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# Mesenchymal Stem Cells: Mechanisms of Inflammation

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## Keywords

immune modulation

## Abstract

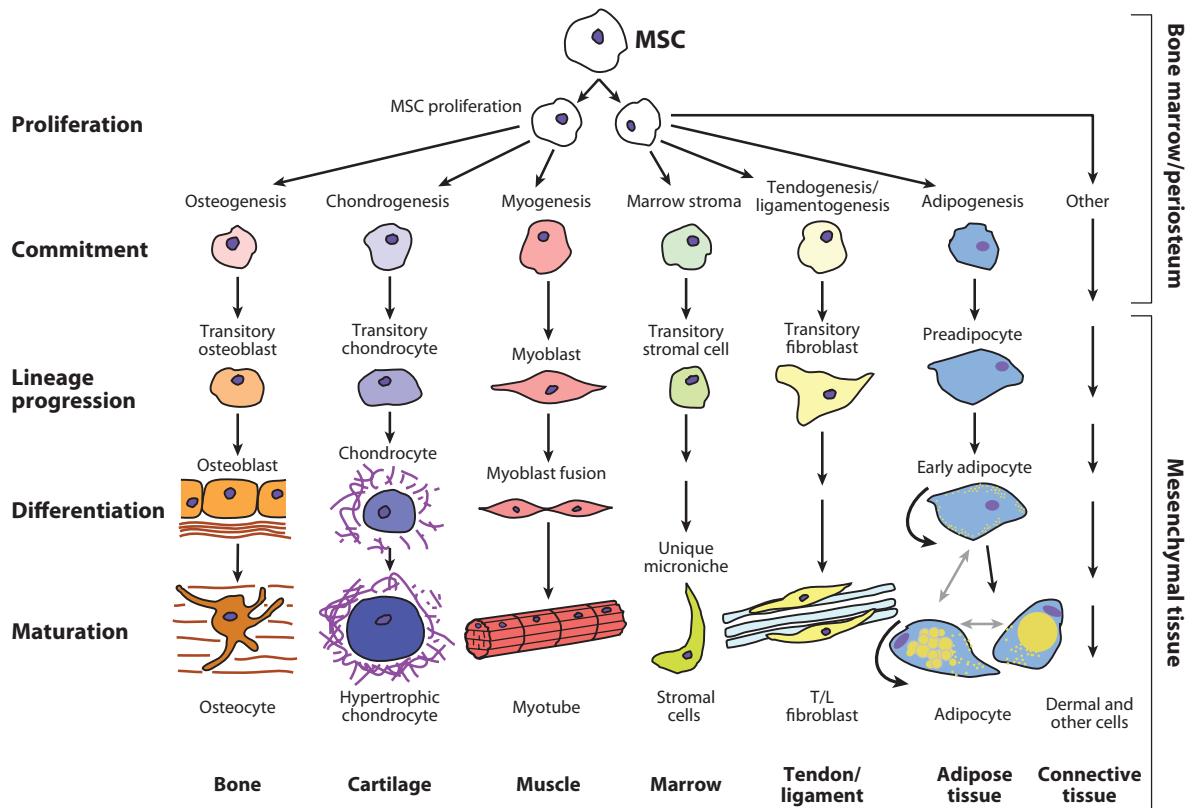
In adults, human mesenchymal stem cells (hMSCs) are found in vivo at low frequency and are defined by their capacity to differentiate into bone, cartilage, and adipose tissue, depending on the stimuli and culture conditions under which they are expanded. Although MSCs were initially hypothesized to be the panacea for regenerating tissues, MSCs appear to be more important in therapeutics to regulate the immune response invoked in settings such as tissue injury, transplantation, and autoimmunity. MSCs have been used therapeutically in clinical trials and subsequently in practice to treat graft-versus-host disease following bone marrow transplantation. Reports of successful immune modulation suggest efficacy in a wide range of autoimmune conditions, such as demyelinating neurological disease (multiple sclerosis), systemic lupus erythematosus, and Crohn's disease, among others. This review provides background information about hMSCs and also describes their putative mechanisms of action in inflammation. We provide a summary of ongoing clinical trials to allow (*a*) full comprehension of the range of diseases in which hMSC therapy may be beneficial and (*b*) identification of gaps in our knowledge about the mechanisms of action of therapeutic MSCs in disease.

## INTRODUCTION

**hMSCs:** human mesenchymal stem cells

Adult human mesenchymal stem cells (hMSCs) can be isolated from a variety of tissues, including bone marrow, muscle, fat, and dermis. Depending on the stimulus and the culture conditions employed, these cells can form bone, cartilage, muscle, fat, and other connective tissues (Figure 1) (1–3). These observations originally suggested that MSCs were responsible for the normal turnover and maintenance of adult mesenchymal tissues, but over the past decade it has become clear that all MSCs are pericytes (1) and that it is their pleiotropic nature that allows them to sense and respond to an event in the local environment, be it injury or inflammation. This is their most important biological property.

The original mesengenic process pathway (Figure 1) was hypothesized and fashioned after the hematopoiesis lineage diagrams of the 1980s. We built our original hypotheses about the regenerative potential of MSCs for damaged bone and cartilage based on our knowledge that osteoblasts and chondrocytes are derived in lineage-progression pathways, as shown in studies from our own and other laboratories (4, 5). This suggestion arises from the facts that all cells have half-lives and that their natural expiration must be matched by their replacement; MSCs are the proposed source of these new replacement cells. This replacement hypothesis mimics the known sequence of events involved in the turnover and maintenance of blood cells that are formed from hematopoietic stem cells (HSCs) (6).



**Figure 1**

The mesengenic process. Human mesenchymal stem cells (hMSCs) from bone marrow may develop into bone, muscle, or adipose tissue, depending on the stimuli to which they are exposed in vitro. Shown is a hypothesis of the mesengenic process that hMSCs undergo both in vitro and in vivo. Reproduced from Reference 21 with permission.

The replacement and turnover role for MSCs is also supported by our data showing that, if  $(10-20) \times 10^6$  cells from the light cell fraction of a bone marrow aspirate were seeded into a 100-mm Petri dish, colonies of fibroblastic cells (CFU-F) would be observed by days 10–12 (7). A dramatic decrease in MSCs per nucleated marrow cell can be observed when the results are grouped by decade: There is a 10-fold decrease from birth to the teenage years and another 10-fold decrease from the teenage years to old age. In comparison, titers of HSCs in marrow remain constant throughout life at approximately 1 per  $10^4$  nucleated marrow cells.

