_h3 and T_{reg} . Additionally, MSC demonstrate the ability to prevent cytotoxic T and natural killer cell (NKC) functions. Overall, MSC dictate T cell functions which suppress the adaptive immune response potentiating their application in T cell associated disorders

MSC effects on destructive T cell behaviors could be harnessed. MSC transplantation in animal models of experimental autoimmune encephalomyelitis (EAE) has resulted in a marked decrease in myelin degeneration [7]. MSC reduce T cell expansion in vivo and provide neuroprotection as seen by preserved axons and reduced CNS inflammation [9]. Also, MSC have been found to promote differentiation of naïve T cell into T_h2 , providing protection against demyelination and axon loss [17–19]. MSC have been explored as a potential therapy for rheumatoid arthritis (RA), controlling T cell-mediated degradation of collagen [8]. In all of these disease states, MSC control T cell-mediated autoimmunity. We now discuss the proposed mechanisms which drive the observed phenomena (summarized in Fig. 2.1).

It has been shown that MSC do not promote T cell apoptosis, but rather induce T cell anergy [7, 16]. Gonzalez et al. reported MSC suppression of activated $CD4^+$ and $CD8^+$ T cell proliferation and simultaneous promotion of T-regulatory responses as measured by enhanced interleukin (IL)-10 secretion and an increase in Foxp3-expressing $CD4^+CD25^+$ T cells [20]. These regulatory T cells (T_{reg}) were found to suppress collagen-specific T cell responses in RA models. This same group observed an identical phenomena in an experimental model of colitis [21]. Others have similarly observed MSC promotion of T_{reg} , but also T_h3 phenotypes [22]. To date there have been several reports of the ability of MSC to reduce T_h1 activities and simultaneously promote T_{reg} phenotypes [23, 24]. Najar et al. reported that this capability is

dependent on cell–cell contact as well as specific MSC to T cell ratios, with low ratios resulting in enhanced rather than reduced proliferation [25]. However, the percentage of T_{reg} in the population with a low MSC to T cell ratio was still elevated. Therefore, it will be important to consider the number of MSC required to achieve a desired effect. This will be regulated not only by the actual number of injected MSC, but also by successful cell homing and persistence. Beyth et al. similarly found that MSC facilitate T cell unresponsiveness in a cell contact and ratio-dependent manner, which is dictated by the microenvironment [26]. It was also determined that MSC reduced interferon (IFN)- γ secretion in PHA activated T cell cultures. However, this could be partially restored when lipopolysaccharide (LPS) or CD40 was used as stimulators. MSC modulation of T cell immunity may also involve other immune cells. Beyth et al. demonstrated the MSC effect on T cells to be dependent on monocytes in a dose-dependent manner [26]. This suggests that multiple cells may be involved in the overall mechanism of MSC-mediated modulation of T cell functions.

While the effects of MSC on T cell subsets is well established from in vitro and in vivo studies, the precise mechanism driving these responses is not well understood. There is evidence that Toll-like receptor (TLR)-2 stimulation augments MSC immunosuppressive behaviors by increasing secretion of indoleamine 2, 3-dioxygenase 1 (IDO1) and thus elevating the levels of kynurenines in the environment [27]. IDO1 is the rate limiting enzyme in kynurenine-dependent catabolism of tryptophan, which will halt T cell proliferation. It was shown that this was dependent on autocrine secretion of IFN- β , which was dependent on protein kinase R (PKR), but independent of IFN- γ [28]. DeLaRosa et al. also identified IDO to be essential to MSC T cell immunosuppression amongst several candidate mediators including IL-10, hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), transforming growth factor-beta 1 (TGF- β 1), and nitric oxide (NO) [29]. IFN- γ was found to be an inducer of MSC IDO1 secretion, but alternative modes of IDO1 activation were not excluded. Ryan et al. identified a similar phenomenon; however, HGF, TGF- β 1, and PGE2 were found to partially mediate immunosuppression [30, 31]. In fact, PGE2 was recently identified to play a significant role in effecting T lymphocyte subset functions [32, 33]. Therefore it is likely that not one mediator and molecular pathway is solely responsible for MSC immunosuppression but rather a synergistic effect results in maximal immunosuppression. Interestingly, the IDO1 mechanism of immunosuppression has been found to be specific to human MSC. Rat and murine MSC do not exhibit IDO1-mediated immunosuppression [34]. Instead, NO secretion in rodent models has been shown to mediate MSC T cell immunosuppression [28, 35, 36]. Ren et al. showed that MSC immunosuppression is driven by IFN- γ and tumor necrosis factor (TNF)- α mediated stimulation of NO, which locally promotes T cell anergy. MSC secretion of chemotactic factors attracts T cells and NO is secreted locally to promote anergy. In addition, several investigators have highlighted human leukocyte antigen G (HLA-G) as a mediator of MSC T cell immunosuppression [37]. MSC HLA-G secretion was found to be IL-10 dependent and maximum when in direct contact with the T cells [38]. HLA-G blocking reversed MSC immunosuppressive effects. Others have observed that MSC secretion of

