Mesenchymal Stromal Cells in Transplantation Rejection and Tolerance

Karen English[1] and Kathryn J. Wood[2]

[1]Cellular Immunology Group, Institute of Immunology, National University of Ireland Maynooth, County Kildare, Ireland

[2]Transplantation Research Immunology Group, Nuffield Department of Surgical Sciences, John Radcliffe Hospital, University of Oxford, Oxford 0X3 9DU, United Kingdom

Correspondence: karen.english@nuim.ie

Mesenchymal stromal cells (MSCs) have recently emerged as promising candidates for cell-based immunotherapy in solid organ transplantation (SOT). In addition to immune modulation, MSCs possess proreparative properties and preclinical studies indicate that MSCs have the capacity to prolong graft survival and in some cases induce tolerance. Currently, the application of MSCs in SOT is being evaluated in phase I/II clinical trials. Whereas the mechanisms of action used by MSC immunomodulation have been somewhat elucidated in vitro, the data from preclinical transplant models have been unclear. Furthermore, the optimal timing, dose, and route of administration remain to be elucidated. Importantly, MSCs have the ability to sense their environment, which may influence their function. In this article, we discuss the impact of the local microenvironment on MSCs and the mechanisms of MSC immunomodulation in the setting of SOT.

Mesenchymal stromal cells (MSCs) are a subpopulation of multipotent cells originally identified in the bone marrow (Friedenstein et al. 1976). MSCs are characterized by their fibroblast-like appearance, colony forming unit capacity, and their rapid adherence to tissue culture plastic. Although MSCs are relatively easy to isolate, culture, and expand (from a number of tissues), the lack of a unique marker to identify MSCs has impacted the advancement of this research field as difficulties arise in comparing data using different MSC populations. In 2006, the International Society for Cellular Therapy proposed a set of phenotypic and functional criteria to define MSCs (Dominici et al. 2006), however, the discovery of new markers that specifically identify MSCs are eagerly awaited. MSCs have the capacity to differentiate into adipocytes, chondrocytes, and osteoblasts in vitro and in vivo (Pittenger et al. 1999). Based on the differentiation potential of MSCs, initially studies focused on the regenerative capacity of these cells (Mahmood et al. 2003; Murphy et al. 2003); however, over time, it became clear that MSCs mediated their effects predominantly through the production of trophic factors (Caplan and Dennis 2006; Prockop 2009). Indeed, some of these trophic

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factors facilitate MSC modulation of immune responses.

One of the first reports describing MSC immunosuppressive capacity was in fact a transplant model that showed that allogeneic (donor derived) MSCs prolonged allogeneic (donor and third-party-derived) skin graft survival (Bartholomew et al. 2002). Around the same time, Di Nicola et al. (2002) showed that MSCs mediated their suppressive effect through secretion of soluble factors. A significant body of data now supports an immunosuppressive capacity for MSCs both in vitro and in vivo. At the outset, studies focused primarily on MSC suppression of the adaptive immune response showing that MSCs can directly inhibit T-cell function, shift the T-helper lymphocyte balance, induce T-cell apoptosis, and induce functional regulatory T cells (Treg) (Kong et al. 2009; Ge et al. 2010; Akiyama et al. 2012). With respect to B cells, the available data are sparse and in some cases contradictory, but some studies suggest that MSCs can also suppress B-cell proliferation and function (Comoli et al. 2008). Recent findings convincingly show that MSCs modulate multiple components of the innate immune system including complement, toll-like receptor (TLR) signaling, macrophages, dendritic cells neutrophils, mast cells, and natural killer cells (Spaggiari et al. 2006; English et al. 2008; Kim and Hematti 2009; Nemeth et al. 2009; Cutler et al. 2010; Choi et al. 2011). Therapeutic efficacy of MSC anti-inflammatory effects has been established in a number of preclinical models including graft versus host disease, sepsis, inflammatory bowel disease, and allergic airway disease (Polchert et al. 2008; Ren et al. 2008; Nemeth et al. 2009; Kavanagh and Mahon 2011; Akiyama et al. 2012). In the case of solid organ transplantation (SOT), MSCs exert their effects on two fronts through attenuation of ischemia reperfusion injury (Liu et al. 2012a) and through the prevention of allograft rejection (Casiraghi et al. 2008; Ding et al. 2009; Ge et al. 2010). Moreover, in some cases, MSC induce a state of tolerance (Ge et al. 2010; Casiraghi et al. 2012). The in vitro immunosuppressive capacity, combined with the proven therapeutic efficacy of MSCs in preclinical models, has paved the way for MSCs in clinical application. Further evidence of a protective role for MSCs in preclinical models of organ transplantation in combination with the reported safety of MSCs in clinical trials has prompted the evaluation of safety and efficacy of MSCs in SOT (Tan et al. 2012). Herein, we will discuss the underlying mechanisms of MSC immunomodulation in the context of ischemia reperfusion injury, prevention of allogeneic graft rejection, and induction of tolerance.