## MESENCHYMAL STEM CELLS

For the purposes of this review, cells that are isolated from adult bone marrow and are multipotent for skeletal phenotype are referred to as hMSCs because their reparative function appears to extend beyond the integumentary and musculoskeletal system. Unlike HSCs, hMSCs can be culture expanded *ex vivo* in up to 40 to 50 cell doublings without differentiation (8). Whether native hMSCs are abnormal is not entirely clear, although at least one study suggests that rheumatoid arthritis (RA) may be associated with premature senescence of hMSCs (9). RA has been associated with increases in lymphocyte proliferation and accumulation of lymphoid aggregates in synovium, which are sometimes referred to as tertiary lymphoid organs. We and others have demonstrated that hMSCs and their secreted products can inhibit T-lymphocyte activation and proliferation induced by mitogens, recall antigens, and alloantigens *in vitro* (10–16). *In vitro* hMSCs require activation (as do, presumably, those *in vivo*) (17), and activating stimuli appear to include secretion of transforming growth factor  $\beta 1$  (TGF- $\beta 1$ ) and interaction with CD14<sup>+</sup> monocytes and involve the proinflammatory cytokine interleukin-1 $\beta$  (IL-1 $\beta$ ). hMSCs modulate cytokine production by the dendritic and T cell subsets DC1/Th1 and DC2/Th2 (18), block antigen presenting cell (APC) maturation and activation (19), and increase the proportion

of CD4<sup>+</sup>CD25<sup>+</sup> regulatory cells in a mixed lymphocyte reaction (20). Recent data suggest a role for hMSCs in angiogenesis and that hMSCs may sense and respond to their environment (21). This feature of hMSCs calls into question whether the effects observed on allogeneic mixed lymphocyte reactions *in vitro* and on graft-versus-host disease (GVHD) *in vivo* should be categorized as immune suppressive, which would imply a nonspecific downregulation of immune responses or as inducing immune tolerance—an active and specific suppression of aberrant immune responses. Finally, all hMSCs may be pericytes and, thus, would be present in all vascularized tissues. Because vascular density decreases by one or two orders of magnitude with age, the local availability of hMSCs or pericytes likewise decreases substantially with age (22). The presence of activated hMSCs at sites of inflammation or injury is understood, given that pericytes would be released from their endothelial interactions in such vascularized locations, although the relative number and source of hMSCs that are mobilized from remote sites, versus those that are derived from proximate vascularized sites, are topics of ongoing investigation.

The original observation that hMSCs can reduce T cell proliferation *in vitro* is mirrored by the *in vivo* finding that infusions of hMSCs can control GVHD following bone marrow transplantation. However, no correlation between the measured effects of MSCs *in vitro* and their effect *in vivo* has been demonstrated, because there is no universally accepted *in vitro* method for determining the potential *in vivo* therapeutic capacity of MSCs. The basic mechanisms of MSCs are depicted in **Figures 2** and **3**.

## T CELL PROLIFERATION CAN BE SUPPRESSED BY ACTIVATED BUT NOT UNACTIVATED HUMAN MESENCHYMAL STEM CELLS IN VITRO

*In vivo* use of hMSCs for therapeutic indications does not require priming of MSCs.

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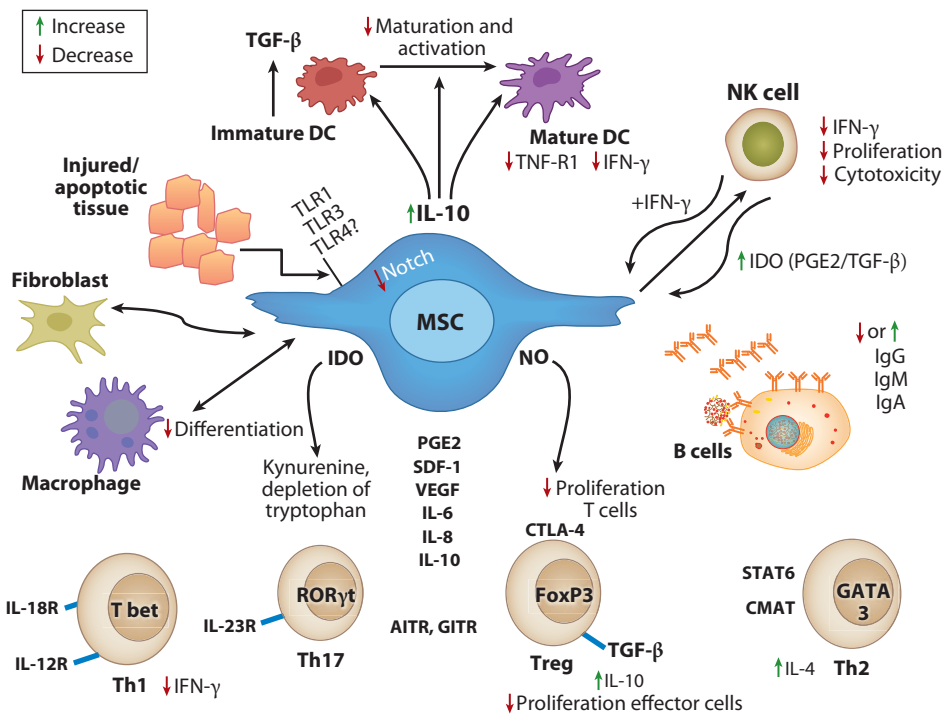
**RA:** rheumatoid arthritis

**TGF- $\beta$ :** transforming growth factor  $\beta$

**APC:** antigen presenting cell

**GVHD:** graft-versus-host disease

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**Figure 2**

A summary of the range of soluble and cell-surface proteins that may both mediate the effects of human mesenchymal stem cells (hMSCs) and provide information to hMSCs about the local environment. hMSCs may be able to both sense and respond to their immediate environment, which makes them ideal cells to tune the response to injury and/or inflammation. Abbreviations: CLTA4, cytotoxic T-lymphocyte antigen 4; DC, dendritic cell; IDO, indoleamine-2,3-dioxygenase; IFN, interferon; Ig, immunoglobulin; IL, interleukin; NK, natural killer; NO, nitric oxide; PGE2, prostaglandin E2; SDF, stromal cell-derived factor; TGF, transforming growth factor; Th, T helper cell; TLR, Toll-like receptor; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.