galectin 1 and 3 mediates their immunosuppressive behaviors [39, 40]. siRNA knockdown of these proteins completely abolished MSC T cell immunosuppression [39, 40]. Most recently the stress protein Heme oxygenase (HO)-1 has been found to promote the MSC-mediated adoption of the T_{reg} phenotype [22]. However, when implemented in an inflammatory T cell reaction, HO-1 effects were trumped by other mediators. At the transcriptional level, signal transducer and activator of transcription 3 (STAT3) has been shown to play a crucial role in MSC effects on antigen presenting cells (APC); when STAT3 was blocked, attenuation of pro-inflammatory T cell secretion ceased [41].

It is clear that there is conflicting data supporting the exact mediators of MSC T cell immunosuppression. These discrepancies may be attributed to varying MSC isolation and culture techniques. One must also keep in mind that the mechanisms used to stimulate and isolate T cells will result in variable MSC responses and could therefore lead to differing observed mechanisms. Lastly, it may be important to distinguish the functions used to assess specific MSC factor effects. For example, Aggarwal et al. utilized T cell IFN- γ secretion as the functional outcome to prove that PGE2 is responsible for MSC immunosuppression [42]. However, Ren et al. evaluated proliferation as the T cell output parameter and identified that IDO was the primary MSC secreted factor responsible for immunosuppression [35]. In fact, it may very well be that both factors contribute to the overall response. One factor may be responsible for promoting T cell anergy (IDO) and another for promoting T_{reg} (PGE2) phenotypes. It is important to note these distinctions in the literature to fully appreciate the mechanisms MSC exploit to carry out immunosuppression.

2.2.1 MSC Inhibit T_h17 Naïve T Cell Differentiation

The recently identified $CD4^+ T_h17$ subset secretes IL-17 and has been implicated in several models of autoimmunity as an integral component of disease progression [43]. These cells are essential for effective microbial elimination through secretion of several cytokines which facilitate microbial clearance [44]. However, with respect to their role in autoimmunity, they contribute to a persistent inflammatory response and recently MSC have been shown to control T_h17 inflammatory functions. Ghannam et al. investigated the role IFN- γ and TNF- α have on enhancing MSC CD54 expression, thus permitting T_h17 adhesion to MSC via the CCR6-CCL20 interaction [45]. It was observed that $CD4^+$ T cells could not differentiate into T_h17 cells when cultured in direct cell contact with MSC. There was decreased secretion of IL-17, IL-22, IFN- γ , and TNF- α , hallmark T_h17 secretion patterns, and this effect was partially mediated by PGE2. Also, there was enhanced IL-10 secretion as well as epigenetic alteration leading to T_{reg} expression of Foxp3. All these phenotypes were enhanced when MSC were pre-incubated with IFN- γ and TNF- α . Duffy et al. observed a similar phenomenon. When indomethacin (PGE2 blocker) and selective COX-2 inhibitor were added to the coculture, MSC inhibition was reversed [46]. It was then shown that PGE2 binding of the prostaglandin

E receptor 4 (EP4) was responsible for mediating the modulatory effects of MSC in preventing T_H17 differentiation. Recent studies by Tatara et al. indicate that MSC prevent naïve T cell T_H17 , but not T_{reg} , differentiation and that the inhibition was partly attributed to MSC PGE2 and IDO secretion [47]. These in vitro findings have been found to be consistent with in vivo observations. Park et al. identified reduced T_H17 and elevated T_{reg} populations in an experimental model of autoimmune arthritis after MSC transplantation [48]. Rafi et al. demonstrated the same in vivo result, but in a model of EAE. They found that IL-17 and TNF- α levels were reduced as was CD4⁺ T cell infiltration into the spinal cord [49]. While these data are very encouraging, other reports suggest that MSC promote an opposing phenomenon, where they enhance T_H17 proliferation and function [50]. It is important to note that this study incorporated MSC at a 1:10 ratio. It has been observed that at lower ratios MSC promote inflammatory responses [25]. These types of findings convey the importance of understanding the cell doses needed to elicit desired MSC effector functions. Overall, it appears that MSC exert their control over T cell function both by preventing inflammatory phenotypes and simultaneously promoting the differentiation of anti-inflammatory T cell subtypes.

