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REVIEW

Mesenchymal stem cells and their immunosuppressive role in transplantation tolerance

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Since they were first described, mesenchymal stem cells (MSCs) have been shown to have important effector mechanisms and the potential for use in cell therapy. A great deal of research has been focused on unveiling how MSCs contribute to anti-inflammatory responses, including describing several cell populations involved and identifying soluble and other effector molecules. In this review, we discuss some of the contemporary evidence for use of MSCs in the field of immune tolerance, with a special emphasis on transplantation. Although considerable effort has been devoted to understanding the biological function of MSCs, additional resources are required to clarify the mechanisms of their induction of immune tolerance, which will undoubtedly lead to improved clinical outcomes for MSC-based therapies.

Keywords: MSC; transplantation; T cells; tolerance; immunosuppression; therapy

Transplantation tolerance: a historical and cellular view

Originally, the concept of *immune tolerance* was defined as the absence of immunity. However, it later became clear that immune tolerance does involve a response, just not of the type early immunologists expected or knew. This type of response was eventually characterized as a suppressor response that required specific cells to play a role in keeping the immune system under control.

The tolerance achieved by the immune system is key in every transplant setting, where the absence of rejection to a graft yet simultaneously retaining the capacity to respond to an infection is the holy grail. Since the 1950s, transplantologists have been working to understand how this particular area of immunology functions to accomplish graft tolerance.[1–3]

The first clue about the existence of tolerance as an immunological process was observed by Owen in 1945.[2] In this study, he noticed in the blood of twin cattle that there was a percentage of cells that belonged to the sibling; that is, the twins were blood chimeras. Simultaneously, Medawar and Bellingham observed a lack of graft rejection when performing skin transplants in twin cattle. Later, the same observations were made in other experimental animal models, including mice and rats.[1,2] It is now known that this phenomenon takes place because the cattle cells are exposed early on to antigens from the twin, and subsequently do not recognize the cells as strange or foreign; in other words, they became tolerant to the antigens. Several years later, Medawar, Billingham, and Brent demonstrated that early exposure to antigens in subjects with an immature immune system would generate immunological tolerance, also called *acquired immunological tolerance.*[1]

From previously described work, it can be inferred that immunological tolerance may be achieved more easily in young subjects compared with adults. Early on, several techniques were used

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to establish immune tolerance, including the generation of bone marrow chimeras, as observed in Owen's cattle decades ago, or using cells with anti-inflammatory or suppressive activity.[4,5] In this regard, several cell populations with regulatory activity—including regulatory T (T_{reg}) cells, regulatory B (B_{reg}) cells, tolerogenic dendritic cells (tol-DCs), and regulatory macrophages—have been studied.[6–10]

Immune responses to graft transplantation begin when alloantigens present in a graft are recognized by recipient cells as foreign molecules, triggering a chain of tolerogenic activation/reaction from immune cells. First, antigen-presenting cells (typically dendritic cells (DCs)) capture antigens from the graft and "present" them to naive T cells ($CD4^+$ and/or $CD8^+$) in lymph nodes. The T cells become activated when the T cell receptor interacts with a major histocompatibility complex (MHC) II loaded with the alloantigen, leading to activation and polarization of $CD4^+$ T cells (now called T helper cells). T helper 1 (T_H1) cells activate macrophages and migrate to a graft site and secrete lytic enzymes, helping to eliminate the graft. On the other hand, T_H2 cells respond to nonself-antigens of the transplant and "help" B cells to mature in plasma cells that secrete antibodies against the antigens. Subsequent activation of the complement pathway and natural killer (NK) cells, which recognize the graft through Fc receptors, results in lytic activity against the graft. Activation of T cells in the lymph node also induces the proliferation of $CD8^+$ T cells, which can then secrete cytotoxic molecules to induce the destruction of the graft. Two cytokines involved in rejection response include IFN-γ and interleukin-17 (IL-17) secreted by T_H1 and T_H17 cells, respectively.

However, almost all of the immune "activating" cell populations have counterparts that function as *regulators* of their effects. The activites of $CD4^+$ T cells (T_H1, T_H2, or T_H17) can be moderated by regulatory T cells (T_{reg} cells), a tolerogenic population of T cells that, among other mechanisms, produce anti-inflammatory cytokines such as IL-10, transforming growth factor β (TGF-β), and/or IL-35, which "suppress" the effector activity of other cells. Additionally, macrophages can also be polarized to at least two phenotypes, referred to as M1 and M2, where M2 is a regulatory (suppressing) type that can minimize the response of other cells. A similar phenomenon occurs with B cells and the production of B_{reg} cells and with DCs and the production of tol-DCs, both of which can suppress activating immune responses. The cells mentioned above are targets for inducing tolerance in transplant patients and for withdrawing the use of immunosuppressive drugs, with the aim of ameliorating the broad secondary effects associated with activating immune responses.

The general protocol for using immune-suppressor cells as therapy involves the isolation of a patient's cells, differentiating them *in vitro,* and then infusion of the suppressor cells into the patient. An obvious limitation associated with this procedure is immune compatibility between donor and recipient.

In addition to the above cells and cell types, other cells have been shown to have regulatory characteristics, including mesenchymal stem cells (MSCs).[11,12] MSCs will be the main focus of the rest of our discussion.

MSCs: the new player in transplantation immunology

MSCs were first described in the 1960s by Friedenstein and colleagues, who discovered the existence of stromal cells and bone-forming cells within the bone marrow.[13] The cells displayed osteogenic potential and were characterized by their prompt adherence to plastic, a fibroblast-like characteristic, and colony-forming unit capacity.[14] In terms of origin, several studies have shown that MSCs can be found in numerous sites, including muscle, liver, adipose tissue,[15] endothelium,[16] and body fluids.[17] Owing to the lack of specific cell surface markers, the International Society of Cellular Therapy established three main criteria for defining MSCs[15] (depicted in Fig. 1): (1) adhesion to tissue culture–treated plastic; (2) capacity to differentiate into mesodermal lineages (adipocytes, osteoblasts, and chondrocytes); and (3) expression of CD105, CD73, and CD90, as well as lack of expression of CD45, CD34, CD14, CD11b, CD79a, CD19, and HLA-DR surface molecules. These criteria are used to characterize the cells, although the combination of surface markers is not yet definitive.

MSCs were thought to facilitate tissue and organ repair by direct replacement of damaged cells. However, recent studies indicate that this is highly improbable, as it is now known that in response to tissue injury MSCs migrate to the site of