REJECTION

Mechanisms of Transplantation Rejection

Despite the significant achievements accomplished during the past 60 years in SOT, rejection remains the greatest barrier (Wood and Goto 2012; Wood et al. 2012). Whereas, the advent of immunosuppressive drugs has facilitated improved outcomes in graft survival and long term function, the toxicity and associated complications of nonspecific immunosuppression are substantial limiting factors (Halloran 2004). Thus, there is a significant unmet need for nontoxic immunosuppressive therapies.

The immune response to an allograft is an ongoing process involving both innate and adaptive components starting from the moment of reperfusion. In fact, the tissue injury associated with organ retrieval (ischemic injury) initiates the production of damage-associated molecular patterns (DAMPs) and subsequent activation of the complement system and innate immune cells (macrophages and neutrophils) through pathogen recognition receptors (PRRs) (Eltzschig and Eckle 2011) after reperfusion and initiates a local inflammatory environment. Activation of the innate immune response orchestrates the adaptive immune response. Although, graft rejection is multifactorial, alloantigen specific induction of T-cell proliferation and activation of T-cell effector functions is the major player in graft destruction. Antibody mediated rejection is triggered by alloantibody binding and complement activation and also significantly contributes to graft loss. Here, we discuss the effect of MSC on ischemia reperfusion injury and innate and

adaptive components in the context of solid organ transplantation.

ISCHEMIA REPERFUSION INJURY

Effect of Mesenchymal Stromal Cells on Ischemia Reperfusion Injury

Ischemia reperfusion injury causes sterile inflammation and results in the production of a number of DAMPs including necrotic cells, cellular debris, heat shock proteins (HSPs), and high mobility group protein box-1 (HMGB-1) (Eltzschig and Eckle 2011). DAMPs activate PPRs such as TLRs, C-type lectin receptors, nucleotide-binding oligomerization domain (NOD) and NOD-like receptors, receptor for advanced glycation endproducts (RAGE), and retinoic acid inducible gene-I receptors. Signaling through these receptors results in activation of the inflammasome (Ogura et al. 2006) and the complement system, upregulating gene transcription and production of micro-RNAs (Eltzschig and Eckle 2011) involved in the inflammatory response. Together, these factors lead to the production of proinflammatory cytokines, the activation of platelets and endothelial cells, tissue hypoxia, and the recruitment of innate and eventually adaptive immune cells (Eltzschig and Eckle 2011).

MSCs are known to express a number of PRRs, including TLR1-9 (Pevsner-Fischer et al. 2007; Tomchuck et al. 2008; Opitz et al. 2009; Romieu-Mourez et al. 2009), NOD receptors (Kim et al. 2010; Sioud et al. 2010), and RAGE (Kume et al. 2005). These receptors are functionally active on MSCs, and binding to their respective ligands leads to alterations in MSC functions. For example, stimulation of NOD-like receptors on MSC leads to the production of interleukin (IL)-8 and vascular endothelial growth factor (Kim et al. 2010; Sioud et al. 2010). HMGB-1 signaling through RAGE induced MSC migration and inhibited MSC production of indoleamine 2,3-dioxygenase (IDO) (Lotfi et al. 2011). TLR3 and TLR4 activation of MSCs resulted in differential effects with TLR4 priming inducing a proinflammatory phenotype and secretion of IL-6, IL-8, and transforming growth factor β (TGF-β), whereas TLR3 priming induced anti-inflammatory MSCs producing IDO, prostaglandin E-2 (PGE-2), IL-4, and IL-1RA (Waterman et al. 2010). In regard to MSC immunosuppressive function, TLR3 and TLR4 enhanced MSC immunosuppression in vitro through IDO induction via IFN-β and protein kinase R signaling (Opitz et al. 2009). In addition, TLR2 but not NOD-1 activation of human MSCs resulted in the upregulation of the immune suppressive protein galectin-3 (Sioud et al. 2010). In contrast, Liotta et al. (2008) showed that TLR3 and TLR4 ligand binding attenuated MSC immunosuppressive effects. It is clear that MSCs are receptive to environmental cues that may be present during ischemia reperfusion injury, however, further research is required to understand how these DAMPs effect MSCs and whether or not they play a role in determining the function of MSCs.