2.2.2 MSC Modulation of Natural Killer Cells and Cytotoxic T Cells

Natural killer (NK) cells are granular cytotoxic lymphocyte effector cells belonging to the innate immune system. The most well-known function of NK cells is the lysis of foreign or infected cells via release of cytotoxic granules or death receptor activating molecules [51]. This is accomplished by the complex interaction of stimulatory and inhibitory signals on target cells with NK receptors [52]. The MSC NK cell interaction is a very interesting one. Spaggiari et al. demonstrated that coculture of autologous or allogeneic MSC with IL-2 activated NK cells resulted in MSC lysis [53]. NK cell cytotoxic activity has been attributed to receptors NKp30, NKp44, and NKG2D, whose ligands, ULBPs, PVR, and Nectin-2, are expressed on MSC. Interestingly, when MSC were pre-activated with IFN- γ , NK cells no longer exhibited cytolytic activity [53]. More recently MSC have been found to prevent IL-2-induced NK cell proliferation as well effector functions [54]. In addition, MSC reduce cytotoxic activity and cytokine production as well as surface expression of the activating NK receptors NKp30, NKp44, and NKG2D. It was determined that MSC secretion of IDO1 and PGE2 was responsible for regulating this effector function. Others have suggested that HLA-G5 may also promote MSC effects on NK cell effector functions [38]. Seemingly, MSC would have to be present while NK cells are stimulated. Rasmusson et al. observed that MSC could not prevent NK cell functions when implemented post-NK cell activation; however, these studies were performed in a mixed lymphocyte reaction (MLR) [55]. Sotiropoulou et al. also observed similar MSC NK cell interactions. They claimed that certain MSC effects on NK cells are dependent on cell number, where low ratios of MSC to NK

cells can modulate NK cell function most effectively [56]. They reported that PGE2 and TGF- β 1 both have roles in mediating MSC effects on NK cell cytotoxic function.

Cytotoxic T cells (CTL) are a subset of CD8⁺ lymphocytes which are primarily responsible for inducing somatic and tumor cell lysis. They have been implicated in the progression of autoimmune diseases as well as other tissue pathologies [57, 58]. MSC effects on CTL functions are dependent on the time of implementation. MLR assays have been employed to assess MSC effects on CTL temporally. MLRs are initiated with allogenic T cell cocultures leading to proliferation and after approximately 48 h, the formations of CTL with cytotoxic capabilities. If MSC are added in the beginning of the MLR, they reduce CTL lysis by 70 %, in the absence of cell–cell contact [55]. However, they did not affect cell lysis when added 3 days into the reaction. In contrast to NK cells, direct CTL lysis of MSC has not been observed [55, 59]. Others have observed that MSC failed to modulate CTL proliferation and IFN- γ secretion once CTLs were exposed to the pathogenic viruses CMV or EBV [60]. It appears that MSC modulation of NK cells and CTL will depend upon their state of activation, which will make the timing and persistence of MSC administration critical for controlling immune cytolytic behaviors.

2.3 MSC Modulation of B Cells

B cells play an essential role in adaptive immunity. They are directly responsible for the humoral immune response via the secretion of antibodies against pathogenic or foreign antigens. A subset of B lineage cells differentiates into memory B cells, which can mediate a rapid response upon secondary exposure to that same antigen. Aberrant antibody production by B cells has been implicated in several autoimmune diseases, including systemic lupus erythematosus (SLE), and MSC have been found to modulate these responses. Stimulated B cells were arrested in the G0/G1 phase of the cell cycle when cocultured with MSC [61]. Reduction in IgM, IgG, and IgA production indicated a decrease in B cell differentiation. Furthermore, B cell responses to chemotactic ligands, specifically SDF-1 and BCA-1, were reduced as well as their expression of several membrane expressed chemotactic receptors [62]. Tabera et al. also observed B cell arrest in the G0/G1 phase of the cell cycle when cocultured with MSC, independent of cell–cell contact [63]. Furthermore, dendritic cell (DC) promotion of B cell differentiation, as seen by an increase in CD38⁺CD138⁺, as well as increased immunoglobulin secretion, was inhibited in the presence of MSC. Asari et al. reported similar findings with MSC and LPS-stimulated B cell contact-independent cell cultures [64]. Schena et al. suggest that this inhibitory effect was augmented in the presence of IFN- γ ; however, IDO1 was not the mediator of this response as it was with T cells [65]. They claim that cell–cell contact enhances MSC function and that the interaction between programmed death 1 (PD-1) receptor and the PD-1 ligand mediates the inhibitory effects of MSC (Fig. 2.2). This has been observed previously by Augello et al. [66]. Interestingly,