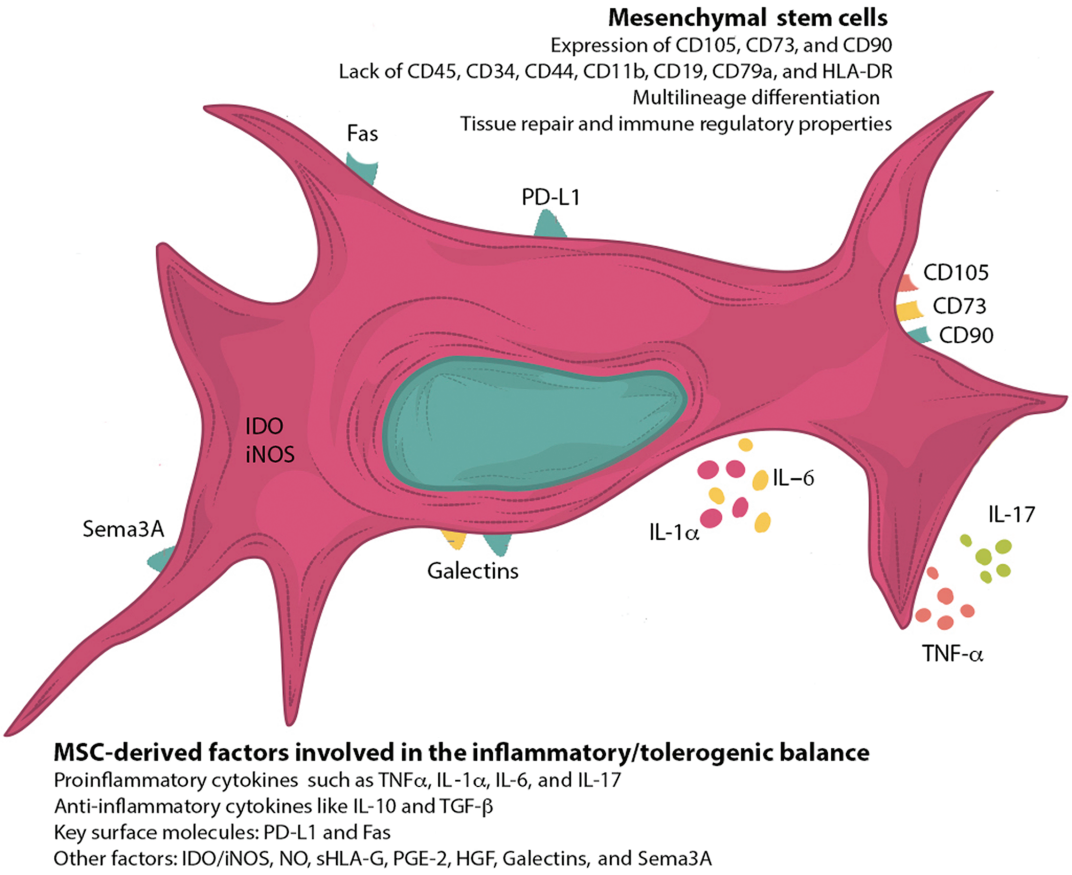

Figure 1. **MSC primary properties and functions. To identify MSCs among other cell types, the following criteria have been established: expression of CD105, CD73, and CD90; absence of expression of CD45, CD34, CD44, CD11b, CD19, CD79a, and HLA-DR; capacity to differentiate toward multiple cell lineages; high ability to repair and regenerate tissues; and the potential to modulate the immune system. All the above is accomplished by expressing and producing molecules corresponding to pro- and anti-inflammatory cytokines and chemokines, surface molecules with inhibitory and proapoptotic roles, enzymes, metabolites, and other compounds.**

damage and facilitate tissue repair by producing trophic factors, including growth factors, cytokines, and antioxidants.[16] Thus, MSCs collaborate in the recruitment of other cells, including immune cells (detailed below), in response to tissue injury.

There is a pressing need to elucidate the mechanisms underlying the favorable actions of MSCs *in vivo*. Recent studies have helped uncover their various properties and have led to them being considered a very promising option for the treatment of several diseases. Some of the multifactorial characteristics with biomedical potential that MSCs display include (1) tissue-repairing abilities;[14] (2) multilineage differentiation capacity toward mesodermal, endodermal, or neuroectodermal cell lineages under specific conditions;[16] and (3) broad immune-regulatory properties owing to their plasticity.[18] The immediate environment of MSCs is the most important factor determining their fate: whether to induce inflammation or tolerance. This particular plastic property of MSCs is also referred to by a process termed "licensing,"[19] which means the MSCs commit (by environmental stimulus from other cells and/or cytokines) to one type of function or the other.

We focus on the immune-regulatory property of MSCs. Below we present and discuss pertinent advances in the transplantation field related to how tolerance is accomplished and how MSCs can interact with other cells *in vivo* to moderate the immune response in this clinical setting.

MSCs and immune cell cross talk

In recent years, strong evidence has pointed to the capability of MSCs to interact with and modulate effector immune cells. Several groups have claimed that the communication between MSCs and with

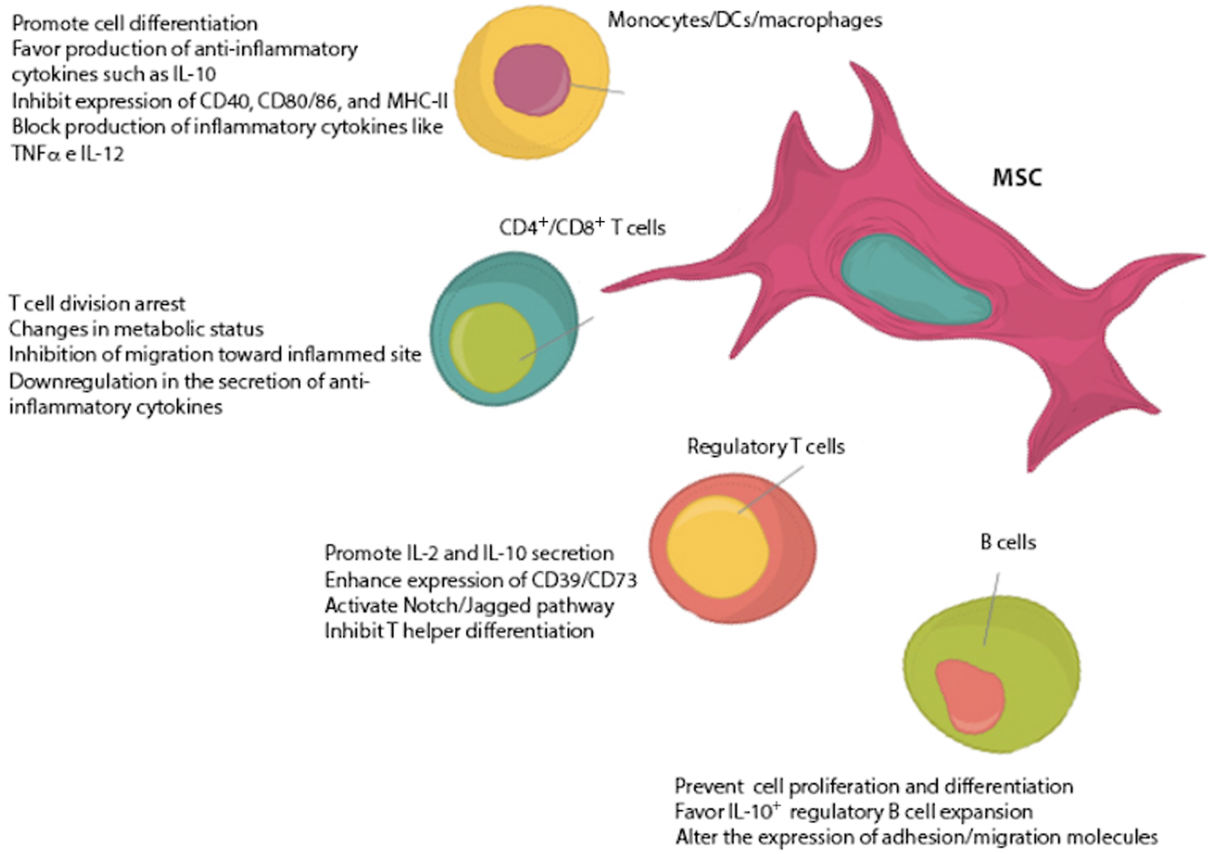

Figure 2. **Effects of MSCs on different leucocyte populations. To date, several studies have reported the ability of MSCs to affect the biology of different immune cell subsets, including monocytes, DCs, macrophages, T cells (both $CD4^+$ and $CD8^+$ T cell compartments), and B cells. For $CD4^+$ T cells, many reports have characterized the mechanisms by which MSCs interact with effector, memory, and regulatory T cell subsets.**

cells of the innate and/or adaptive immune systems can take place both through cell–cell contact and via secreted soluble elements[20–23] (Fig. 2).

Below, we summarize studies describing the interaction between MSCs and different cell populations of the immune system and generation or establishment of tolerance.