MSCs are also responsive to complement and migrate in response to C1q, C3a, and C5a (Schraufstatter et al. 2009; Qiu et al. 2012), and high levels of C3 activation correlate with enhanced immunosuppressive capacity of MSCs (Moll et al. 2011). Importantly, MSCs express CD59, a complement regulatory protein, and also release complement factor H that protects them from complement lysis (Tu et al. 2010; Moll et al. 2011). In addition, stimulation through C3aR and C5aR protect MSCs from oxidative damage (Schraufstatter et al. 2009) and MSCs produce a number of antioxidants including hemeoxygenase-1 and superoxide dismutase (Kemp et al. 2010; Mougiakakos et al. 2011), and have been shown to suppress oxidative stress and inflammation in ischemia reperfusion injury models in vivo (Chen et al. 2011, 2012; Sun et al. 2011; Du et al. 2012). The exact mechanisms of action are unclear; however, MSC protection in these models was associated with increased expression of IL-10, heme oxygenase-1 (HO-1), and hepatocyte growth factor, decreased expression of the proinflammatory cytokines IL-1β, TNF-α, and interferon γ (IFN-γ), reduced reactive oxygen species, reduced apoptosis and decreased numbers of activated T cells, and infiltrating immune cells (Hara et al. 2011; Sun et al. 2011; Chen et al. 2012; Du et al. 2012).

MSC derived microvesicles combined with soluble factors have been shown to protect against ischemia reperfusion induced acute and chronic kidney injury through inhibition of apoptosis and stimulation of tubular epithelial-cell proliferation (Gatti et al. 2011). Importantly, a protective effect of MSCs in ischemia reperfusion transplant models has been reported. Administration of MSCs reduced intragraft inflammatory gene expression and recruitment of antigen presenting cells into the allograft in a prolonged cold ischemic kidney transplant model (Hara et al. 2011) and provided long-term protection from chronic allograft nephropathy (Franquesa et al. 2012a). Ischemia reperfusion also plays a key role in the recruitment of MSCs to transplanted organs (Casiraghi et al. 2012). MSC recruitment mediated by ischemia reperfusion injury in combination with an ongoing alloreactive response leads to premature graft dysfunction and fails to prolong graft survival (Casiraghi et al. 2012). This study showed that although MSCs administered posttransplant promoted neutrophil infiltration and complement deposition, infusion of MSCs pretransplant induced significant allograft survival through a Treg dependent mechanism (Fig. 1) (Casiraghi et al. 2012). The key observation of this study is that MSCs infused pretransplant localize in the lymphoid organs whereas MSC administered posttransplant are recruited to the graft (syngeneic or allogeneic). Overall, it seems that MSCs can exert protective effects in ischemic reperfusion injuries through anti-inflammatory and paracrine factors and this likely plays an important part in MSC enhancement of allograft survival. However, there is still a lot to learn with regard to the effect of the ischemic environment on MSC functions and how this might impact MSC therapy in solid organ transplantation.

TOLERANCE

Mesenchymal Stromal Cell Modulation of Macrophages

Macrophages and neutrophils are generally the first innate immune cells to infiltrate the graft postischemia reperfusion injury. Whereas neutrophils are present only in the graft during inflammatory episodes, macrophages are present throughout the life of the graft, infiltrating in response to ischemia reperfusion injury, and maintained in reduced numbers after resolution of tissue injury in the absence of rejection. Neutrophils and macrophages have been shown to play a role in graft rejection through tissue damage induced by effector function, production of proinflammatory cytokines, and activation of antigen specific T cells (Wyburn et al. 2005). On the other hand, macrophages are also known to play a role in tissue repair and can be repolarized from pro to anti-inflammatory (Sica and Mantovani 2012). Although it has not been shown in a SOT setting, MSCs have the capacity to re-educate monocytes/macrophages. MSCs induce alternatively activated macrophages down-regulating the production of TNF-α, IL-1α, IL-6, and IL-12p70, and increasing the production of IL-10 and enhancing phagocytic activity (Kim and Hematti 2009; Nemeth et al. 2009; Cutler et al. 2010; Choi et al. 2011) through production of IDO and PGE-2 (Maggini et al. 2010; Francois et al. 2012).