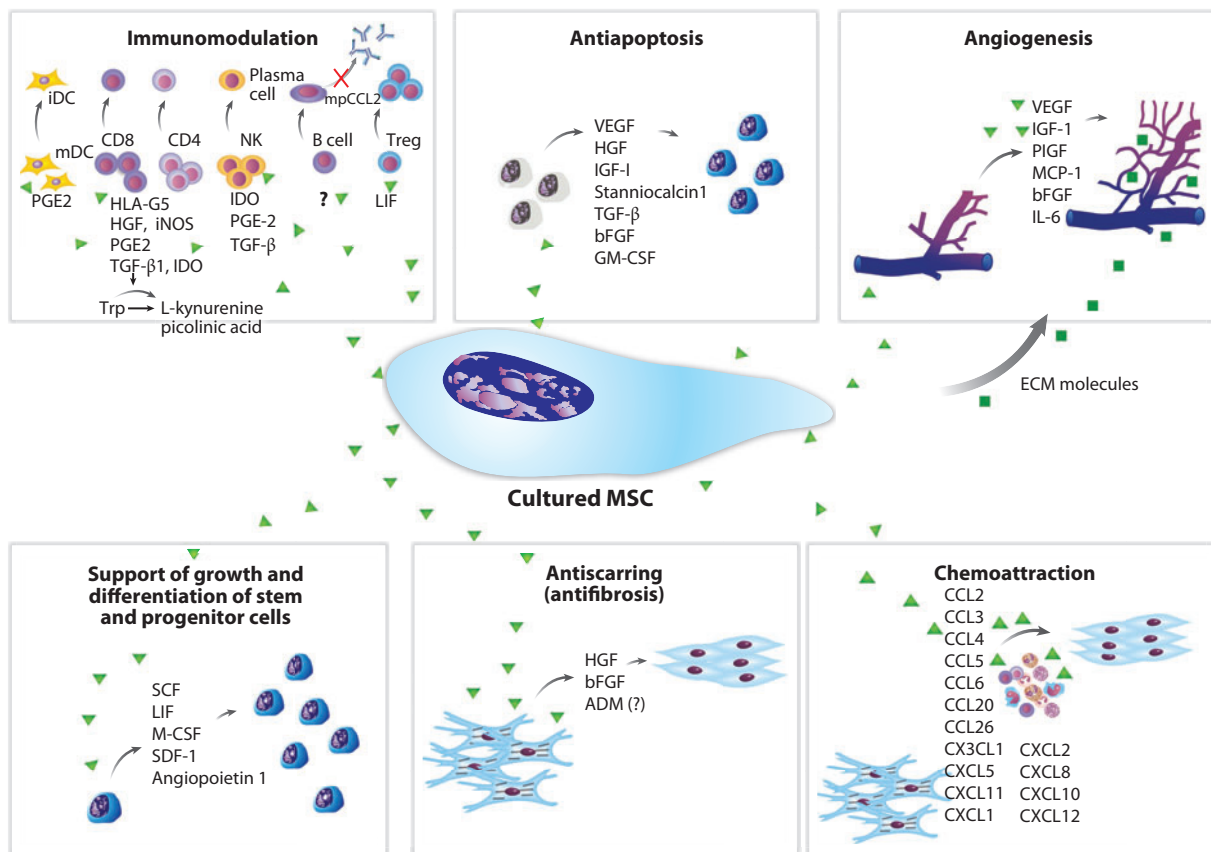
However, *in vitro*, MSCs must be activated in order for them or for conditioned medium from such cultures to suppress T cell proliferation. MSC effects on T cell proliferation *in vitro* appear to have both contact-dependent and contact-independent components (10). This conclusion resulted from observations that conditioned medium from MSCs activated either singly or in combination with cytokines [IL-1 $\beta$ , tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interferon- $\gamma$  (IFN- $\gamma$ )] or by coculture with allogeneic peripheral-blood mononuclear cells (PBMCs) can reduce T cell proliferation of (a) PBMCs stimulated with mitogens such as phytohemagglutinin, (b) PBMCs stimulated through the T cell receptor (TCR) complex

using a combination of antibodies directed against CD3 and CD28, (c) T cell lines created by the stimulation conditions given in items a and b, and (d) antigen-specific T cell clones (17, 24, 25). The greatest impediments to interpreting how these data relate to the effectiveness of any single group of culture-expanded MSCs are as follows:

1. There is no established manner in which to correlate MSCs' capacity to reduce T cell proliferation with their potential therapeutic effects (discussed in Reference 1).
2. Individual batches of MSCs may display variation in suppressive ability *in vitro*,

**TNF- $\alpha$** : tumor necrosis factor  $\alpha$

**IFN- $\gamma$** : interferon- $\gamma$



**Figure 3**

Paracrine effects of cultured mesenchymal stem cells (MSCs). The secretion of a broad range of bioactive molecules is now believed to be the main mechanism by which MSCs achieve their therapeutic effect. This mechanism can be divided into six main actions: immunomodulation, antiapoptosis, angiogenesis, support of the growth and differentiation of local stem and progenitor cells, antiscarring, and chemoattraction. Although the number of molecules known to mediate the paracrine action of MSCs increases every day, only a few factors that are secreted by cultured MSCs are shown. The immunomodulatory effects of MSCs consist of inhibition of the proliferation of CD8<sup>+</sup> and CD4<sup>+</sup> T lymphocytes and natural killer (NK) cells, suppression of immunoglobulin production by plasma cells, inhibition of maturation of dendritic cells (DCs), and stimulation of the proliferation of regulatory T cells. The secretion of prostaglandin E2 (PGE2), human leukocyte antigen G5 (HLA-G5), hepatocyte growth factor (HGF), inducible nitric oxide synthase (iNOS), indoleamine-2,3-dioxygenase (IDO), transforming growth factor β (TGF-β), leukemia-inhibitory factor (LIF), and interleukin (IL)-10 contributes to this effect. MSCs also limit apoptosis, and the principal bioactive molecules responsible for this process are HGF, TGF-β, vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), stanniocalcin 1, and granulocyte macrophage colony-stimulating factor (GM-CSF). MSCs stimulate local angiogenesis by secretion of extracellular matrix (ECM) molecules, VEGF, IGF-1, phosphatidylinositol-glycan biosynthesis class F protein (PIGF), monocyte chemoattractant protein 1 (MCP-1), basic fibroblast growth factor (bFGF), and IL-6; they also stimulate mitosis of tissue-intrinsic progenitor or stem cells by secretion of stem cell factor (SCF), macrophage colony-stimulating factor (M-CSF), stromal cell-derived factor (SDF-1), LIF, and angiopoietin 1. Moreover, HGF and bFGF (and possibly adrenomedullin) produced by MSCs contribute to inhibition of scarring caused by ischemia. Finally, a group of at least 15 chemokines produced by MSCs can elicit leukocyte migration to the injured area, which is important for normal tissue maintenance. Adapted from Reference 23 with permission.

but whether or not that is predictive of their potency *in vivo* is largely unknown.

3. The effects of MSCs *in vivo* appear to be pleiotropic and influenced by their local environment, whereas that dimension of cell-cell interaction is lacking *in vitro*.

Soon after MSCs were first observed to reduce T cell proliferation *in vitro*, immune-activated MSCs or conditioned medium from immune-activated MSCs was found to reduce the frequency of IFN- $\gamma$ - and IL-2-secreting T cells (10). These data used enzyme-linked immunosorbent spot assays to assess the frequency of T cell response to both mitogens and TCR ligation *in vitro* (26). Complementary data obtained via enzyme-linked immunosorbent spot assays suggest that the absolute amounts of T cell cytokines produced under similar stimulation conditions are also reduced (10).

Some controversy exists regarding whether, under all circumstances, MSC effector function *in vitro* occurs in tandem (i.e., proliferation and production of IFN- $\gamma$  by T cells are reduced) (27, 28). This controversy fuels the debate as to what measurement of MSC potency would best reflect the *in vitro* potential of any particular expanded population of MSCs. The observation that under some but not all circumstances the amounts of T cell proliferation and cytokine secretion are similar or dissimilar probably depends on the source of MSCs. (hMSCs and mouse MSCs are not equivalent, but hMSCs may be used in, for example, mouse models of autoimmune disease, where they exert immunosuppressive effects or may induce actual immune tolerance.) The absolute amount of cytokine production may not be the most relevant measurement, and ratios of cytokine production (which are not yet fully understood) may be a better barometer for predicting the *in vivo* function of MSCs.

## MESENCHYMAL STEM CELLS AND CYTOKINES

Like IL-1 $\beta$ , IFN- $\gamma$  appears to be one of the cytokines that can prime MSCs *in vitro*. However, IL-1 $\beta$  may not require combinations of

cytokines and may alone be sufficient to prime hMSCs, whereas the effect of IFN- $\gamma$  may be amplified in the presence of other proinflammatory cytokines, such as IL-1 $\beta$  and TNF- $\alpha$ . Theoretically, IFN- $\gamma$  may also interfere with hMSC function. One of the reasons that hMSCs appear to be immune privileged is that constitutively freshly isolated and cultured MSCs lack MHC class II expression. In the presence of IFN- $\gamma$ , culture may induce MHC class II expression and transform MSCs into cells that can act as APCs rather than as immune-modulating cells (29).