Monocytes/macrophages

Monocytes that circulate through the bloodstream and migrate to other tissues can differentiate into macrophages or DCs. Depending on the microenvironment and presence of stimulation signals, these macrophages may adopt an M1 phenotype (classical, proinflammatory) or an M2 phenotype (alternative, anti-inflammatory). A set of published reports suggests that MSCs are able to interfere with the acquisition of an M1 phenotype while favoring an M2 phenotype. For example, Kim *et al.* showed that macrophages cocultured with human bone marrow–derived MSCs increased their expression of CD206 and exhibited high levels of IL-10, with low IL-12, production.[24] Additional studies have corroborated these observations *in vitro*, showing that macrophages cultured with MSCs have lower production of proinflammatory cytokines (i.e., TNF-α, IL-1α, and IL-6), increased IL-10 production, and higher phagocytic capacity,[24–27] which is a positive environment for the establishment of tolerance.

The importance of MSCs driving monocyte modulation became apparent in a couple of reports that indicated that depletion of monocytes from stimulated peripheral blood mononuclear cells (PBMCs) diminished both the immunosuppressive capacity of MSCs[28] and their induction of $FOXP3^+$ T_{reg} cells.[27] Moreover, monocytes isolated from these cocultures exhibited higher expression of CD206 and CD73, and lower levels of HLA-DR, consistent with their reduced allostimulatory function.[28]

Similarly, in a mouse model of skin wound healing, Zhang *et al.* showed that infused human gingiva–derived MSCs migrated to the injury site and interacted closely with host resident macrophages, where they contributed to M2 polarization and tissue repair.[26] Such evidence supports a model in which MSCs interacting with monocytes/macrophages favor a suppressive phenotype of the latter. Such a strategy could be used as a means to either induce or enhance tolerogenic properties of monocytes/macrophages.

Dendritic cells

DCs are the main orchestrators of immune responses, serving as a bridge between the cells of the innate and adaptive immune systems. DCs capture antigens from an inflammation zone and migrate to secondary lymphoid organs, where they activate naive T cells and mount an immune response. DCs can also interact with other types of immune cells, including B cells and NK cells. The capacity of MSCs to impair both monocytes and DC differentiation from $CD34^+$ precursors could exert a considerable impact on the outcome of the immune response.[29–31]

At the same time, MSCs can hinder the recruitment and function of DCs in a variety of immune settings. TNF-α–exposed DCs cocultured with MSCs downregulate the expression of MHC class II and costimulatory molecules, such as CD40, CD80, and CD86,[31–33] hence leading to an immature phenotype (iDCs). This phenotype can be maintained despite exposure to the strong inflammatory stimulus, lipopolysaccharide (LPS).[34] Also, MSCs cocultivated with DCs cause the latter to adopt an anti-inflammatory secretory profile, with lower production of TNF-α and IL-12, and higher secretion of IL-10, especially in the $NRP1^+$ plasmacytoid DC compartment.[31,34–36] In a study performed by Aldinucci *et al.*, MSCs displayed a novel mechanism of DC modulation in which human monocyte–derived DCs, after coculture with MSCs, were unable to form stable immune synapses with lymphocytes in a cell–cell contact manner (the consequences on the T cell–dependent response were not studied).[37] The modulated DCs maintained the expression of costimulatory molecules, cytokine production, and endocytosis capacity after LPS stimulation. Thus, similar to the influence of MSCs on monocytes and macrophages, DCs can also be stimulated by MSCs to display a tolerogenic phenotype. Since DCs are one of the major players in the initiation of the immune response, the use of MSCs to target this cell subset may be relevant in *in vivo* settings.

T cells

The inhibitory effect of MSCs on T and B cells has been a matter of interest. Even though MSCs were first thought to suppress T cell activation, it was later observed that MSCs only minimally affect the activation markers CD25 and CD69 on T cells.[38] It is widely accepted that both human and murine MSCs (hMSCs and mMSCs), regardless of their source, can suppress activated, proliferating T cells,[39,40] and that this type of suppression is not restricted to an individual T cell subpopulation. Whether T cells are $CD4^+$, $CD8^+$, γδ, or $CD4^+CD8^+$ central or effector memory cells, MSCs can inhibit them in a dose-dependent, nonantigen-specific or non-MHC-restricted manner,[38,41–45] but the mechanisms underlying this function have not been fully elucidated. Glennie *et al.* found that the inhibition of T cell proliferation caused by mMSCs was due to their capacity to induce T cell division arrest or anergy.[46] These cells remained at the early G_1 phase of the cell cycle (a similar effect was seen in activated B cells) that was partly mediated through the inhibition of cyclin D2, whose expression is associated with the G_1 phase.[46,47] Although in this study the effect of MSCs was described as irreversible, it has been widely reported that it is not, since the restimulation of T cells with IL-2 and cognate peptide resulted in reversing the effect.[45] Additionally, a high MSC:T cell ratio ($<$1:10) in cocultures has been shown to be important for efficient suppression,[43,48] while lower MSC:T cell ratios were shown to stimulate T cell proliferation.[40] Determination of the mechanisms driving inhibition of T cell proliferation by human and mouse MSCs must take into consideration secreted or soluble factors, although cell–cell contact seems to enhance the efficiency of suppression, most likely owing to the presence of additional signals, such as interactions between inhibitory molecules. Among the factors described are indoleamine 2,3 dioxygenase (IDO),[49] soluble histocompatibility locus antigen (sHLA)-G,[50,51] prostaglandin E2 (PGE-2),[36] hepatocyte growth factor (HGF), TGF-β,[45] nitric oxide (NO),[52] galectin-1 (Gal-1),

and semaphorin-3A (Sema-3A).[53] The roles of these different factors may vary among species, because MSCs from monkeys, pigs, and humans use IDO to exert their immunosuppressive functions, while MSCs from mice, rats, rabbits, and hamsters use iNOS.[54]

In mMSCs, iNOS produces NO, but it does not seem to have activity in hMSCs.[54] NO acts through phosphorylation of the signal transducer and activator of transcription 5 (STAT5),[52] which is critical for cell cycle regulation of T cells[55] and thus their proliferation. On the other hand, the role of IDO in the inhibition of proliferation is related to its actions in depleting tryptophan,[49] an amino acid that is necessary for T cell expansion.[56] Tryptophan depletion caused by MSC-secreted IDO can affect metabolic pathways in T cells, whose shift from glycolysis toward oxidative phosphorylation can cause T cell arrest.[57,58]

The inhibition of T cell proliferation through soluble Gal-1 and Sema-3A secreted by hMSCs seems to occur via their binding to NRP1,[53] which is constitutively expressed on the T cell surface,[59] and its binding causes T cells to arrest in the G_0/G_1 cell cycle phase.[60] On the other hand, PGE-2 appears to be a common inhibitory factor secreted by both hMSCs and mMSCs; blocking it causes a similar level of restoration of T cell proliferation in both species upon mitogen stimulation.[50,52,61]

Finally, contradictory results with TGF-β, HGF, and HLA-G make it difficult to define the role of these factors in the inhibition of T cell proliferation.[45,50,52] However, TGF-β and HGF seem to be essential for this blockade when T cells are allogeneically stimulated.[45] Therefore, with hMSCs, upregulation of the production of the soluble factors PGE-2 and Gal-1, or the expression of Sema-3A and IDO, could be promising routes for enhancing the immunosuppressive activities of hMSCs and further applications in inflammatory states.