Two important studies build a picture of how MSCs orchestrate macrophage polarisation and the influence the local microenvironment has on that process. Nemeth and colleagues show that MSCs ameliorate sepsis through alternative activation of macrophages, showing that lipopolysaccharide (LPS) and TNF-α activate TLR4 and tumor necrosis factor receptor 1 on MSCs to activate NF-κB signaling. This, in turn, leads to the expression of cyclooxygenase (COX)-2 and synthesis of PGE-2 by MSCs that bind E-prostanoid 2/4 (EP2/EP4) receptors on macrophages resulting in increased production of IL-10 and facilitating the resolution of inflammation (Nemeth et al. 2009). In the second study, Prockop's group used a zymosan induced peritonitis model to show that MSCs exerted anti-inflammatory effects through the production of tumor necrosis factor α (TNF-α)-induced protein 6 (TSG-6), which subsequently limits TLR2/NF-κB signaling through direct interaction with CD44 expressed on the macrophage to initiate a negative feedback loop

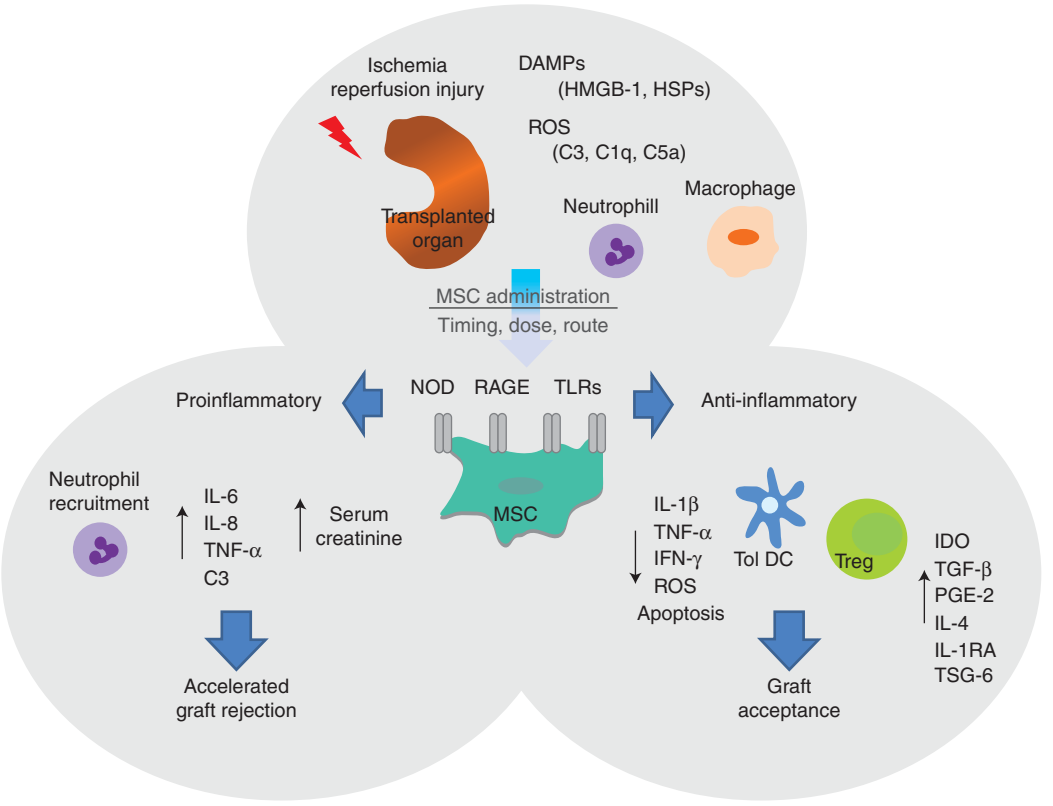

Figure 1. Influence of ischemia reperfusion injury on MSC function. Ischemia reperfusion injury (associated with organ retrieval) leads to the production of DAMPs including HMGB-1 and HSPs among others. Signaling through PRRs including TLRs, NOD receptors, and RAGE results in the activation of the complement system, recruitment of innate immune cells (neutrophils and macrophages), and the production of reactive oxygen species (ROS). Depending on the timing, dose, and route of administration, MSC given to patients in the context of SOT may encounter an ischemia reperfusion microenvironment. MSCs express a number of PRRs including NOD, RAGE, and TLRs, and activation of these receptors through DAMPs like HMGB-1 and HSPs may determine MSC function. Signaling through RAGE, NOD-like receptors, or TLR4 on MSC may lead to the promotion of a proinflammatory environment leading to the production of the proinflammatory cytokines IL-6, IL-8, and TNF-α, neutrophil recruitment, and complement deposition resulting in elevated serum creatinine and accelerated graft rejection. Alternatively, activation of TLR3 on MSC may induce an anti-inflammatory milieu, reducing the production of proinflammatory cytokines (IL-1β, TNF-α, and IFN-γ), decreasing ROS and apoptosis, and generating tolerogenic dendritic cells and Treg facilitating graft acceptance.

inhibiting the inflammatory response (Choi et al. 2011). Although MSC secretion of TSG-6 has been implicated in corneal allograft survival (Oh et al. 2012), it remains to be determined whether or not MSC derived TSG-6 can modulate macrophages in this setting. On the whole, microenvironmental cues present at the site of MSC activation seem to determine the particular mechanism of actions deployed by MSCs in modulating the immune response and resolving inflammation.