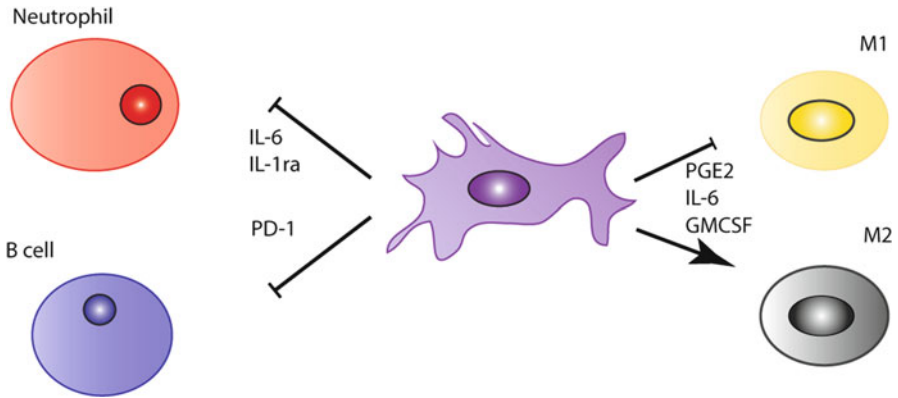


Fig. 2.2 Modulation of macrophage, B and neutrophil cell pro-inflammatory functions. MSC direct macrophages toward an M2 phenotype and simultaneously suppress M1 functions. MSC inhibit B cell differentiation via the PD-1 surface receptor, which in turn reduces immunoglobulin and chemokine production. MSC also provide effector function on neutrophils via HLA-g and IL-6, preventing pro-inflammatory secretion and migration

TLR-9 activation of B cells did not induce an MSC inhibitory response, but B cell receptor (BCR)-dependent activation was inhibited by MSC [65]. Furthermore, there is no conclusive *in vivo* data establishing an MSC ability to affect B cell functions. Schena et al. observed that MSC, in a model of lupus, reduce nephron glomerulosis [65]. However, neither reduced immunoglobulin levels nor other changes in B cell phenotypes were detected. Others have observed no effect whatsoever on B cells post MSC transplantations. Youd et al. reported that MSC do not have therapeutic potential in lupus models driven by type II inflammation-associated disorders [67]. However, this study used one transplanted dose of MSC and did not titrate or evaluate multiple injections. Also, this group waited until disease onset to implant MSC [67]. While the *in vitro* data on the MSC effect on B cells is suggestive, more studies need to be done to reveal the potential of MSC in treating B cell-mediated disorders.

2.4 MSC Modulation of Macrophages: Promotion of the M2 Phenotype

Macrophages are phagocytic cells of the myeloid lineage, differentiated from monocytes and present in essentially all tissues. They play major roles in adaptive and innate immunity and are able to perform pathogen clearance in the absence of phagocytic labels for pathogen ingestion/destruction (opsonization) and act as APC. Considering their abundance throughout the body, the macrophage is an essential player in tissue damage as well as the overall immune response.

Macrophage behaviors have been implicated in pathology after organ trauma [68], allograft organ rejection [69] and atherosclerosis [70]. Over the past several years, the complexity of the macrophage response has been documented as well as the role of phenotypic plasticity in macrophage responses. The major implication of these observations has been the distinction of classically (M1) and alternatively (M2) activated macrophages [71]. M1 macrophages represent the pro-inflammatory arm of the macrophage response while M2 is the anti-inflammatory arm. Intriguingly, MSC secrete several of the factors found to promote M2 phenotypes either constitutively or in the presence of certain soluble cues (Fig. 2.2). Furthermore, considering the tremendous amount of data supporting the ability of MSC to modulate immune responses, it is no surprise that MSC have been found to promote M2 macrophage phenotypes in the presence of stimuli which normally lead to M1 phenotypes. Kim and Hematti were the first to observe that macrophages cultured in the presence of MSC adopted phenotypes indicative of M2 macrophages (CD206^{high}, IL-10^{high}, IL-12^{low}) after 48 h of culture [72]. These studies were performed in the absence of cell–cell contact, suggesting that soluble factors were responsible for the phenomenon. Gonzales et al. cocultured colitis-derived macrophages with MSC and found that the pro-inflammatory secretion of TNF- α and IL-12 was diminished [21]. Anti-inflammatory IL-10 secretion was found to be elevated and, when PGE2 blocking antibodies were introduced, inflammatory functions were partially reverted. Reports by Cutler et al. suggested that MSC can modulate monocyte functions, which ultimately resulted in the suppression of T cell proliferation [73]. They suggested that this response was dictated by MSC secretion of PGE2. Similarly, Maggini et al. observed that thioglycolate-treated peritoneal macrophages cultured with MSC adopted a regulatory phenotype [74]. These macrophages exhibited reduced secretion of pro-inflammatory mediators and enhanced secretion of anti-inflammatory mediators [74]. Furthermore, LPS-dependent upregulation of major histocompatibility complex (MHC) class II and co-stimulatory CD86, factors which are responsible for macrophage antigen presentation, were mitigated [74]. They claimed that MSC secretion of PGE2 was responsible for these changes. Zhang et al. also observed that macrophages assumed M2 phenotypes in the presence of MSC. Macrophages expressed mannose receptors (CD206) and secreted IL-10, hallmarks of M2 macrophage phenotypes. This was observed with a concomitant reduction in M1 secretion of TNF- α as well as the ability to stimulate T_H17 expansion [75]. It was suggested that MSC drive macrophage phenotype through the synergistic interaction between granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-6, which when blocked, reduced macrophage expression of CD206 [75]. The studies of Barminko et al. support these findings as well. They observed that THP-1 pro-inflammatory secretion of IL-1 β , TNF- α , IP-10, and MIP1- α was reduced in the presence of MSC. These macrophages exhibited elevated CD206 expression as well as IL-10 secretion [76]. Collectively, these observations strongly suggest that MSC can dictate macrophage plasticity toward regulatory M2 behavior. MSC promote similar phenomena in vivo. In an animal model of sepsis, Nemeth et al. found that MSC reprogrammed macrophages to secrete IL-10 and this was dependant on MSC secretion of PGE2 [77].