The expression of erythropoietin-producing hepatocellular (EPH) receptor B2 (EPHB2) and ephrinB2 on MSCs, and EPHB4 and ephrinB1 on T cells, seems to be a key for the establishment of interactions between T cells and MSCs.[38] Blocking EPHB2/ephrinB1 and ephrinB2/EPHB4 interactions leads to a decreased ability of hMSCs to inhibit T cell proliferation in a mixed lymphocyte reaction (MLR) experiment. Furthermore, IDO, TGF-β, and iNOS expression have been shown to be upregulated upon activation of EPHB2 and ephrin B2 by EPHB4 and ephrinB1 on IFN-γ–licensed MSCs. Additionally, TNF-α, IL-2, and IL-17 expression levels were shown to be downregulated in human T cells following stimulation with EPHB2 and ephrinB2.[62]

On the other hand, PD-L1 expression in hMSCs was found to be significant in inhibiting the expression of CD69 in $CD4^+$ T cells and, together with FasL stimulation, the progression of T cells into the G_0/G_1 cell cycle phase.[63,64] HLA-G1 expressed on hMSCs was found to be involved in the inhibition of T cell proliferation, in a contact-dependent manner, by inducing the blockage of the G_0/G_1 phase.[47] This blockage is partly caused by a downregulation of phosphoretinoblastoma (pRb), cyclin D1, and cyclin A, as well as upregulation of cyclin-dependent kinase inhibitor 1B ($p27^{Kip1}$), which plays a key role in controlling cell cycle progression.[47,65–67]

T helper cell subsets

It has been proposed that MSCs promote an immune-suppressive microenvironment by changing the cytokine secretion profiles of T_H1 and T_H2 cells.[36] This may occur by favoring T_H2-type cytokine secretion and inhibiting the production of the proinflammatory cytokines IFN-γ, TNF-β, and IL-1β.[36] MSCs can also promote the secretion of T_{reg} cell–differentiating cytokines IL-2 and IL-10 in already differentiated T_H1 cells, thus repressing their differentiation.[48,68–70] MSCs can also inhibit the expression of IL-6 from T_H2 cells, which plays a significant role in the differentiation of T_H17 cells.[48,71] Specifically, the inhibition of T_H1 cells does not require cell–cell contact and it is effective even at low MSC:T cell ratios.[72] A T_H2 cell phenotype with higher IL-4 expression was found to be characteristic of a tolerant response in mice receiving kidney allografts and treatment with MSCs.[73] This effect seemed to be mediated through the secretion of IDO, which causes depletion of tryptophan or tryptophan metabolites leading to metabolite-induced apoptosis in T_H1 cells.[74]

Additionally, many reports have indicated that MSCs may also inhibit T_H17 cells. Their differentiation from naive $CD4^+$ T cells and the production of inflammatory cytokines such as IL-17, IL-17F, IL-21, and IL-22 by fully differentiated T_H17 cells are inhibited in the presence of MSCs, while the production of IL-10 is upregulated.[75] MSCs also

seem to affect the expression of CCR6, a chemokine receptor that mediates the migration of T_H17 cells to inflammatory sites, thus affecting their tissue-infiltration ability.[75,76]

Conversely, one T_{reg} cell phenotype is promoted by the presence of MSCs probably through the secretion of PGE-2, TGF-β, and IL-10.[20,75,77] However, these effects vary depending on when MSCs come in contact with fully differentiated/activated T cells, promoting the expansion of T_H17 cells in some cases, and T_{reg} cells, via the secretion of IL-6 and IL-1, in other cases.[48] Thus, early addition of MSCs into culture diminishes the generation of T_H17 cells, while a late addition expands them.[78]

On the basis of the above data and of the previously characterized influence of MSCs in T cell migration, various factors should be considered when MSCs are administered in human studies, as the differentiation of specific T cell subsets will direct the immune response. The mechanism that determines which phenotype MSCs will promote, T_{reg} or T_H17, depends considerably on the cytokine secretion profile of the MSCs.[23,77] The fact that MSCs can polarize differentiated T_H17 cells to a T_{reg} phenotype is not surprising, since these two T cell subsets share a differentiation pathway, with TGF-β being a common required factor for their differentiation.[79] The plasticity between the two phenotypes has also been documented.[80,81] Which cell phenotype is expressed is modulated at a transcriptional level through the control of two key transcription factors: RORγt and FOXP3. The expression of both proteins is mediated through epigenetic changes that also affect cytokine production.[75] The inhibition of a T_H17 phenotype by MSCs appears to be affected by the suppression of the STAT3 transcription factor through the secretion of a cleaved form of the chemokine CCL2 (mpCCL2) and the activation of the cytokine signaling 3 (SOCS3) pathway.[77,82,83] In addition, STAT3 expression promotes a T_H17 cell phenotype by upregulating the expression of RORγt and activating the expression of the IL-17 gene locus.[84]

While IFN-γ and IL-10 upregulate SOCS3 and inhibit STAT3, thus promoting a T_{reg} cell phenotype, IL-6 activates STAT3 and promotes a T_H17 cell phenotype.[83,85] On the other hand, IL-2 activates STAT5, which binds to FOXP3 and promotes a T_{reg} phenotype.[73] Importantly, the presence of $FOXP3^+$ T_{reg} cells was found to be relevant in inducing and maintaining tolerance to kidney and liver allografts, as shown in mouse and rat models, respectively.[86,87]

Altogether, the reports describing the effects of MSCs on T helper subsets highlight the versatility of MSCs in the sense that the resulting T_H subset can be "controlled" depending on how MSCs were previously manipulated.

It has been reported that MSCs promote the proliferation of T_{reg} cell populations.[36] Interestingly, these MSC-expanded T_{reg} cells express low levels of NRP1 and the transcription factor Helios, suggesting that MSCs may induce T_{reg} cell differentiation rather than promote expansion of already existing T_{reg} cells.[51] Both cell–cell contact and secreted factors, such as PGE-2, TGF-β, HLA-G5, and IL-10, seem to play roles in this activity.[51,88,89]

Furthermore, hMSCs not only promote the differentiation of T_{reg} cells but also induce their function by enhancing the expression of CD39 and CD73.[68,88,89] These molecules participate in the adenosine-producing pathway, which is necessary for T_{reg} cell immunosuppressive activity.[69,89] Upon coculturing with hMSCs, T_{reg} cells also decrease their granzyme B production and secretion, a feature that has been shown to be beneficial for the treatment of graft-versus-host disease (GvHD).[90,91]

Tr1 cells are a $FOXP3^-IL\text{-}10^+$ T_{reg} cell subset that, together with the IL-10–secreting T_H3 cell subset, is considered essential for peripheral tolerance.[92,93] Tr1 and T_H3 cells have proven beneficial in GvHD suppression.[94,95] Additionally, the secretion of IL-1 receptor agonist by mMSCs plays a role in decreasing the ratio of T_H17/Tr1 cells in mice,[96] thus promoting an immunosuppressive microenvironment. The proportions of Tr1 and T_H3 cells have been shown to be increased by MSCs through a pathway that involves the expression of the stress-inducible enzyme heme oxygenase-1.[97]

Complementing the above, the Notch signaling pathway has been shown to be involved in modulating hMSC immunosuppressive properties.[98–100] Del Papa *et al.* demonstrated that activation of the Notch1 pathway was related to the induction of $CD4^+CD25^+FOXP3^+$ T_{reg} cells from $CD4^+$ T cells cocultured with hMSCs. Later, Cahill *et al.* reported that Notch signaling through the ligand Jagged-1 in murine MSCs was essential for the expansion of the T_{reg} cell population in mice.[100] However, the activation of $CD4^+CD25^+FOXP3^+$ T_{reg} cells

via activation of the Notch1 pathway by TLR3- or TLR4-activated hMSCs was reported to occur through the ligand delta-like 1 (DL1) in a cell contact–dependent manner.[101] TLR3- and TLR4-activated MSCs were found to have an enhanced capacity for inducing this T_{reg} cell subset.[101,102]

The androgen receptor (AR) has been proposed to play a role in the regulation of T_{reg} cells by MSCs.[103] Not only do AR-depleted mMSCs generate fewer FOXP3$^+$ T_{reg} cells from CD4$^+$ naive T cells, but the ones generated display impaired suppressive function.[103] This impaired function likely occurs because of a downregulation of TGF-β production in the cocultures, which is known to be an essential factor for T_{reg} cell development.[103,104]

Moreover, it has been reported that several lectins expressed in MSCs might play roles in the immunosuppressive function that MSCs exert on T cells.[105–107] Among these are galectins (Gal) 1, 3, and 9, which might be promising for GvHD treatment, as Gal-1 and -9 have been reported to improve graft rejection in murine models.[108,109] Specifically, Gal-9 on MSCs seems to be key in the inhibition of T cell proliferation through a cell–cell dependent manner, probably by binding to its receptor TIM-3 on activated T cells.[110] TIM-3 is significantly expressed in T_H1, T_H17, and cytotoxic CD8$^+$ T cells, and its binding to Gal-9 leads to apoptosis of these cells.[111–114]

A study by Luz-Crawford *et al.* showed that glucocorticoid-induced leucine zipper (GILZ)–deficient MSCs have impaired immunosuppressive function. They exhibit a lower ability to inhibit CD4$^+$ T cell proliferation and T_H1/T_H17 cell polarization *in vitro.*[115] Similarly, Yang *et al.* showed that the T_{reg} cell phenotype is regulated by GILZ expression in bone marrow–derived MSCs, both *in vitro* and *in vivo,*[116] and a higher expression of GILZ in MSCs was reported to cause a higher proliferation of FOXP3$^+$ T_{reg} cells in MLR experiments.