Mesenchymal Stromal Cell Modulation of Dendritic Cells

Dendritic cells (DCs) are sentinel cells and as such, present alloantigens activating antigen specific T cells. Both donor and recipient

DCs play a critical role triggering graft rejection through the direct, indirect, or semi-direct pathways of allorecognition (Wood and Goto 2012). MSCs can interfere with the key features of DC function: migration, maturation, and antigen presentation (English et al. 2008), and mediate these effects through down-regulation of DC maturation markers including major histocompatibility complex (MHC) class II, CD40, CD80, and CD86 (Nauta et al. 2006a; Djouad et al. 2007; Li et al. 2008b; Zhang et al. 2009), and modulation of the lymph node homing chemokine receptor CCR7 in vitro (English et al. 2008) and in vivo (Chiesa et al. 2011). The soluble factor IL-6 produced by MSCs has been shown to be involved in down-regulation of maturation markers (Nauta et al. 2006a; Djouad et al. 2007; English et al. 2008), whereas contact-dependent Notch signaling but not IL-6 was shown to be required for DC modulation in another study (Li et al. 2008b). Further support of a role for Notch signaling in this scenario has been put forward by Zhang and colleagues (2009) showing contact-dependent Jagged-2 (a Notch ligand) signaling in the generation of regulatory DC. In the context of SOT, MSCs have been shown to block DC maturation and function in a kidney allograft model (Ge et al. 2010); however, the exact mechanisms used by MSCs to achieve this effect remains to be elucidated.

Analogous to the effects of MSCs on macrophage polarization, MSCs can also promote the generation of tolerogenic DCs (Li et al. 2008a; Ge et al. 2009; Spaggiari et al. 2009; Zhang et al. 2009; Liu et al. 2012b) producing anti-inflammatory cytokines and displaying enhanced phagocytic activity (Zhang et al. 2009; Liu et al. 2012b) typical of tolerogenic DCs. MSC educated DCs have the capacity to suppress alloreactive responses and prolong islet allograft survival (Huang et al. 2010) and to induce a state of tolerance in the context of SOT (cardiac allograft) in the presence of low dose immunosuppression (Ge et al. 2009).

The mechanisms of action mediated by MSCs in the generation of tolerogenic DCs are likely influenced by the context in which MSCs see DCs. The key mediator in MSC modulation of DC maturation is IL-6 (Nauta et al. 2006a; Djouad et al. 2007; English et al. 2008), however, the mechanism involved in MSC promotion of tolerogenic DCs has been less clear. Other studies have shown a central role for PGE-2 (Spaggiari et al. 2009) and cell contact-dependent activation of the Notch signaling pathway (Li et al. 2008b; Zhang et al. 2009), but not IL-6 (Li et al. 2008b; Spaggiari et al. 2009) in MSC generation of tolerogenic DCs. Further evidence of a contact-dependent mechanism involving activation of AKT and impaired NF-κB signaling has been proposed (Chiesa et al. 2011). Finally, it has been shown that mouse embryonic fibroblast derived MSCs generate a novel population of IL-10 dependent tolerogenic DCs through an IL-10 activated SOCS3 dependent mechanism (Liu et al. 2012b). MSC induction of tolerogenic DCs is a key mechanism involved in MSC modulation of immune responses; however, significant gaps in our understanding of exactly how MSCs promote the generation of tolerogenic DCs remain.

Mesenchymal Stromal Cell Suppression of Allogeneic T-Cell Responses in Transplantation Rejection

Allogeneic T-cell proliferation and activation are prerequisites for allograft rejection and induction of tolerance in allogeneic organ transplantation is usually associated with Treg (Issa et al. 2011; Wood et al. 2012). A large body of data convincingly show that autologous and allogeneic MSCs modulate T-cell proliferation, activation, and function both in vitro and in vivo (Di Nicola et al. 2002; Glennie et al. 2005; English et al. 2007; Asari et al. 2009; Ding et al. 2009; English and Mahon 2011). Moreover, in vitro assays have also confirmed the capacity for MSCs to inhibit Th17 cell differentiation (Duffy et al. 2011; Tatara et al. 2011) or to shift the T helper cell balance in favor of a more anti-inflammatory phenotype (Batten et al. 2006; Bai et al. 2009; English et al. 2009; Fiorina et al. 2009; Ghannam et al. 2010). The mechanisms used by MSCs in mediating these effects vary between in vitro and in vivo models. However, many of these effects are mediated through soluble factors secreted by MSCs (English 2013).