Gonzalez et al. reported that MSC reduced T_H1 driven histopathology as well stimulated systemic levels of IL-10 in an experimental model of colitis [21]. They claimed that MSC act directly on activated macrophages to partially facilitate these benefits. In skin injury models, MSC transplantation accelerated wound healing by increasing the number of macrophages infiltrating the wound site [78]. Zhang et al. observed that subcutaneous administration of MSC increased M2 macrophages and enhanced wound healing [75]. Ohtaki et al. explored the effects of MSC on inflammation in an animal model of stroke and found that microglia exhibited M2 phenotypes. The implication of these findings is that if appropriately implemented in vivo, MSC could be utilized as a means of driving endogenous macrophage expression of M2 phenotypes. This could potentially provide an approach to enhance resolution of chronic inflammation.

2.5 MSC Modulation of Neutrophils

Neutrophils are phagocytic granulocytes and are among the first cells to arrive at sites of inflammation. They are recruited and activated by chemoattractants such as IL-8, CXCL1, CXCL2, CXCL5, CCL3, and CCL4, produced by tissue-resident and circulating macrophages that have been activated in response to microbial components or tissue damage-associated molecular patterns (DAMPs) [79]. The main targets of neutrophil activity are pathogenic microorganisms. Upon invasion into the inflamed tissue, neutrophils that encounter microbes phagocytize them and fuse the phagosome with intracellular granules containing acidic hydrolases and bactericidal proteins [80]. If no microbe is encountered within a short time activated neutrophils will release these and other granules containing proteolytic enzymes into the extracellular space. This combined with the abrupt release of reactive oxidative species (respiratory burst) can cause further damage to the tissue [81]. MSC have been reported to affect the neutrophil contribution to inflammation by influencing their recruitment and invasion into tissues in several models of inflammatory conditions, including sepsis [77], acute lung injury [82, 83], diabetes [84], and tetrachloride-induced cirrhosis [85]. The mechanism of this effect may be indirect (Fig. 2.2). In a model of sepsis, Nemeth et al. reported that septic mice treated intravenously with MSC had higher numbers of circulating neutrophils, lower levels of myeloperoxidase (a granulocytic enzyme) in the liver and spleen, and higher secretion of IL-10 from macrophages [77]. They hypothesized that the decreased invasion of neutrophils was due to this direct effect of MSC on macrophages, since IL-10 has been reported to inhibit neutrophil migration from the vasculature [86–88]. Ortiz et al. reported that certain subpopulations of MSC produce IL-1 receptor antagonist (IL-1ra), which may block the production of pro-inflammatory cytokines from macrophages and therefore the subsequent expression of adhesion molecules and chemokines by endothelial cells, resulting in decreased neutrophil recruitment [82]. This proposed mechanism was supported by the observation of a reduced number of neutrophils in the bleomycin-injured lungs of mice treated with MSC.

There is also evidence of more direct relationships between MSC and neutrophils. Raffaghello et al. reported that MSC have an anti-apoptotic effect on neutrophils, which is independent of cell–cell contact. Instead, MSC secretion of IL-6 is apparently the key soluble factor responsible for this effect [89]. The neutrophil production of reactive oxidative species was also inhibited, while phagocytosis was unimpaired. Interestingly, activation of MSC through TLR3 and TLR4 may enhance this anti-apoptotic effect as well as enhance the respiratory burst function of neutrophils [90]. No effect on neutrophil adhesion molecules or migration was observed, again supporting the notion that the effect of MSC on neutrophil tissue invasion is indirect in nature [64].