The CD8$^+$CD28$^-$ T_{reg} cell subset is also modulated by MSCs.[68,117,118] Both the frequency and the immune regulatory function of CD8$^+$CD28$^-$$T_{reg}$ cells were shown to be increased in the presence of MSCs, partly by upregulating FasL and IL-10 expression, which enhances their capacity for inducing apoptosis in activated CD4$^+$ T cells.[118] The presence of this regulatory subset was increased in GvHD patients who showed a complete response after treatment with hMSCs and in patients who showed tolerance to transplants.[118–120]

Memory T cell populations

The presence of alloreactive memory T cells before transplantation is linked not only to decreased allograft survival but also to delayed and poorer function.[121–123] The immunomodulation activity that MSCs may exert on memory T cells is of considerable interest since they correspond to long-lived T cells with the ability to become easily reactivated in comparison with other subsets.[43] It has been reported that MSCs may inhibit memory T cell antigen-specific proliferation, IFN-γ production, and cytotoxic activity, and could also induce CD3$^+$CD45 RO$^+$ memory T_{reg} cells.[43,124]

Most preclinical studies generally focus on circulating or lymphoid T cells. However, a nonnegligible number of T cells reside as noncirculating, tissue-resident memory T cells (T_{RM} cells) in multiple peripheral tissue sites, including lungs, intestine, and skin.[125] The potential role of T_{RM} cells in transplantation complications, tolerance, and their interaction with immunosuppressive therapies represents an important emerging interest that needs to be addressed.

Some transplanted organs, including lungs, liver, and skin, contain large numbers of T_{RM} donor cells, which can persist or be replenished by host T cells to varying degrees. T_{RM} cell content is thought to play an important role in long-term graft survival and complication rates as compared with other T_{RM} cell–free organs, such as kidney and pancreas.

Owing to the recent identification of these cells, there are few studies investigating the loss and repopulation of donor and recipient T cells in mucosal allografts by T_{RM} cells. The susceptibility of T_{RM} cells to immune modulators is not known, but evidence from animal models suggests that T_{RM} cells are less accessible to systemically administered agents.[126] Hence, local targeting of immunosuppression to tissue sites would be a more pertinent strategy to follow. The interaction of T_{RM} cells with recipient or administered MSCs has not yet been addressed, opening an important area to pursue in future studies.

B cells

Despite most studies suggesting an immunosuppressive effect for MSCs on T cells, the impact of MSCs on B cells is rather controversial. It seems that the presence of other leukocyte populations in coculture is required for MSCs to suppress Ig

production and B cell proliferation, events that take place during graft rejection.[61,127–130] However, some studies indicate that when B cells alone are in contact with MSCs their proliferation and differentiation to plasma cells are inhibited.[131,132] This indicates that MSCs interact with B cells not only indirectly but also directly. The mechanism that underlies the inhibition of B cell proliferation and differentiation by MSCs is unclear, but many reports describe similar mechanisms that MSCs have on T cells, including cell cycle arrest and blockade of cell differentiation, both driven by cell-to-cell contact (e.g., via PD-1/PD-L1) or via soluble factors (such as the release of PGE-2, mpCCL2, or Sca-1).[95,111,130–139]

The effect of MSCs on nonactivated B cells (transitional, naive, and memory subsets) and plasmablasts seems to be important to their survival.[130,132,140] The presence of these augmented nonactivated B cell subsets could enhance an immunosuppressive phenotype. For example, naive B cells can stimulate the differentiation of T_{reg} cells.[141] Although plasmablasts can produce antibodies, they do so in lower quantities when compared with plasma cells, and they can proliferate to the detriment of plasma cell survival.[142] Even though MSCs can inhibit B cell proliferation and differentiation, this effect does not take place via apoptosis.[131,135] Recently, it was reported that IL-1RA might play a role in increasing the survival of some B cell subpopulations by inhibiting differentiation into plasmablasts when cocultured with MSCs.[95] It has also been proposed that MSCs, through the IL-1RA axis, could induce the proliferation of IL-10–secreting B_{reg} cells.[95,130,143] The expansion of $CD19^+$ B_{reg} cells in addition to naive, transitional, and memory B cells in MSC cocultures could account for the enlargement of the total B cell population observed in some studies in which the analysis did not include the characterization of B cell subsets.[138,144] Since the presence of B_{reg} cells has been linked to a tolerant phenotype in transplants,[145–148] preclinical studies need to be designed to clarify the contributions of MSCs to various B cell populations and their consequent activities *in vivo*.

It is clear that achieving transplant tolerance is not a simple process, as many molecular and cellular mechanisms remain to be elucidated. The variety of cells and molecules that participate in the process is very wide, and it makes the generation of specific therapies more difficult. However, there are several groups testing the efficacy of different therapies in order to improve the survival of transplant patients.

Preclinical transplantation models using MSCs

The first studies using MSCs in solid organ transplantation (SOT) were performed in the early 2000s by Bartholomew *et al.* They observed that administration of MSCs suppressed lymphocyte proliferation and promoted graft survival in a baboon skin transplant model.[149] This report describes for the first time that administration of allogeneic MSCs does not elicit an immune response by alloreactive lymphocytes, but the administration of exogenous IL-2 in *ex-vivo* cell cultures can abrogate this effect.

It has been shown that the administration of donor MSCs in mice promotes semiallogeneic heart transplant survival, along with a decrease in effector T_H1 cell proliferation and function, and encourages the increase in $CD4^+CD25^+FOXP3^+$ T_{reg} cells.[150] It is notable that these MSC-induced T_{reg} cells were donor specific, since transfer of splenocytes from tolerant mice did not prevent the rejection of third-party allografts.[150] Following this finding, Wang *et al.* showed that infusion of autologous, heterologous, or third-party MSCs in a rat model of allogeneic liver transplantation induced a longer graft survival, and that this phenomenon was accompanied by an increase in the number of $CD4^+CD25^+FOXP3^+$ T_{reg} cells and low cell infiltration into the graft.[151] The results of Ding *et al.* showed that the matrix metalloproteinases (MMP)-2 and -9 are involved in the immunosuppressive effect of MSCs administered to grafted mice in a pancreatic islet transplantation model.[152] MMPs reduced the amount of CD25 receptor on the surface of $CD4^+$ T cells by enzymatic cleavage, thus leaving them hyporesponsive to IL-2. This protective effect was reversed in MSC-treated mice when blocking antibodies or specific inhibitors of the MMPs were administered to the animals.[152] Moreover, it has been demonstrated that the proliferation of T_{reg} and tol-DCs mediated by MSC treatment promotes the induction of tolerance in a model of fully allogeneic cardiac transplantation, an effect that was enhanced by the combined treatment of MSCs with a low dose of rapamycin.[153] To facilitate the tracking of MSCs following *in vivo* administration, the authors used GFP^+ reporter MSCs, which showed that

these cells migrated mainly to lymphoid organs (spleen, bone, and lymph nodes), cardiac muscle, and blood vessels of grafts from tolerant mice.[153] Shortly thereafter, another study using an allogeneic kidney graft model established that posttransplant MSC infusion failed to prolong graft survival and caused premature graft function impairment and, conversely, pretransplant MSC infusion induced a significant kidney allograft tolerance through a T_{reg}-dependent mechanism.[154] This result suggested that pretransplant infusion might be beneficial for improving allograft tolerance in patients.