Memory T cell production of IL-2 and IFN- $\gamma$  to both specific antigens and lectins is reduced by coculture with MSCs or by medium of MSCs activated *in vitro*. Boccelli-Tyndall et al. (30) recently demonstrated that, under conditions meant to mimic conditions observed in inflammatory joint disease, exogenous addition of IL-15 and IL-7 may contribute more than IL-2 to proliferation of PBMCs, T cells, and T cell subsets *in vitro*. Furthermore, when low ratios of either MSCs or articular chondrocytes are added, proliferation of most but not all of these subsets may be augmented in a cytokine-dependent fashion. In contrast, at high ratios of MSCs or articular chondrocytes to effector cells, proliferation is suppressed rather than enhanced (30).

IL-10, an anti-inflammatory cytokine that contributes to the downregulation of T cell proliferation and other effector responses, may be produced by mature dendritic cells that have been cocultured either with hMSCs or with conditioned medium from cytokine/PBMC-activated MSCs (18, 27, 31). These data suggest that seemingly antigen-independent proliferation may respond to MSC-mediated effects and that choosing the right dose of MSCs in a therapeutic setting may be critical for optimal results.

## MESENCHYMAL STEM CELLS AND REGULATORY T CELLS

Regulatory T cells (Tregs) suppress the proliferation and cytokine production of effector T cell populations, thereby mediating

peripheral tolerance by targeting autoreactive T cells that arise de novo or escape thymic deletion. Tregs consist of several different subsets with overlapping but distinct actions, particularly in human disease. Tregs may be identified by the coexpression of CD4 and CD25 and by the expression of the transcription factor FoxP3, the production of regulatory cytokines IL-10 and TGF- $\beta$ , and the ability to suppress proliferation of activated CD4<sup>+</sup>CD25<sup>-</sup> T cells in coculture experiments. Purifying Tregs relies largely on their CD4<sup>+</sup>CD25<sup>+</sup> phenotype. Differential expression of CD127 (the  $\alpha$ -chain of the IL-7 receptor) enables flow cytometry-based separation of human CD4<sup>+</sup>CD25<sup>+</sup> Tregs from CD127<sup>+</sup> nonregulatory T cells (32).

TGF- $\beta$  is another anti-inflammatory cytokine that is implicated in mediating MSC responses and that may have T cell-independent effects. For example, TGF- $\beta$  produced in experiments using MSCs alters angiogenesis following injury (33, 34). Tregs secrete TGF- $\beta$ , and when used in vitro, TGF- $\beta$  in combination with IL-2 directs the differentiation of T cells into Tregs in which CD4<sup>+</sup> cells (*a*) express high levels of surface CD25, (*b*) express the nuclear transcription factor FoxP3, and (*c*) suppress the proliferation of TCR-dependent proliferation of effector CD25 null or CD25 low T cells in a nonautologous fashion (reviewed in Reference 35).

A particularly interesting and important observation in the field of arthritis is that TNF- $\alpha$  can downmodulate the function of CD4<sup>+</sup>CD25<sup>+</sup> Tregs; anti-TNF- $\alpha$  antibodies increase FoxP3 messenger RNA and protein expression (36). MSCs downregulate TNF- $\alpha$  as one of their mechanisms of action. Thus, it follows that treatment of RA PBMCs with conditioned MSC medium may increase the amount of Tregs and improve their function. By using MSCs derived from human adipose tissue, Gonzalez et al. (37) demonstrated an increase in Treg numbers in adipose-derived hMSC-treated arthritic mice. In vitro, these cells appear to suppress collagen-specific antigen responses of PBMCs from RA patients, which

suggests that induction of Tregs could be a goal in the treatment of RA (38).

## THE ROLE OF COSTIMULATORY MOLECULES IN MESENCHYMAL STEM CELL REGULATION OF IMMUNE RESPONSE

hMSCs express few to none of the B7-1/B7-2 (CD80/CD86) costimulatory-type molecules; this appears to contribute, at least in part, to their immune privilege. Mechanisms that lead to immune tolerance rely on interrelated pathways that involve complex cross talk and cross-regulation of T cells and APCs by one another. Both soluble mediators and modulation exerted via complex networks of cytokines and costimulatory molecules appear to play a role in MSC regulation of T cells, and these mechanisms appear to invoke both contact-independent and -dependent pathways.

Although many of the studies we discuss herein use MSC-conditioned medium, both contact-independent and -dependent mechanisms are probably invoked in the therapeutic use of MSCs (22, 39). In addition to cytokines, the network of costimulatory molecules is hypothesized to play a prominent role in modulating tolerance and inflammation. MSCs downregulate the expression of costimulatory molecules (12, 15, 40–44). The discovery of new functions for B7 family members, together with the identification of additional B7 and CD28 family members, is revealing new ways in which the B7:CD28 family may regulate T cell activation and tolerance. Not only do CD80/86:CD28 interactions promote initial T cell activation, they also regulate self-tolerance by supporting CD4<sup>+</sup>CD25<sup>+</sup> Treg homeostasis (40–44). Cytotoxic T-lymphocyte antigen 4 (CTLA-4) can exert inhibitory effects in both B7-1/B7-2-dependent and -independent fashions. B7-1 and B7-2 can signal bidirectionally by engaging CD28 and CTLA-4 on T cells and by delivering signals into B7-expressing cells (45). The five B7 family members—inducible costimulator (ICOS) ligand, PD-L1 (B7-H1),

PD-L2 (B7-DC), B7-H3, and B7-H4 (B7x/B7-S1)—are expressed on professional APC cells, and B7-H4 and perhaps B7-H1 are also expressed on hMSCs and on cells within nonlymphoid organs. These observations may provide a new means for regulating T cell activation and tolerance in peripheral tissues (17, 39, 46, 47). ICOS and PD-1 are inducibly expressed on T cells, and they regulate previously activated T cells (48). Both the ICOS:ICOSL pathway and the PD-1:PD-L1/PD-L2 pathway play a critical role in regulating T cell activation and tolerance (48). There is consensus that both CTLA-4 and PD-1 inhibit T cell and B cell activation and may play a crucial role in peripheral tolerance (48, 49). Both CTLA-4 and PD-1 functions are associated with RA and other autoimmune diseases. PD-1 is overexpressed on CD4<sup>+</sup> T cells in the synovial fluid of RA patients (50). Whether or not these costimulatory molecules are critical mediators of MSC-mediated immune suppression and/or tolerance *in vivo* is still under investigation.

### Effect of Mesenchymal Stem Cells on the T Cell Cycle

Several but not all studies suggest that one mechanism to reduce the proliferation of T cells may occur via effects on the cell cycle, particularly in the G1 phase or the G0/G1 transition, which result in the induction of T cell anergy (51). In these studies, Glennie et al. (51) showed that cyclin D2 expression is profoundly inhibited, whereas p27<sup>kip1</sup> was upregulated, consistent with the phenomenon termed division arrest anergy, in which T cells remain in the G1/G1 phase and do not recover their proliferative capacity even after contact with MSCs ceases.