2.6 MSC Modulation of Dendritic Cells

DC are phagocytic APC which link the innate immune system to the adaptive immune system. After differentiation from myeloid progenitor cells in the bone marrow, DC distribute to the blood and many peripheral tissues [91]. DC in non-lymphoid tissues are considered immature and in a state of surveillance characterized by low expression of MHC class II, very low expression of co-stimulatory molecules, and little secretion of IL-12 [92]. Encounter with a bacterial, viral, or parasitic component activates DC, allowing them to phagocytize the antigen, process it, and present it on their cell surface [93]. During this maturation process, surface expression of MHC class II and co-stimulatory molecules are upregulated, as is IL-12 secretion. These activated DC then migrate to lymphoid tissues, where they present their antigen complexes to T and B lymphocytes, thereby initiating the adaptive immune response.

MSC have been shown to affect each aspect of DC participation in inflammation (differentiation, maturation, and function; Fig. 2.3) in numerous in vitro coculture systems [94–100]. MSC are consistently reported to inhibit DC differentiation, decrease the expression of MHC class II and co-stimulatory molecules, decrease the secretion of IL-12 and inhibit the capacity to stimulate T cell proliferation for both CD34⁺-derived and monocyte-derived DC. Several mechanisms for these effects have been proposed. Ramasamy et al. reported that the differentiation of DC from peripheral blood monocytes was inhibited due to a human MSC-derived arrest of cell cycle in G₀ [97]. The reduced expression of MHC class II and co-stimulatory molecules and impaired stimulation of T cell proliferation observed by Djouad et al. were attributed to the secretion of high levels of IL-6 by murine MSC [94]. IL-6 has previously been suggested to be an important regulator of DC differentiation [101]. Action of MSC-derived PGE2 has also been implicated as having a central role in these effects on DC [98, 100].

In addition, MSC have been shown to exert effects on mature DC (maDC). Zhang et al. demonstrated that MSC increase maDC proliferation, which display high endocytic capacity, low immunogenicity, and strong immunoregulatory function [102]. Likewise, Wang et al. observed a reduction in maDC expression of maturation

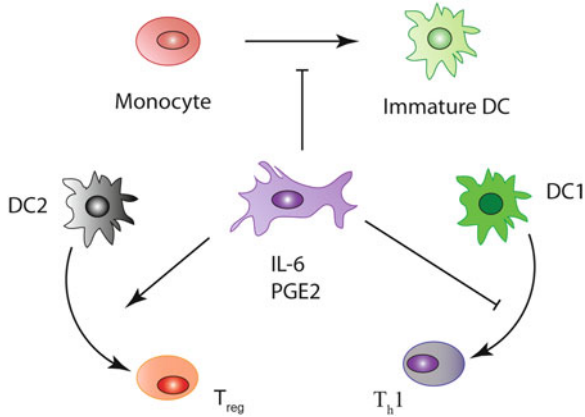


Fig. 2.3 MSC impede DC maturation and augment DC anti-inflammatory functions. Dendritic cells are potent APC, possessing a great deal of control over the adaptive immune response. MSC prevent monocyte commitment to DC differentiation and subsequent maturation. Furthermore, MSC favor DC2 phenotypes within mature maDC populations, while simultaneously inhibiting DC1 phenotypes. The consequence of these observations is that MSC treated DC will favor type II T cell immune functions in vivo

marker CD83 as well as an increase in endocytic activity [103]. These DC orchestrated a shift from pro-inflammatory T_h1 to anti-inflammatory T_h2 , suggesting that MSC can promote DC immunoregulatory phenotypes. Interestingly, DC exhibit type I and II phenotypes as do most cells of the immune system. These cells have been referred to as DC1 (pro-inflammatory) and DC2 (regulatory) [104]. Studies by Aggarwal et al. indicated that MSC enhance DC2 functions, while subduing DC1, within a maDC population [42]. The data suggest that MSC can direct DC to adopt regulatory phenotypes. Considering the tremendous control of DC subpopulations over the immune system, MSC success both in treating autoimmune disorders and overcoming allogeneic organ transplantation, may be partially attributed to their effects on DC.

2.7 Differential MSC Activation

The immunomodulatory potential of MSC has been described by many investigators using numerous in vitro systems and in vivo models of inflammatory diseases/conditions. This is not, however, a constitutive function of MSC since activation by external factors is required to attain MSC immunomodulatory activity [105]. Further, the outcome of MSC activation is dependent upon the types of stimulating factors as well as the order and timing of MSC exposure [106]. Many reports related to this have recently emerged regarding the function of TLR in MSC. TLR are a class of pattern recognition receptors (PRR) which recognize bacterial, viral,

fungal, and protozoal pathogen-associated molecular patterns (PAMPs), and therefore are very important in innate immunity [107]. There is also ample evidence that they can be activated by endogenous danger signals (DAMPs) [108]. Stimulation of these receptors results in activation of MyD88-dependent (NF- κ B) and -independent (IRF) pathways, resulting in the production of pro-inflammatory cytokines and type 1 interferons [109, 110].