Thus, MSC-mediated T_{reg} cell induction seems to be a key mechanism of inducing tolerance in SOT. Many studies, both *in vitro* and *in vivo*, have shown that TGF-β is both a key soluble factor produced by MSCs and required for the generation of T_{reg} cells,[20,155–158] but the underlying mechanisms are dependent on the specific microenvironment and the animal model. For example, in a ragweed asthma mouse model, exposure of MSCs to IL-4 and IL-13 (classic cytokines produced in an allergic environment) results in the activation of the STAT6 pathway and the upregulation of TGF-β production, which help to block the proinflammatory T_H2 response and, at the same time, induce the differentiation of T_{reg} cells.[157] In other cases, MSCs can induce T_{reg} cells indirectly through the modulation of innate immune cells such as macrophages, which produce TGF-β following the phagocytosis of apoptotic effector T cells, resulting in the expansion of T_{reg} cells.[158]

On the other hand, the use of a combined treatment regimen of MSCs with immunosuppressive drugs has been studied as well, since these drugs are usually used in transplant patients to dampen the immune response and promote graft survival. However, in one report using heart-transplanted rats receiving MSCs alone, the animals did not accept the transplant, and coadministration of MSCs with low-dose cyclosporin A treatment accelerated allograft rejection.[159] And although steroid-based anti-inflammatory therapy is administered to decrease severe inflammatory responses in transplant patients in a mouse model of liver fibrosis, the inflammatory inhibition effect of MSCs was abrogated by coadministration of dexamethasone, leading to increased levels of inflammatory mediators (e.g., bilirubin, albumin, and aminotransferases), and IFN-γ^+IL-17^+ T cell infiltration.[160] The detrimental effect of dexamethasone seems to be exerted through impairment of STAT1 phosphorylation and downregulation of iNOS expression.

One of the major complications associated with hematopoietic stem cell transplantation (HSCT) is the development of GvHD. Early studies of MSCs in murine acute GvHD (aGvHD) models showed that a single infusion of MSCs at the same time as HSCT failed to prevent aGvHD;[161] however, this could be improved by the administration of multiple doses of MSCs at a weekly frequency following HSCT. Another report described that MSCs control inflammation more effectively when administered in the presence of high production of IFN-γ in the animals,[162] which could be attributed to the *in vivo* licensing of the administered MSC. These observations support the view that the inflammatory state of the microenvironment determines the response of MSCs. A summary of the above is presented in Table 1.

MSCs in transplantation clinical trials

The wide range of immunomodulatory properties described for MSCs thus far and the results obtained with animal models have led researchers to propose MSCs as a promising therapeutic strategy for improving tolerance after transplantation, which has driven their utilization in clinical trials. We performed a systematic search of the last 5 years of research and selected the most recent and homogenous studies in terms of MSC source, underlying condition of the patients, and their immunosuppressive pharmacological treatment (summarized in Table 2). The majority of the studies investigated the use of bone marrow–derived MSCs to ameliorate GvHD after HSCT for treating hematopoietic malignancies. We also include studies using MSCs after SOT, specifically kidney.

A closer look at the clinical trials presented here highlights the necessity of more rigorous and standardized protocols to assess the patient status before MSC infusions and to evaluate its progression during and after treatment, combining clinical features as well as cellular and molecular characterization. As the reader will notice, some clinical trials report only remission of symptoms and survival rates, while others analyze lymphocyte populations and others focus on inflammation and plasma markers for cell damage.

Table 1. Summary of studies using MSCs as treatment in animal models of transplantation

Type of MSCs used	Organ transplanted	Effects	Animal used	Reference
Allogeneic bone marrow–derived MSCs	Allogeneic skin transplant	There was no response by alloreactive lymphocytes, but, when treated with IL-2, this effect was reversed.	Baboon	149
Allogeneic bone marrow–derived MSCs	Semiallogeneic heart transplant	Decreased function and proliferation of T_H1 cells. Promotes the increase in $CD4^+CD25^+FOXP3^+$ cells.	Mouse	150
Autologous, heterologous, or third-party MSCs	Allogeneic liver transplant	Long-term graft survival, increase in $CD4^+CD25^+FOXP3^+$ T_{reg} cells, and low number of cells infiltrating graft.	Rat	151
Allogeneic bone marrow–derived MSC	Pancreatic islet transplant	MSCs release MMP, reducing the IL-2 receptor (CD25), making T lymphocytes hyporesponsive to IL-2.	Mouse	152
Allogeneic GFP^+ bone marrow–derived MSCs	Allogeneic heart transplant	Proliferation of T_{reg} cells and tol-DCs.	Mouse	153
Allogeneic bone marrow–derived MSCs + rapamycin	Allogeneic kidney transplant	Preadministration of DCs causes allograft survival. Postadministration of DCs failed to prolong graft survival.	Mouse	154
Allogeneic bone marrow–derived MSCs with or without low-dose cyclosporine A	Allogeneic heart transplant	MSCs did not prolong allograft survival. MSCs + cyclosporine A had an accelerated allograft rejection.	Rat	158

A controlled study by Zhao *et al.* with 47 enrolled GvHD patients demonstrated that MSC infusions from HLA-mismatched third-party donors increased resolution of GvHD symptoms after 8 weeks of treatment, with an overall resolution rate of 75% compared with 42% in control groups receiving only corticosteroid treatment. Among the patients responding to MSC treatment, 60% achieved complete response, while 14% showed a partial response. MSC infusions also decreased the mortality rate from 58% to 25% owing to GvHD progression and infections. The same study also showed that the results of MSC treatment depended on GvHD grade and the number of organs compromised, being less effective for patients who had GvHD stage IV and two or more organs involved.[163] Moreover, the 2-year cumulative incidence of cGvHD was significantly lower in MSC-treated patients (31% vs. 79% in control groups). Cellular analysis revealed that MSC treatment diminished the number of $CD3^+CD8^+$ T cells and increased the $CD4^+CD25^+FOXP3^+$ T_{reg} cell subset with respect to baseline measurements before MSC infusion, an effect detected from the first 8 weeks until 6 months posttreatment.[163]

In another study without a control group, 13% of HSCT patients exhibited complete resolution of all clinical manifestations, while 61% showed partial resolution of cGvHD symptoms after a 12-month follow-up period after MSC infusion.[147] Interestingly, the patients who did not respond to MSC treatment showed a decrease in B cell number during the first 6 months after treatment ($CD27^+$ memory B cells and $CD27^+$ naive B cells). Conversely, the patients who partially or completely responded to MSC infusions showed an enrichment of B cell populations.[147] Measurements of plasma levels of B cell–activating factor (BAFF), a key regulator of B lymphocyte homeostasis, and expression of its receptor on B cells (BAFF-R) were performed before and after MSC infusions, revealing that only those patients who responded partially or completely to MSC treatment had higher levels of BAFF-R, while at the same time BAFF plasma levels were decreased.[147] Although this study lacked a control group, it was the first to highlight the relevance of B cell homeostasis during MSC treatment, suggesting that combining treatments could enhance or potentiate MSC therapy and increase its success.