### Effect of Mesenchymal Stem Cells on B Cells

The initial reports of the effect of MSCs on B cells came from studies in mice; they suggested that MSCs have dampening effects on the proliferation of B cells. However, the events that

determine whether MSCs augment or inhibit immunoglobulin production appear to be dependent on the dose of MSCs used and on the activation state of the B cells themselves; some differences between MSC effects on human peripheral B cells versus splenic B cells retrieved postmortem have been observed (52–54).

### Effects of Mesenchymal Stem Cells on Natural Killer Cells

Studies of the effect of MSCs on natural killer (NK) cells are particularly controversial, given that most data suggest that MSCs do not inhibit the cytotoxicity of NK cells. Furthermore, any attenuation on NK function that might occur when MSC ligands bind to NK-stimulatory receptors occurs at such a low level that the binding of MHC class I molecules to Kir family-inhibitory NK receptors probably tips the balance in favor of tolerance (rather than cytotoxicity) of NK cells toward MSCs (16, 55).

### Mesenchymal Stem Cell Response to Injury

Another characteristic of hMSCs is their ability to quell inflammation resulting from injury, allogeneic solid organ transplants, and autoimmune disease. hMSCs can effectively suppress the normal growth and expansion of stimulated T cells (13). Consistent with *in vitro* studies, murine allogeneic MSCs are effective cellular therapies in the treatment of murine models of human disease (24, 56–60). Several studies have documented the dramatic clinical improvements observed in animal models using systemically introduced hMSCs as a therapy in mouse models of multiple sclerosis (24, 56–60), inflammatory bowel disease (61–63), infarct, stroke, and other devastating neurologic diseases (64, 65), as well as diabetes (66). These findings strongly suggest that xenogeneic hMSCs are not immunologically recognized by various immunocompetent mouse models of disease. hMSCs may be able to home to sites of inflammation, where dampening of inflammation can begin and regeneration of injured



tissues leading to normal, scarless healing can proceed. However, the precise mechanisms behind these actions have not yet been elucidated.

NO produced by T cells may mediate its effect through phosphorylation of Stat-5 with the effect being lower levels of T cell proliferation. Inhibition of NO synthase or inhibition of prostaglandin synthesis was able to obviate MSC-dependent effects on proliferation. Although indoleamine-2,3-dioxygenase (IDO) has been hypothesized to be important in mediating the effect of NO, neutralizing IDO by using a blocking antibody did not interfere with NO's suppressive effects (67, 69). In vivo, the current paradigm is that injury, inflammation, and/or foreign cells can lead to T cell activation, and those T cells produce proinflammatory cytokines including, but not limited to, TNF- $\alpha$ , IFN- $\gamma$ , IL-1 $\alpha$ , and IL-1 $\beta$ . Combinations of cytokines may also induce cell production of chemokines, some of which bind to CXCR3R-expressing cells (including T cells) that colocalize with MSCs. MSCs then produce NO, which inhibits Stat-5 phosphorylation, thereby leading to cell-cycle arrest (and thus halting T cell proliferation) (67). In addition, iNOS appears to be important in mouse MSC in vivo effects: MSCs from mice that lack iNOS (or IFN- $\gamma$ R1) are unable to suppress GVHD (**Figure 4**). In contrast to mouse MSCs that use NO in mediating their immune-suppressive effect, hMSCs and MSCs from nonhuman primates appear to mediate their immune-suppressive effects via IDO (68). There is some controversy about whether the effect of IDO results from local depletion of tryptophan itself, from the accumulation of tryptophan metabolites (which is suggested by data showing that use of a tryptophan antagonist, 1-methyl-L-tryptophan, restored alloreactivity that would otherwise have been suppressed by MSCs), or from some combination thereof (**Figure 5**) (69). In addition to affecting the JAK-STAT pathway, NO may also influence mitogen-activated protein kinase and nuclear factor  $\kappa$ B, which would cause a reduction in the gene expression of proinflammatory cytokines.

## ANIMAL MODELS

Consistent with results from in vitro studies, murine allogeneic MSCs are effective in the treatment of murine models of human disease (58, 60, 70, 71). Several studies have documented the dramatic clinical improvements observed in animal models by using systemically introduced xenogeneic hMSCs rather than allogeneic MSCs as a therapy in mouse models of multiple sclerosis and amyotrophic lateral sclerosis (65), inflammatory bowel disease, infarct, stroke, diabetes (66), and GVHD. A major advantage of demonstrating success using hMSCs in mouse models of human disease is that the possibility of gathering mechanistic data—beyond measuring biomarkers from readily obtainable body fluids or using noninvasive imaging technology—may be realized, perhaps in the course of a clinical trial.

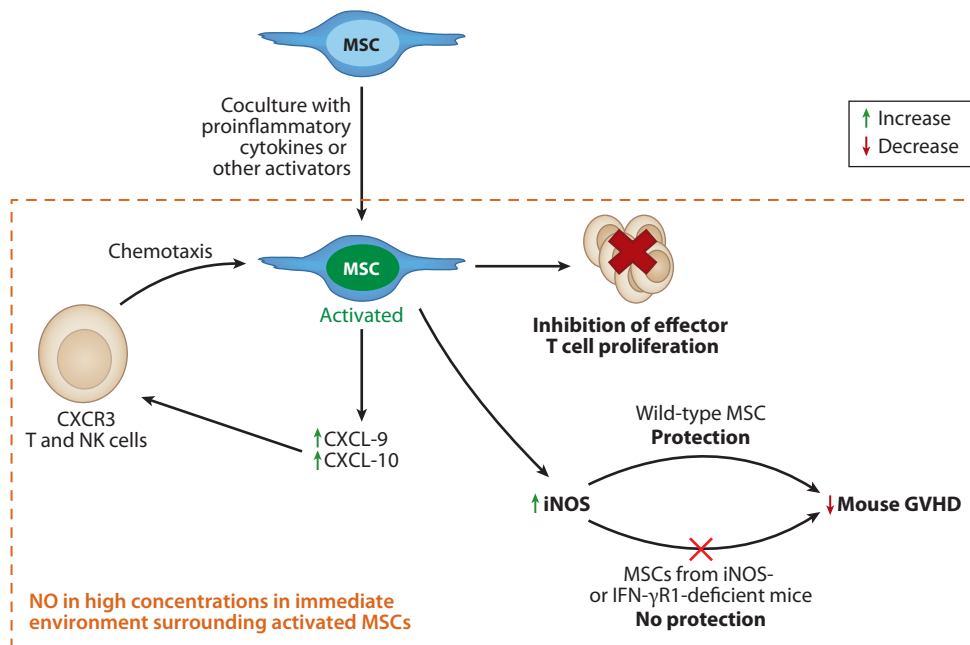
### Mesenchymal Stem Cells in Mouse Models of Arthritis