Human MSC have been reported to express TLR1, 2, 3, 4, 5, and 6 mRNA and TLR2, 3, 4, 7, and 9 protein [111]. The effect of TLR3 and TLR4 activation on MSC functions, including migration, differentiation, and immunomodulation, has been the particular focus of many recent studies, sometimes describing contradictory results [27, 90, 112–116]. Liotta et al. observed that activation of MSC TLR3 and TLR4 resulted in NF- κ B activity, the secretion of inflammatory cytokines and chemokines (IL-6, IL-8, CXCL10), and the inhibition of the suppression of T cell proliferation [112]. This was attributed to a downregulation of Jagged-1 in MSC after TLR3 and TLR4 ligation, which resulted in impairment of MSC signaling to T cell Notch receptors. Contrary to these findings, Opitz et al. reported that engagement of TLR3 and TLR4 enhanced the MSC-mediated suppression of T cell proliferation by inducing an IFN- β autocrine signaling loop that led to the MSC production of IDO1 [27]. The findings of Waterman et al. fall in the middle of these two contradictory reports. MSC were able to suppress T cell activation after TLR3 priming, but were unable to have this effect after priming of MSC TLR4 [116]. There is further evidence of the anti-immunosuppressive effect of TLR4 activation in vivo. Wang et al. investigated whether MSC from TLR4 knock-out mice could have a therapeutic effect after myocardial ischemia/reperfusion injury [115], a disease model in which MSC have been shown to impart therapeutic benefit [117, 118]. They reported that TLR4-deficient MSC were better able to impart cardioprotection due in part to increased production of angiogenic factors and increased activation of the STAT3 pathway.

Despite some contradictory reports, there is evidence that MSC can have differential states of activation with different immunomodulatory outcomes based on which molecules they are exposed to. Due to this apparent plasticity in MSC phenotype, it has been suggested the MSC be considered as adopting either MSC1 or MSC2 phenotype, following the paradigm used in the monocyte literature [116]. Differential MSC activation may prove to be important when considering their therapeutic use. Further investigation of MSC activation and related underlying mechanisms of immunomodulation may also prove to be a valuable therapeutic tool in that MSC can be preprogrammed/pre-activated to the particular phenotype that will be the most beneficial for the specific disease/condition under consideration.

2.8 MSC Homing

Just as understanding MSC mechanisms of action are important in designing an effective therapy regimen, ensuring that MSC will target the proper tissue is equally as important. MSC have been heralded for their ability to specifically home to areas

of tissue damage. The ability to noninvasively transplant MSC and then have them specifically home to areas of tissue injury is an intriguing and controversial concept. Much can be gleaned from leukocyte and hematopoietic stem cell (HSC) homing, which is a multistage process of (1) chemotaxis, (2) tethering and rolling, (3) firm adhesion, and (4) diapedesis. Most MSC targeting studies attempt to evaluate potential mechanisms of homing in the context of what is known about leukocyte extravasation. We begin our discussion with molecular cues that initiate MSC migration to areas of tissue trauma.

2.8.1 Chemotaxis

Post injury, chemokines activate local endothelial cells to increase expression of cell surface P-selectin, E-selectin, and vascular cell adhesion protein 1 (VCAM-1). These chemokines are also released into the systemic circulation and selectively activate specific leukocyte subsets. Chemokines potentially secreted post-trauma are CXCL12 (SDF-1), CCL2, CCL3 CCL4, CXCL8 (IL-8), CXCL1, and CXCL-10. MSC express receptors for several of these chemoattractant proteins [119–122]. The stromal cell-derived factor 1 (SDF-1)/CXCR4 axis has been described to induce MSC mobilization [123, 124]. Others have found that SDF-1 acts synergistically with other factors, such as HGF, to potentiate MSC targeting [125]. In vitro transmigration assays identified that MCP-1, MIP-1 α , IL-8 as well as ischemic brain tissue extract enhance MSC migration [126]. Platelet-derived growth factor (PDGF) [127] and vascular endothelial growth factor A (VEGF-A) [128] have displayed similar functions in vitro. These factors have also been implicated in directing MSC into the injury site locally, once they have adhered to the endothelial lumen. However, in vivo studies directly linking these factors to extravasation efficiency have not been reported.