In an uncontrolled trial that combined both pediatric and adult patients, the authors showed that as early as 28 days after MSC infusion 25% of the patients experienced complete response, while 50% achieved complete resolution of symptoms for at least one consecutive month.[164] Moreover, overall survival after one year for all patients included in the study was 44%. In addition, a panel of plasma biomarkers, including IL-2Rα, TNFR1, HGF, IL-8, elafin, and REG3α levels, was indeed predictive of the obtained overall survival.[164,165]

Another study, also assessing plasma GvHD biomarkers and cytokines as predictive tools for

Table 2. List of clinical studies in which transplantation patients received MSCs as cellular therapy

Type of MSC	Dosage and prophylaxis	Organ transplanted	Underlying pathological condition	Type of clinical trial	Reference
Allogeneic MSC from unrelated donors, derived from peripheral blood, bone marrow, and umbilical chord	2 doses of 1–2 × 10^6 MSCs per kg of body weight at days 0 and 8. A third infusion was performed on partial responders at day 22. In combination with cyclosporine and prednisolone.	Allogeneic hematopoietic stem cells (HSCs)	Hematological malignancies (myeloid and lymphoid neoplasms) and nonmalignant disorders.	Phase II Uncontrolled 7 pediatric patients 43 adult patients	164
Autologous bone marrow–derived MSCs	0.2 × 10^6 MSCs per kg of body weight, IV. In combination with melphalan or a mixture of BCNU, melphalan, etoposide, and cytarabine. Coinfusion with HSCs.	Autologous HSCs expanded *in vitro*	Hematological malignancies (non-Hodgkin lymphoma, Hodgkin lymphoma, and multiple myeloma).	Phase II Controlled Nonrandomized Unblinded Single center 162 patients Age 7–62 years	170
Allogeneic bone marrow–derived MSCs from HLA-identical siblings	1.2 × 10^6 MSCs per kg of body weight, IV. In combination with cyclosporine and methotrexate. Some patients also received mycophenolate mofetil or prednisolone. Infusion after blood cell reconstitution.	Allogeneic bone marrow	Hematological malignancies (not specified).	Phase II Controlled Randomized Single center 77 patients Age 17–63 years	171
Allogeneic bone marrow–derived MSCs from HLA-mismatched third party	2–8 doses of 1 × 10^6 MSCs per kg of body weight, weekly, IV. In combination with methylprednisolone and calcineurin inhibitors. Some patients also received methotrexate, mycophenolate mofetil, antithymocyte globulin, cyclophosphamide, and CD25 monoclonal antibody.	Allogeneic HSCs	Acute GvHD after hematopoietic transplantation.	Phase II Controlled Nonrandomized Unblinded Multicenter 47 patients Age 14–54 years	163
Allogeneic bone marrow–derived MSCs from unrelated donors	2 doses of 1 × 10^6 MSCs per kg of body weight at 4-week intervals, IV. In combination with prednisone, mycophenolate mofetil, tacrolimus, cyclosporine, or rapamycin.	Allogeneic HSCs	Extensive chronic GvHD involving two or more organs after HSCT therapy for hematological malignancies (acute lymphoblastic leukemia, acute monocytic leukemia, and chronic monocytic leukemia).	Phase II Uncontrolled 38 patients Age 20–47 years	175
Allogeneic bone marrow–derived MSCs from unrelated donors	3 doses of 2 × 10^6 MSCs per kg of body weight (once a week); in combination with immunosuppressive therapy (tacrolimus, sirolimus, and cyclosporine) before MSC administration and during the whole trial.	Allogeneic HSCs	Acute GvHD after HSCT therapy for hematological malignancies (acute leukemia, myelodysplastic syndrome, severe aplastic anemia, diffuse large B cell lymphoma, chronic granulomatous disease, and cutaneous T cell lymphoma).	Phase I 9 GVHD patients 1 patient with tissue injury Age 20–71 years	166
Allogeneic bone marrow–derived MSCs from unrelated donors	4 doses of 1.1 × 10^6 MSCs per kg of body weight at days 0, 4, 11, and 18, IV; with paracetamol and dexchlorpheniramine before MSC administration. In combination with tacrolimus, rapamycin, methotrexate, cyclosporine, or mofetil mycophenolate.	Allogeneic HSCs	Chronic GvHD after HSCT therapy for hematological malignancies (acute myeloid leukemia, myelodysplastic syndromes, and Hodgkin lymphoma).	Phase II Uncontrolled Multicenter 25 patients Age 20–65 years	176

Continued

Table 2. *Continued*

Type of MSC	Dosage and prophylaxis	Organ transplanted	Underlying pathological condition	Type of clinical trial	Reference
Allogeneic bone marrow–derived MSCs from unrelated donors	1–13 doses of 1–2 × 10^6 MSCs per kg of body weight. In combination with cyclosporine A, methotrexate, and antithymocyte globulin.	Allogeneic HSCs donor lymphocyte infusion (DLI)	GvHD after HSC or DLI therapy for hematological malignancies (acute lymphoblastic leukemia, acute myeloid leukemia, myelodysplastic syndrome, and juvenile myelomonocystic leukemia), immune deficiency, and nonmalignant disorders.	Phase II Uncontrolled 37 pediatric patients Age 0–18 years	168
Allogeneic bone marrow–derived MSCs from unrelated donors	2 × 10^6 MSCs per kg of body weight. In combination with methotrexate, antithymocyte globulin, and cyclosporine.	Allogeneic HSCs	GvHD or hemorrhages after HSC therapy for hematological malignancies (acute myeloid leukemia, chronic myelomonocytic leukemia, Hodgkin lymphoma, myelofibrosis, and chronic lymphatic leukemia) and immune deficiency.	Phase II Controlled Unblinded Nonrandomized 11 patients Age 27–66 years	167
Allogeneic bone marrow–derived MSCs from unrelated donors	2 doses of 1–2 × 10^6 MSCs per kg of body weight at days 12 and 26 after initiating steroids, IV; some patients received a third MSC infusion at day 50. Six patients received high-dose steroids, tacrolimus, or mofetil mycophenolate before MSC infusions.	Allogeneic HSCs	Acute gastrointestinal GvHD after HSC therapy for hematological malignancies and nonmalignant disorders	Phase I Uncontrolled Single center 22 pediatric patients Age 0–18 years	169
Allogeneic adipose tissue–derived MSCs (AD-MSCs) from the same organ donor, HLA compatible	0.33 × 10^4 MSCs per kg of body weight, portal coinfusion with 8.8–10.4 × 10^8 HSCs per kg of body weight 5 days before same donor renal transplantation. In combination with rabbit antithymocyte globulin, tacrolimus, and methylprednisone or cyclophosphamide. Previous radiotherapy (200 cG) from days 1 to 5.	Kidney	End-stage renal disease due to chronic glomerulonephritis, chronic tubulointerstitial nephritis, or diabetic nephropathy.	Phase II Controlled Unblinded Randomized Three-armed 285 patients Age 20–47 years	177
Autologous bone marrow–derived MSCs	2 doses of 1–2 × 10^6 MSCs per kg of body weight, 7 days apart. In combination with basiliximab, prednisone, tacrolimus, or cyclosporine and mycophenolate mofetil. In addition, patients were treated routinely with oral valganciclovir prophylaxis for 3 months.	Kidney	Subclinical rejection of kidney transplantation for treating nephrosclerosis, acute kidney injury, hypertensive nephropathy, and adult polycystic kidney disease.	Phase II Controlled Unblinded Nonrandomized 15 patients Age 18–70 years	178
Autologous bone marrow–derived MSCs	2 × 10^6 MSCs per kg of body weight, the day before kidney transplantation. In combination with low-dose antithymocyte globulin, cyclosporine mycophenolate mofetil, and steroids.	Kidney	End-stage renal disease	Phase II Controlled 14 patients (2 treated) Age 27–64 years	173

Continued

Table 2. *Continued*

Type of MSC	Dosage and prophylaxis	Organ transplanted	Underlying pathological condition	Type of clinical trial	Reference
Autologous bone marrow–derived MSCs	2 doses of 1–2 × 10^6 MSCs per kg of body weight, the first dose at the moment of kidney transplantation and the second one 2 weeks later. In combination with steroids, mycophenolate mofetil, tacrolimus, or cyclosporine and methylprednisolone. Only control groups received anti-IL-2 receptor antibody.	Kidney	End-stage renal disease	Phase II Controlled Unblinded Randomized 159 patients Age 18–61 years	174
Autologous bone marrow–derived MSCs	2 doses of MSCs, the first (5 × 10^6) at the moment of kidney transplantation and the second (2 × 10^6 per kg of body weight) 1 month later. In combination with tacrolimus, cyclosporine, cytoxan, mycophenolate mophetil, and methylprednisolone. Tacrolimus dose was high in control group and low in MSC group.	Kidney	Chronic glomerulonephritis	Pilot study Controlled Nonrandomized 12 patients Age 18–60 years	175

MSC treatment outcome, showed that five out of seven evaluable patients with GvHD achieved complete response to MSC infusion, detecting lower plasma levels of the epithelial apoptosis marker CK18 and also exhibiting survival rate of 100% at a median of 300 days after MSC infusion.[166] Importantly, on the basis of a Levine panel and CK8 levels, the authors were able to define a pattern for complete responding and nonresponding patients: high levels of TNFR1, IL-2Rα, CK18, IL-18, and REG3α in patients who did not achieve MSC response and ultimately died of sepsis or multiple organ failure. The study also showed that complete-responding patients were younger and had lower GvHD grades, less prolonged immunosuppressive therapy, and higher levels of memory lymphocytes.