In experiments designed to study the trafficking of hMSCs, we used mice that contain a single copy of a transgene (Tg) with an altered 3' regulatory region that causes chronic TNF- $\alpha$  overexpression, which leads to the development of severe erosive polyarthritis (15–16). The TNF-Tg mice resemble RA patients in terms of their arthritis, and the arthritis phenotype is obviated by blocking with the anti-TNF- $\alpha$  monoclonal antibody infliximab. The primary reason for using these mice is to determine whether exogenous MSCs localize to lymph nodes, joints, and/or lung as is observed in the ovalbumin model of chronic asthma and in models of acute lung injury (72; T. Bonfield, M. Kolozem, D. Lennon, B. Zuchowski, S. Yang & A.I. Caplan, manuscript submitted). To determine where hMSCs localize in arthritic mice, we injected hMSCs into five-month-old TNF-Tg mice via the carotid artery. The hMSC-infused mice showed a reduction in ankle arthritis; ankle swelling was reduced from 4.5 mm to 3.5 mm in one

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**IDO:** indoleamine-2,3-dioxygenase

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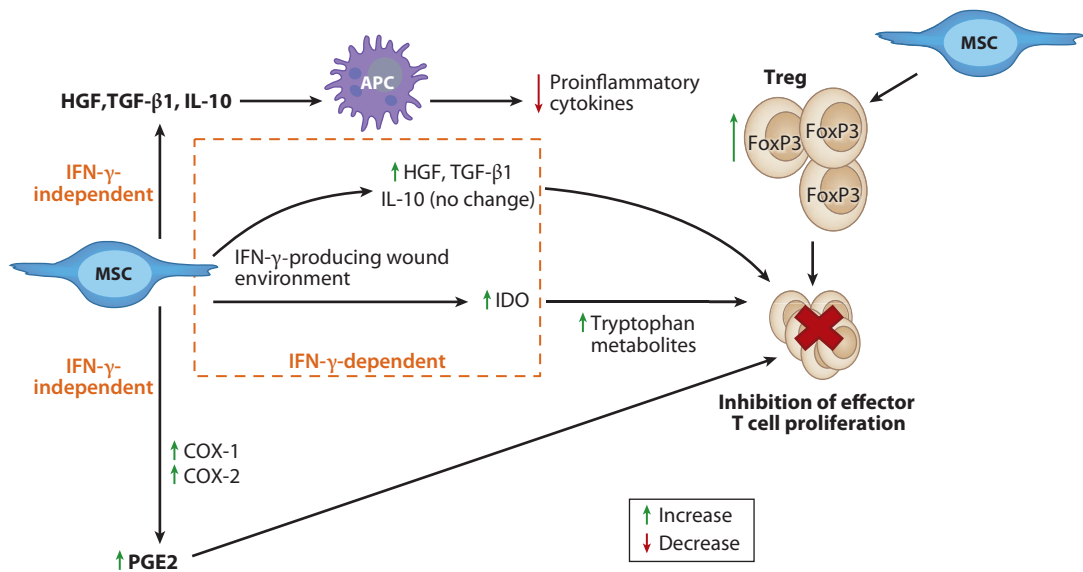


**Figure 4**

Mesenchymal stem cell (MSC) immunomodulation via nitric oxide (NO) and chemokines. MSCs are activated by coculturing with  $\alpha$ CD3-stimulated splenocytes, their supernatant, or a combination of one or more of IFN- $\gamma$ , TNF- $\alpha$ , IL-1 $\alpha$ , or IL-1 $\beta$  (presumably similar activation occurs in vivo). In the absence of coculture with T cell cytokines, MSCs do not suppress T cell proliferation. *Inducible nitric oxide synthase* (*iNOS*) messenger RNA (mRNA) is upregulated in MSCs, but neutralization of cytokines during activation of MSCs prevents *iNOS* mRNA upregulation. Activated MSCs produce large numbers of chemokines, including CXCL-9 and CXCL-10. MSCs from *iNOS*<sup>-/-</sup> or IFN- $\gamma$ RI<sup>-/-</sup> mice still attract T cells but cannot induce immune suppression of graft-versus-host disease (GVHD). Thus, in mice, NO production is required for the suppression of pathological immune response in vivo. In the absence of NO, MSC-mediated T cell recruitment enhances inflammation. The *iNOS* inhibitor N5-[imino(methylamino)methyl]-L-ornithine, citrate (L-NMMA) attenuates the capacity of mouse MSCs to be immunosuppressive: When *iNOS* is inhibited, T cell proliferation is not susceptible to suppression by MSCs, and MSCs fail to confer the protection from GVHD that is observed with the use of wild-type MSCs. Abbreviations: IFN, interferon; NK, natural killer.

ankle and from 4.0 mm to 3.0 mm in the other. Reduction in swelling was associated with decreased erythema in hind and front paws. Magnetic resonance imaging findings demonstrated reduction in inguinal lymph node size in MSC-treated mice, and bioluminescence showed localization of MSCs to the ankle joints, which were the most clinically affected joints. Our hypothesis that bone marrow MSCs are plastic and act through several different mechanisms to induce tolerance is supported by studies of adipose-derived MSCs. These studies demonstrate the induction of Tregs in cultures of RA

PBMCs and in mouse models of arthritis (20, 37). Differences in the capacity of MSCs to downregulate collagen-induced arthritis, and whether or not Tregs are induced in these studies, depend on whether mouse MSCs or hMSCs are used and whether the cells are primary isolates or MSC lines, whose function may differ from that of primary expanded MSCs (73). Studies of RA T cells performed using adipose-derived MSCs, which may differ somewhat from bone marrow-derived MSCs, support our hypothesis that MSCs can downregulate effector responses in RA T cells (74–76).



**Figure 5**

Mesenchymal stem cell (MSC) immunomodulation through prostaglandin E2 (PGE2) and indoleamine-2,3-dioxygenase (IDO) production. Both interferon- $\gamma$  (IFN- $\gamma$ )-independent pathways and IFN- $\gamma$ -dependent pathways appear to be important for immune suppression by MSCs. Mechanisms of MSC-mediated effects that appear to be independent of IFN- $\gamma$  include COX-1 and COX-2 expression and production of PGE2. PGE2 secretion decreases dramatically when MSCs are treated with indomethacin (a nonsteroidal anti-inflammatory drug that blocks PGE biosynthesis by inhibiting COX). PGE2 effects may be mediated at least in part via soluble factors. Human MSCs cultured *in vitro* constitutively express messenger RNA for hepatocyte growth factor (HGF), interleukin-10 (IL-10), and transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), all of which can be detected in MSC supernatants. IL-10 and TGF- $\beta$ 1 inhibit proinflammatory cytokine secretion by antigen presenting cells (APCs) and are involved in the induction of regulatory T cells (Tregs). HGF, a cytokine with antiapoptotic and hematopoietic activity, is a potent immunomodulator that plays a role in MSC-mediated effects. IFN- $\gamma$  secretion leads to increased HGF and TGF- $\beta$ 1 but has little effect on the level of IL-10. MSCs do not constitutively express IDO, although IFN- $\gamma$  stimulation of MSCs induces IDO expression. IDO appears to suppress T cell activity either through accumulation of tryptophan metabolites or by depletion of tryptophan, or both. Addition of 1-methyl-L-tryptophan, a chemical antagonist of IDO, restores T cell proliferation *in vitro*, which suggests that the accumulation of tryptophan metabolites such as kynurenine, rather than tryptophan depletion, is more important for MSC-mediated immunosuppressive effects.