2.8.2 Tethering and Firm Adhesion

Once a leukocyte migrates to its destination, adhesion molecules on endothelial cells bind leukocyte receptors to facilitate tethering. Tethering decelerates the leukocyte flow and permits strong adherence to the luminal wall. Several protein interactions have been identified to govern this phenomenon and include endothelial P-, L-, and E-selectin binding to carbohydrates on leukocyte transmembrane glycoproteins [129]. Leukocyte firm adhesion is mediated by β 1 integrins, particularly α 4 β 1 (VLA-4) and α 5 β 1 (VLA-5) [130]. Ruster et al. indicated that MSC adhere to endothelial cells via P-selectin and VCAM-1/VLA-4, similar to HSC and peripheral blood mononuclear cell (PBMC) [131]. Firmness of adhesion was increased upon TNF- α endothelial stimulation, which likely increased cell surface expression of

integrin adhering proteins. There is conflicting evidence that MSC do not utilize P- or any other selectins as an endothelial tethering mechanism [122]. However, in that study MSC expression of VLA-4 was detected and found to bind VCAM-1. Likewise, Steingen et al. showed that antibody blocking of either VCAM-1 or VLA-4 significantly diminished MSC-endothelial cell adhesion [132]. In vivo, pre-blocking MSC with an antibody against integrin $\beta 1$ before transplantation significantly reduced MSC homing to myocardial infarct sites [133]. Semon et al. published a thorough analysis of MSC integrin expression. They indicated that integrin subunits $\beta 1$, $\beta 2$, and $\alpha 3$ were expressed on over 80 % of the MSC population [134]. While the authors highlight the many discrepancies regarding integrin expression on MSC, integrin $\beta 1$ has unequivocally been detected on these cells. While the data supporting MSC use of classical selectin tethering mechanisms are debatable, the VCAM-1/VLA-4 axis has consistently been found to play a major role in homing. Some have suggested that since MSC are larger than HSC, both passive and active homing mechanisms may be involved in successful MSC targeting [122, 135]. Therefore, MSC may not need to exhibit classical tethering mechanisms to attach to the endothelium. Furthermore, endothelium from different tissues utilize distinct subsets of these integrins to facilitate adhesion [132, 134], suggesting that MSC homing efficiency will depend on the specific nature of the targeted tissue. Others have suggested that clotting factors such as fibronectin could potentially bind integrin subunits on MSC [136].

2.8.3 Diapedesis

The final step in MSC extravasation is trans-endothelial migration into the targeted tissue. Unlike rolling and adhesion, the mechanisms driving diapedesis are not well understood. There is evidence for para- and trans-cellular routes of tissue entry [137]. However, considering the size of an MSC, transcellular entry would be an unlikely route as MSC would need to transverse the basement membranes of these tissues. Son et al. demonstrated MSC matrix metalloproteinase 2 (MMP-2) and membrane type 1 matrix metalloprotease (MT1-MMP) mediated transmigration across a Matrigel basement membrane [125]. Similarly, Becker et al. also described the MMP-2 mediated transmigrations of MSC [138]. Others have described the role of the MMP1/protease-activated receptors (PAR1) axis in MSC migration, as blocking of this interaction resulted in reduced migration in a glioma model [139]. These findings suggest that MSC would degrade the endothelial layer to enter the tissue.

The disparity in the mechanisms proposed to dictate MSC homing capabilities may be attributed to several factors. MSC expression of homing mediators begin to decline over passage number and is dependent upon culture conditions [120]. Therefore, depending on the isolation techniques and cultures conditions, MSC from different laboratories will display varying homing potential and mechanisms. To complicate the issue further, MSC isolated from different tissues may also express these homing mediators differently [140]. Some investigators have suggested

that MSC homing mechanisms are trumped by their tremendous size [122], as MSC will get trapped in nonspecific locations and will therefore display reduced homing efficiencies which are chemotaxis-independent [135]. This may explain why localized delivery of MSC enhances engraftment efficiencies [141–143] since intravenously injected MSC have been observed to be systemically delivered to unintended tissues, mainly the lung and liver [141, 144]. Furthermore, MSC have not been found to persist at a tissue site long term and are sometimes undetectable as early as 1 week post transplantation [145]. To date MSC homing potential and the mechanisms which govern homing control continue to be debated.

2.9 Conclusion

It is clear that MSC have a tremendous effect on the immune system. MSC seem to exert their effects by controlling white blood cell differentiation into regulatory phenotypes as well as attenuating pro-inflammatory functions. Also, MSC appear to affect differentiated immune cell functions; however, the degree of regulation appears to be dependent upon the particular cell type and the activation state of MSC. The specific mediators which control these unique cell–cell interactions vary, and it is likely that several mediators synergistically contribute to the overall effects. In addition, the homing mechanisms governing MSC targeting are poorly understood and are also extremely controversial. It appears that MSC targeting is not as efficient as once thought. Furthermore, since persistence may not be long term, MSC therapeutic potential may be maximized with continuous implantations. Considering the complexity of MSC immunomodulation and the factors or cell–cell interactions necessary for effector functions to be activated, maximal immune regulation may require constant MSC surveillance. Therefore, approaches to prolong MSC persistence will be crucial in translating their immunotherapeutic potential.

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