A more complete analysis of the patient status is presented in the study of Jitschin *et al.*, in which clinical, molecular, and cellular features are assessed. MSC infusion produced a 40% reduction of CK18 levels after 30 days.[167] On the other hand, MSC treatment also increased the proportion of $CD4^+$ T cells over $CD8^+$ T cells and lowered T cell activation and the IFN-γ:IL-4 ratio, suggesting that MSC treatment favored a T_H2 phenotype. Moreover, MSC treatment also increased the frequency of T_{reg} cells at 30 to 90 days after treatment by 4% and decreased T_H17 frequency without any detectable differences in memory T cell subsets.[167]

Two different studies focused on pediatric patients showed similar results on responsiveness to MSC treatment. The study by Ball *et al.* indicated that complete response to MSC treatment was achieved by 59% of children, while 21.6% showed partial response.[168] With a median follow-up of 2.9 years, 51% of patients survived; however, 25% of patients who had achieved complete response with MSC infusions died. Unfortunately, the work does not provide any other information about T cell count or plasma biomarkers. However, the study by Calkoen used the same biomarkers mentioned above, adding a molecular context to the survival data. After 28 days of MSC infusion, 50% of the patients reached complete response with an overall survival rate of 80% after a 2-year follow-up, while 27% achieved a partial response with a 30% survival rate after 2 years.[169] Four patients with signs of GvHD (and not responding to MSC infusions) received additional MSC administration, and 70% reached complete response afterward. The authors find that the best correlation corresponds to complete response to MSC treatment and lower levels of TNFR and REG3α at the onset of GvHD. Unlike other reports, the authors did not find any correlation between GvHD grade and MSC response.

Importantly, the study proposes the use of CK18 and REG3α as a less invasive alternative to endoscopic and histological analysis for gastrointestinal GvHD.[169]

Finally, the studies of Batorov *et al.* and Shipounova *et al.* tested the infusion of MSCs before GvHD symptoms as a prophylatic approach. The former study showed that early lymphocyte recovery, a predictive factor for HSCT survival, was higher in patients receiving MSC infusions. Furthermore, MSC-treated patients showed a recovery of total lymphocyte numbers, including naive and memory $CD4^+$ and $CD8^+$ T cells, within a month. MSC infusion also induced antiapoptotic effects in naive $CD4^+$ T cells and an increased proliferation rate of $CD8^+$ memory T cells.[170] Although these results are indicative of more effective immune reconstitution after MSC treatment, the study did not provide information about GvHD onset or resolution. Moreover, the authors showed cellular analysis with a maximum follow-up of 12 months without giving details regarding the success of HSC survival or patient survival beyond that period.[170] The latter study showed that MSC infusion decreased by half the development of GvHD symptoms and lethality. In addition, after a 5-year follow-up (the longest period of any study documented here), MSC infusion lowered lethality due to relapse from 73% to 55%.[171] Although this study did not provide data regarding immune cell frequencies, the authors describe an MSC expression profile that could predict a favorable outcome, determining that both elevated expression of FGFR1 and diminished expression of peroxisome proliferator gamma receptor (PPARG), a regulator of MSC metabolism, increase the probability of success of MSC transplantation.[171]

With regard to the use of MSCs as a cell therapy for SOT, advances have been made for renal engraftment survival and functional recovery. In this field, preclinical studies have shown the use of autologous MSC to be feasible, apparently safe, and, most importantly, therapeutic;[172] however, in most of them, the reduced number of patients or the short follow-up timing and lack of cellular and molecular analysis do not allow a complete overview of the real impact of MSCs on patient recovery.

A study by Perico *et al.* described the results obtained with only two patients who received autologous MSCs a day before kidney transplantation. In one patient, they observed no sign of rejection and improved renal function after 540 days. In the other patient, 17 days after transplantation, biopsy analysis showed signs of acute rejection, which was overcome by administrating intravenous pulses of methylprednisolone, reaching normal renal function after 360 days of engraftment.[173] The number of memory and effector $CD8^+$ T cells was lower in patients receiving MSCs compared with untreated patients, who exhibited higher numbers at days 180 and 360 posttransplant. In contrast, T_{reg} cell number was reduced in MSC-treated patients, which later reached comparable levels to control groups.[173]

Another study showed that MSC infusion before kidney transplant resulted in acute rejection in 7% of the treated patients versus 21% in the nontreated group.[174] MSCs also improved histological changes after transplantation. After one year, patient and graft survival rate was similar in both treated and control groups, but MSC infusion diminished the occurrence of opportunistic infections.[174] Despite the large number of patients analyzed in this study, there was no information regarding T or B cell status or other immunological parameters.

Peng and colleagues provided cellular and molecular analysis from MSC-treated kidney transplant patients in a small pilot study with a follow-up of 12 months. The authors stated that after one year of kidney transplantation 16% of the control group experienced acute rejection, while none of the patients treated with MSCs did.[175] All patients and grafts survived one year of follow-up. Peripheral blood lymphocyte analysis showed no differences in $CD4^+CD8^+$ T cells and NK cells between controls and treated groups at different time points. However, memory B cells increased in the treated group at 3 and 12 months after transplantation; however, their frequency in control groups gradually diminished. Production of proinflammatory cytokines was also assessed by intracellular staining of patient PBMCs, detecting no differences in the frequency of cells producing IFN-γ, TNF-α, IL-4, or IL-10 in control and treated groups.[175]

Clinical trials using MSCs for inducing tolerance after HSCT or kidney transplantation show promising results in terms of patient and graft survival; however, it is still evident that MSC infusion timing and combination with immunosuppressive drugs are still matters of controversy that need further homogenization. Since the immunomod-

ulatory properties of infused MSCs have not yet been fully determined, longer follow-up times and immune monitoring should be considered in future clinical trials; thus, testing tolerance achievement may be performed over time.

Concluding remarks

Immune tolerance is a major goal in transplantation, enabling graft survival without depleting infection-related immune responses. In recent years, MSCs have gained great attention in the effort to define new therapies for transplant tolerance.

The diverse immunomodulatory properties of MSCs present an exciting opportunity to develop new approaches for cellular therapy in the transplantation field. In this review, we discussed how MSCs are capable of interacting with and modulating key effector immune cells, such as macrophages, DCs, T cells, and B cells, by both cell–cell contact and the secretion of soluble regulatory elements.

Even though there is a large amount of evidence concerning the general properties of MSCs and their immune regulation capabilities, very little has translated into transplantation-related clinical use. Most clinical studies have investigated the use of MSCs to ameliorate GvHD after HSCT for treatment of hematopoietic malignancies, and have shown promising results thus far.

Undoubtedly, future studies that address the still-pending questions about the immune-modulatory nature of MSCs, and how they respond to different environmental settings, are necessary to promote safe and effective clinical trials of these cells in the organ transplantation field.

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Competing interests

M.K. is the chief science officer of Cells for Cells and Consorcio Regenero. The other authors declare no competing interests.

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