Other investigators (22) have reported that in the collagen-induced model of arthritis, mice infused with MSCs have increased numbers of CD4<sup>+</sup>CD25<sup>+</sup> cells that express FoxP3 and therefore have a Treg phenotype. We are interested in exploring the effect of hMSCs in a model that is thought to be incited not by abnormalities in T cells per se, but rather by the unregulated secretion of TNF- $\alpha$ , as may occur in some RA and inflammatory bowel disease patients. Recent data obtained from the collagen-induced arthritis model, in which mouse MSCs did not protect against arthritis *in vivo* but did reduce T cell proliferative responses *in vitro*, also suggest that (a) we do not yet have

appropriate *in vitro* measures that reflect the potential therapeutic utility of MSCs *in vivo* and (b) mouse MSCs do not have the same immunoregulatory capacity as hMSCs (28).

### Mesenchymal Stem Cells in Mouse Models of Inflammatory Lung Disease

Mice induced to develop both acute and chronic asthma with ovalbumin (72; T. Bonfield, M. Kolozem, D. Lennon, B. Zuchowski, S. Yang & A.I. Caplan, manuscript submitted) are susceptible to immune regulation by hMSCs and show inhibition of the autoimmune and allergic phenotype, respectively. In

**Table 1** Clinical trials registered as of April 19, 2010, at <http://clinicaltrials.gov>

Sponsor	Indication	Phase	Number of patients and study identification number
<b>Completed</b>			
Osiris Therapeutics	Moderate to severe Crohn's disease	2	10, NCT00294112
Shaheed Beheshti Medical University, Iran	Improvement of liver function in liver cirrhosis patients	—	30, NCT00420134
Osiris Therapeutics	Acute GVHD	2	33, NCT00136903
Osiris Therapeutics	Treatment-resistant moderate to severe Crohn's disease	3	200, NCT00543374
Osiris Therapeutics	Steroid treatment-refractory GVHD	3	240, NCT00366145
University of Tehran, Iran	Homozygous familial hypocholesterolemia	1	1, NCT00515307
Osiris Therapeutics	MSC intra-articular injection following meniscectomy	1-2	60, NCT00225095
Translational Research Informatics Center Kobe/Hyogo, Japan	Adult periodontitis	1-2	10, NCT00221130
St. Jude Children's Research Hospital	Severe osteogenesis imperfecta by allogeneic bone marrow transplantation	1	9, NCT00705120
Osiris Therapeutics	Treatment-refractory acute GVHD	2	30, NCT00284986
St. Jude Children's Research Hospital	Marrow mesenchymal cell therapy for osteogenesis imperfecta (pilot study)	?	14, NCT00187018
Rigshospitalet, Denmark	Vasculogenesis in patients with severe myocardial ischemia	1-2	40, NCT00260338
Cairo University, Egypt	Chronic spinal cord injury—autologous bone marrow transplant	1-2	80, NCT00816803
Ruhr University of Bochum, Germany	Diabetic patients with critical limb ischemia	2	30, NCT01065337
St. Jude Children's Research Hospital	Stromal therapy of osteodysplasia after allogeneic bone marrow transplantation	1	8, NCT00186914
<b>Active, closed to enrollment</b>			
Case Comprehensive Cancer Center	Acute or chronic GVHD after donor stem cell transplant	1	49, NCT00361049
Medipost Co., Ltd., United Kingdom	Promote engraftment of unrelated hematopoietic stem cell transplantation	1-2	10, NCT00823316
Osiris Therapeutics	Chronic obstructive pulmonary disease	2	60, NCT00683722
Osiris Therapeutics	Chondrogen intra-articular injection following meniscectomy	1-2	50, NCT00702741
Osiris Therapeutics	Acute myocardial infarction	1	48, NCT00114452
Osiris Therapeutics	Treatment-resistant moderate to severe Crohn's disease	3	270, NCT00482092
Stempeutics Research, Pvt. Ltd., Malaysia	Critical limb ischemia	1-2	20, NCT00883870
Stempeutics Research, Pvt. Ltd., Malaysia	Acute myocardial infarction	1-2	20, NCT00883727
Sun Yat-sen University, China	MSCs via hepatic artery for liver cirrhosis	2	50, NCT00976287
Sun Yat-sen University, China	MSCs via portal vein for liver cirrhosis	2	60, NCT00993941

(Continued)

**Table 1 (Continued)**

Sponsor	Indication	Phase	Number of patients and study identification number
Cambridge University, United Kingdom	Multiple sclerosis	1–2	10, NCT00395200
Osiris Therapeutics	Treatment of recently diagnosed type 1 diabetes mellitus	2	60, NCT00690066
<b>Active, recruiting</b>			
Third Military Medical University, China	Diabetic foot disease	2–3	80, NCT00955669
Juan Jesús Bandera, Fundacion Progreso y Salud, Spain	Secondary progressive multiple sclerosis	1–2	30, NCT01056471
Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School, China	Primary Sjögren’s syndrome	1–2	20, NCT00953485
University of Liège, Belgium	Prevention for graft rejection and GVHD	2	30, NCT00504803
Digestive Disease Research Center, Medical Sciences and University of Tehran, Iran	Decompensated cirrhosis	2	50, NCT00476060
The Children’s Hospital of Philadelphia	Osteogenesis imperfecta	1	12, NCT01061099
Cell and Tissue Therapies, Australia	Crohn’s disease	2	20, NCT01090817
Christian Medical College, India	GVHD	1–2	25, NCT00314483
Nantes University Hospital, France	MSC genome	Observational	25, NCT00836732
University of Liège, Belgium	GVHD or poor graft function	2	100, NCT00603330
Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School, China	Systemic sclerosis	1–2	20, NCT00962923
Fuzhou General Hospital, China	Type 1 diabetes mellitus	1–2	30, NCT00646724
Fundación Progreso y Salud, Spain	Limb ischemia in diabetes mellitus	1–2	36, NCT01079403
University Hospital of Salamanca, Spain	GVHD	1–2	15, NCT00447460
University Hospital Dresden, Germany	Osteoarthritis	Observational	30, NCT01038596
Royan Institute, Iran	MSC collagen scaffold in full-thickness articular cartilage	1	6, NCT00850187
Angioblast Systems	Heart failure	2	60, NCT00721045
Ullevaal University Hospital, Norway	Articular cartilage defects	1	50, NCT00885729
University Hospital Toulouse, France	Myocardial infarction	1–2	10, NCT01076920
Hadassah Medical Organization, Israel	Distal tibial fractures	1–2	24, NCT00250302
Yonsei University, Korea	Multisystem atrophy	2	Not specified, NCT00911365
Hadassah University Hospital, Israel	Amyotrophic lateral sclerosis	1–2	24, NCT01051882
University of Miami	Cardiac surgery	1–2	45, NCT01051882
Nanjing University Medical College–Affiliated Drum Tower Hospital, China	Refractory systemic lupus erythematosus	1–2a	20, NCT00698191

(Continued)