IDO and PGE-2 have been implicated in MSC inhibition of Th17 differentiation (Duffy et al. 2011; Tatara et al. 2011). In the case of PGE-2, the steps involved in the process require contact-dependent COX-2 induction of PGE-2 and direct inhibition through EP4 (Duffy et al. 2011). MSCs can also mediate this effect through suppressing the Th17 transcription factor RORγt and upregulating Foxp3 to induce a Treg phenotype producing IL-10 (Ghannam et al. 2010). MSC derived TGF-β has been shown to play a partial role in shifting the balance of Th1/Th2/Th17 and Treg in an autoimmune disease model (Kong et al. 2009).

With regard to SOT, MSCs impaired alloreactive T-cell responses (Casiraghi et al. 2008, 2012; Ge et al. 2010) and inhibited the migration of activated T cells into the allograft (Eggenhofer et al. 2011b; Hara et al. 2011; Franquesa et al. 2012a). In particular, matrix metalloproteinase (MMP)2 and MMP9 secreted by MSCs facilitated cleavage of CD25 expressed on $CD4^+$ T cells inhibiting alloantigen driven proliferation and preventing islet allograft rejection (Ding et al. 2009). Other evidence suggests that MSC derived MMPs also cleave CC chemokine ligand (CCL2), which subsequently inhibits Th17 activation via a STAT3 dependent pathway (Rafei et al. 2009). In addition, MSCs mediated their effects in part through shifting the balance of T helper 1 cell phenotype to a more anti-inflammatory Th2 phenotpye producing IL-4 and IL-10 (Ge et al. 2010; Jia et al. 2012). Importantly, MSCs also have the capacity to expand or induce Treg in the setting of SOT (Casiraghi et al. 2008, 2012; Wang et al. 2009; Jia et al. 2012) and in some cases to generate a state of Treg-dependent tolerance (Ge et al. 2010; Casiraghi et al. 2012). Both of these studies elegantly show the importance of Treg in MSC induced tolerance using Treg depletion studies. Apart from the study by Ge et al. (2010), which identifies an important role for MSC derived IDO in the generation of Treg, the mechanisms of action mediated by MSCs remain to be elucidated. In vitro, the factors required for MSC induction of Treg are thought to involve cell contact, PGE-2, and TGF-β (English et al. 2009). Further evidence for a contact-dependent role was provided by Selmani and colleagues, showing that cell contact-dependent production of HLA-G5 was required for the expansion of Treg (Selmani et al. 2008). In vivo, MSC derived TGF-β was required for the generation of antigen specific Treg and overall, TGF-β seems to be the major soluble factor involved in MSC promotion of Treg in vivo (Zhao et al. 2008; Kong et al. 2009; Nemeth et al. 2010; Akiyama et al. 2012). MSC generation of Treg involves a number of different steps that are dependent on the specific environment or disease model. For example, Nemeth and colleagues (2010) showed that MSCs exposed to IL-4 and IL-13 (typically produced in allergic environment) produced TGF-β through an IL-4R/STAT6 dependent pathway. Alternatively, MSCs may induce Treg indirectly through modulation of innate immune cells. A study performed by Akiyama et al. examining the effect of MSCs in a mouse model of dextran sodium sulfate-induced colitis, unravelled a complex course of events that ultimately led to the expansion of Treg. Specifically, MSCs induced T effector cell apoptosis through FAS/FASL facilitated by MSC MCP-1 chemoattraction of T cells. Subsequently, macrophages produce TGF-β following phagocytosis of the apoptotic cell debris resulting in the expansion of Treg (Akiyama et al. 2012).

INTERACTION OF MESENCHYMAL STROMAL CELLS WITH ALLOREACTIVE B CELL RESPONSES

The triggering of antibody mediated rejection by alloantibody binding and complement activation is increasingly associated with graft loss (Wood and Goto 2012). The reported effects of MSCs on B-cell activation, proliferation, and function have been variable and in some cases contradictory. MSCs have been shown to inhibit B-cell proliferation (Augello et al. 2005; Corcione et al. 2006; Asari et al. 2009; Schena et al. 2010) and immunoglobulin (Ig) production (Corcione et al. 2006; Comoli et al. 2008; Rafei et al. 2008) in vitro. The mechanisms of action involve contact-dependent factors (Schena et al. 2010) including programmed death-1 [PD-1]/programmed death-ligand [PD-

L1]/PD-L2 (Augello et al. 2005) as well as soluble factors like MMP cleaved CCL2 (Rafei et al. 2008). MSC inhibition of plasma cells induced by LPS or plasmacytoid DCs was shown to be mediated through extracellular signal-related kinases (ERK) 1/2 and phosphorylation of p38 (Tabera et al. 2008; Asari et al. 2009). Moreover, MSCs induced cell cycle arrest in B cells (Corcione et al. 2006), and particularly under highly proliferative conditions (Traggiai et al. 2008). In contrast, MSCs also have the capacity to promote B-cell proliferation and survival (Tabera et al. 2008; Traggiai et al. 2008; Youd et al. 2010) and enhance Ig production (Rasmusson et al. 2007; Youd et al. 2010). Considerable variations are reported in these findings with MSCs increasing IgM but not IgA or IgG in another study (Traggiai et al. 2008) with differences in the mechanisms involved (contact-dependent versus soluble factors) depending on the source of B cells (purified versus peripheral blood mononuclear cells or mononuclear cells) (Rasmusson et al. 2007) and the ratio of MSCs to B cells in co-cultures (Franquesa et al. 2012b). These differences may well be resolved by ensuring purity of B cells (excluding T-cell help) and by further understanding the effect that TLR ligands (LPS, cytosine phosphodiester guanine, polyinosinic:polycytidylic acid) have on MSC activation and function. The effect of MSCs has also been examined in the B cell driven pathology systemic lupus erythematosus (SLE) both in mouse models and in patient samples. MSC enhanced survival and reduced serum creatinine, blood urea nitrogen, proteinuria, C3 deposition, and decreased circulating double-stranded deoxyribonucleic acid (dsDNA) antibodies as well as antigen specific IgM and IgG secretion (Zhou et al. 2008; Asari et al. 2009; Choi et al. 2011). In contrast, Schena et al. (2010) reported no effect of MSCs on survival, proteinuria, or dsDNA antibodies. Furthermore, MSCs were shown to negatively impact SLE through enhancing pathology, autoantibody production, and proteinuria (Youd et al. 2010). In the setting of SOT, much of the focus has been on the effect of MSCs on T cells (discussed above), and to our knowledge, no study has rigorously examined the impact of MSC on alloreactive B cells. Nonetheless, there are reports that suggest that MSCs reduce intragraft IgG deposits as well as circulating donor specific antibodies (Ge et al. 2009; Franquesa et al. 2012a) providing protection from injury (Franquesa et al. 2012a) and inducing allograft tolerance in the presence of immunosuppression (Ge et al. 2009). Notably, failure of MSCs to prolong allograft survival has been associated with MSC promotion of intragraft B cell infiltration (Seifert et al. 2012), and although administration of donor MSCs posttransplant lead to sensitization and premature graft dysfunction, this was not thought to be associated with antibody mediated humoral rejection (Casiraghi et al. 2012). Rather, syngeneic MSCs administered posttransplant (but not pretransplant) localized in the transplanted kidney in response to ischemia reperfusion injury and subsequently produced IL-6 and TNF-α promoting a proinflammatory environment facilitating neutrophil infiltration and C3 deposition (Casiraghi et al. 2012). These studies highlight the significant gap in our understanding of the effect of the microenvironment on MSC activation and function and particularly the effect this has on how MSCs see B cells and vice versa.

Immunogenicity of Allogeneic Mesenchymal Stromal Cells

Although, allogeneic MSCs were thought to be immune privileged, evidence now suggests that allogeneic MSCs are recognized by the innate and adaptive immune system (Griffin et al. 2013). A clear understanding of how recognition of allogeneic MSCs impacts their capacity for modulating immune responses in vivo is hampered by the lack of appropriate experimental data measuring antidonor T-cell and antibody responses to allogeneic MSC. Nevertheless, a small number of studies show that allogeneic MSC evoke antidonor T-cell and antibody responses in vivo in healthy animals as well as models of myocardial infarction and bone marrow transplantation (Eliopoulos et al. 2005; Beggs et al. 2006; Nauta et al. 2006b; Badillo et al. 2007; Poncelet et al. 2008; Zangi et al. 2009; Isakova et al. 2010; Schu et al. 2012).

In the SOT setting, allogeneic MSC have been shown to accelerate graft rejection (Inoue et al. 2006; Popp et al. 2008; Renner et al. 2009; Eggenhofer et al. 2011a,b; Seifert et al. 2012). In some cases, accelerated graft rejection was attributed to administration of allogeneic MSC pretransplant (Eggenhofer et al. 2011b; Seifert et al. 2012) or syngeneic MSC posttransplant (Casiraghi et al. 2012). In other cases, allogeneic or syngeneic MSC were shown to attenuate immunosuppressive drugs like cyclosporine A (Inoue et al. 2006). Importantly, allogeneic MSCs have the capacity to work in synergy with immunosuppressive drugs including mycophenolate mofetil (Eggenhofer et al. 2011a,b) and rapamycin (Ge et al. 2009), and in these studies promote graft survival. Perhaps these studies emphasize the impact that different microenvironments may have on allogeneic MSC immunogenicity as well as the immunosuppressive function of both syngeneic and allogeneic MSCs in vivo. These conflicting reports highlight the significant need for further research in this area.

CLINICAL APPLICATION OF MESENCHYMAL STROMAL CELLS IN SOLID ORGAN TRANSPLANTATION

The application of MSC therapy in conjunction with a reduced immunosuppressive regimen is theoretically very appealing as MSCs not only promote the resolution of inflammation and enhance graft repair, but also may facilitate the induction of tolerance. Based on the safety and efficacy data generated in preclinical models and clinical trials utilizing MSC therapy for acute graft versus host disease (Casiraghi et al. 2008; LeBlanc et al. 2008; Ge et al. 2010), MSC therapy is currently being evaluated in SOT (Hoogduijn et al. 2010). Perico and colleagues (2011) provided the first report on MSCs in kidney transplant patients in a pilot study examining safety and feasibility. Culture expanded autologous bone marrow derived MSCs (1.7×10^6–2.0×10^6/kg body weight) were administered intravenously on day 7 postkidney (living donor) transplant in addition to T-cell depletion induction therapy in two patients. This study reported an increase in serum creatinine in both patients 7–14 days after MSC infusion. In addition, a focal inflammatory (granulocyte) infiltrate was observed in a graft biopsy taken from one of the patients, but acute graft rejection was ruled out. Importantly, both patients maintained stable graft function, which was associated with decreased memory $CD8^+$ T cells and increased Treg (Perico et al. 2011). Notably, similar effects were observed in a mouse model of kidney allograft transplantation, and this study elegantly showed the correlation of premature graft injury with posttransplant (but not pretransplant) syngeneic MSC infusion and localization of the MSCs primarily in the injured graft (Casiraghi et al. 2012). Indeed these studies may help to resolve the disparate findings with regard to MSC efficacy in prolonging graft survival (Inoue et al. 2006; Popp et al. 2008; Renner et al. 2009; Eggenhofer et al. 2011a,b; Seifert et al. 2012). This highlights the importance of timing of MSC administration and the requirement for a better understanding of the influence of the transplanted graft microenvironment (ischemia reperfusion, for example) on MSC function (Fig. 1). A large randomized controlled trial (106 patients over three arms) investigating the safety and efficacy of autologous bone marrow derived MSCs (in kidney transplantation) in combination with standard dose calcineurin inhibitors (CNI), or low dose CNI were compared with control groups receiving anti-IL-2 receptor antibody therapy in combination with standard dose CNI (Tan et al. 2012). This trial showed safety and efficacy with decreased incidence of acute rejection and glucocorticoid-resistant rejection, and increased estimated glomerular filtration rate levels and faster recovery of renal function in the first month post transplant as well as better estimated renal function at year 1. In addition, MSC treated groups revealed significantly reduced risk of opportunistic infections than the control group (Tan et al. 2012). Importantly, this large randomized controlled trial did not observe increased creatinine levels in MSC treated patients and this may be associated with the dose and/or timing ($1–2 \times 10^6$ on day 0 and day 14 versus $1.7–2 \times 10^6$ on day 7 posttransplant) of

MSC administration or with differences in immunosuppressive regimen (Perico et al. 2011; Tan et al. 2012). These are important factors that may significantly impact MSC efficacy in SOT and require careful consideration.

CONCLUDING REMARKS

Although the capacity for MSCs to modulate immune responses in vitro has been undeniable, the in vivo immunosuppressive function of MSCs has, at times, been ambiguous. Over time it has become clear that MSCs are receptive to microenvironmental cues, and indeed evidence has emerged to suggest that the interactions between MSCs and the local environment are fundamental in determining MSC activation and function. In vivo models have shown that MSCs are responsive to ischemia reperfusion injury and can provide protection in this setting. Moreover, MSCs have the capacity to prolong graft survival and, indeed, may induce tolerance. Together, these characteristics make MSCs an ideal candidate for application in SOT, and early data from clinical trials suggest that MSCs are safe and efficacious, but highlight the gap in our understanding of how exactly MSCs mediate their protective effects in vivo. To this end, we must endeavor to fill these gaps and enhance MSC therapeutic efficacy.

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