**Table 1 (Continued)**

Sponsor	Indication	Phase	Number of patients and study identification number
National Heart, Lung and Blood Institute; University of Miami	Percutaneous stem cell injection delivery effects on neomyogenesis (pilot study)	1–2	30, NCT01087996
Fuzhou General Hospital, China	Recipients of living kidney allografts	Not specified	Not specified, NCT00658073
Osiris Therapeutics	Acute GVHD, expanded access	—	NCT00826046
Fuzhou General Hospital, China	Combination with hyperbaric oxygen therapy in type 2 diabetes mellitus	1–2	100, NCT00767260
University of Miami, Miller School of Medicine	Ischemic heart failure trial	1–2	60, NCT00768066
Children’s Health System, Alabama	Nonmalignant red blood cell disorders	2	35, NCT00957931
The Ohio State University	Thermal burn	Not specified	50, NCT00609661
Osiris Therapeutics	Acute myocardial infarction	2	220, NCT00877903
The Third Affiliated Hospital of Sun Yat-sen University, China	Liver failure	1–2	92, NCT00956891
Jaslok Hospital and Research Center, India	Parkinson’s disease	Not specified	5, NCT00976430
Universitair Medisch Centrum Utrecht, Netherlands	GVHD	1–2	10, NCT00827398
Osiris Therapeutics	Pediatric patients who have failed to respond to steroid treatment for acute GVHD	Expanded access	Not specified, NCT00759018
National Institutes of Health Clinical Center	Marrow collection for MSCs	Observational	250, NCT01071577
Medipost Co., Ltd., Korea	Microfracture in patients with knee articular cartilage injury or defect	3	104, NCT01041001
Helsinki University Hospital, Finland	Coronary artery bypass graft and MSCs in congestive heart failure	2	60, NCT00418418
Masonic Cancer Center, University of Minnesota	Epidermolysis bullosa	2	75, NCT01033552
Kanazawa University, Japan; Cyton Biosciences, Ltd., United Kingdom	Liver regeneration therapy using autologous adipose tissue–derived stromal cells	Not specified	9, NCT00913289
Emory University	Effects of thiazolidinediones on human bone marrow stromal cell differentiation capacity in vitro and in vivo (pilot study)	Not specified	12, NCT00927355
TCA Cellular Therapy, LLC	Severe coronary ischemia	2	60, NCT00790764
Cardio 3 BioSciences	Heart failure secondary to ischemic cardiomyopathy	2–3	240, NCT00810238
Kanazawa University, Japan	Liver regeneration	Not specified	4, NCT01062750
Pluristem Therapeutics, Inc., Israel	PLX-PAD cells for the treatment of critical limb ischemia	1	12, NCT00951210
Pluristem Therapeutics, Inc., Israel	PLX-PAD cells for the treatment of critical limb ischemia	—	15, NCT00919958

(Continued)

**Table 1 (Continued)**

Sponsor	Indication	Phase	Number of patients and study identification number
Orthofix, Inc.	Novel allogeneic, cancellous, bone matrix containing viable stem cells (Trinity Evolution Matrix) in subjects undergoing tibiotalar arthrodesis with or without hind foot fusion	—	100, NCT00988338
Angioblast Systems	NeoFuse in subjects requiring posterolateral lumbar fusion	1–2	24, NCT00810212
University Hospital, France	Grafting for open tibial shaft fracture	Not specified	186, NCT00512434
Mesoblast, Ltd., Australia	Subjects six weeks after an anterior cruciate ligament reconstruction	1–2	24, NCT01088191
Hospital Universitario Reina Sofia, Spain	Idiopathic dilated cardiomyopathy	2	30, NCT00629096
Cairo University School of Medicine, Egypt	Cartilage defects	2	Not specified, NCT00891501
<b>Not yet recruiting</b>			
Leiden University Medical Center, Netherlands	Subclinical rejection	1–2	15, NCT00734396
Angioblast Systems	Myocardial infarction	1–2	25, NCT00555828
The Heart Center, Rigshospitalet, Denmark	Heart failure	—	60, NCT00644410
University Hospital Grenoble, France	Ischemic stroke	2	30, NCT00875654
Fuzhou General Hospital, China	Chronic allograft nephropathy	1–2	20, NCT00659620
Guangdong General Hospital, Guangdong Academy of Medical Sciences, China	Chronic GVHD	2	52, NCT00972660
Fuzhou General Hospital, China	Osteonecrosis femoral head	1–2	30, NCT00813267
Fuzhou General Hospital, China	Lupus nephritis	1–2	20, NCT00659217
Cleveland Clinic, Mellen Center and University Hospitals (cosponsor)	Multiple sclerosis	1	24, NCT00813969
Universitair Ziekenhuis, Belgium	Coinfusion with umbilical cord transplant	1–2	20, NCT01092026
Mario Negri Institute for Pharmacological Research, Italy	Under basiliximab/low-dose RATG to induce renal transplant tolerance	1–2	6, NCT00752479
M.D. Anderson Cancer Center, University of Texas	Cord blood expansion on MSCs	1	100, NCT00498316
National Heart, Lung, and Blood Institute; Mount Sinai School of Medicine; Angioblast Systems	Effect of intramyocardial injection of mesenchymal precursor cells on myocardial function in LVAD bridge to transplant patients	2	80, NCT00927784
University of Liège, Belgium	MSC and HSC coinfusion in mismatched minitransplants	2	120, NCT01045382
Hadassah Medical Organization, Israel	GVHD	1–2	20, NCT00749164

(Continued)

**Table 1 (Continued)**

Sponsor	Indication	Phase	Number of patients and study identification number
Department of Orthopaedic Surgery, Emory University	Bone void defects in patients with benign bone lesions	2–3	50, NCT00851162
Stempeutics Research, Pvt. Ltd., Malaysia	Ischemic cerebral stroke	1–2	78, NCT01091701
Institut National de la Santé et de la Recherche Médicale, France	Postprostatectomy erectile dysfunction	1–2	31, NCT01089387
Ljubljana University Medical Center, Slovenia	Dilated cardiomyopathy	2	50, NCT00629018
University of California, Irvine	After stroke	2	33, NCT00908856
Veterans Affairs Medical Center, Washington, DC	Lipoaspirate injection in the treatment of diabetic lower-extremity wounds and venous stasis ulcers	Not specified	250, NCT00815217

Abbreviations: GVHD, graft-versus-host disease; HSC, hematopoietic stem cell; LVAD, left ventricular assist device; MSC, mesenchymal stem cell.

these studies, the investigators used hMSCs to treat ovalbumin-induced, immune-mediated lung injury in immunocompetent mice; the mice were cured with one exogenously infused dose of hMSCs given at  $1 \times 10^6$  per mouse (T. Bonfield & A.I. Caplan, personal communication). Serum assays show reduced IFN- $\gamma$  levels in bronchoalveolar lavage fluid following hMSC treatment of mice in which ovalbumin was used to induce immune injury in the lungs. Goblet cell hypertrophy and the severity of the disease were significantly reduced following treatment with hMSCs. In addition, these studies documented the lack of a major immune response to hMSCs in the absence of asthmatic lung inflammation. Furthermore, systemic IFN- $\gamma$  induction in this asthma model was significantly suppressed following treatment with hMSCs, which suggests that systemic immune modulation took place.

## HUMAN STUDIES

The first therapeutic uses of allogeneic MSC transplants were in osteogenesis imperfecta and in breast cancer, in which MSCs were used to increase engraftment following

transplantation with autologous peripheral-blood progenitor cell grafts (77, 78). These experiments were followed by studies of patients with inborn errors of metabolism, such as metachromatic leukodystrophy and Hurler syndrome, who received allogeneic MSCs without adverse effects and showed some improvement in nerve-conduction velocity but not in overall clinical status (79, 80). Studies of human leukocyte antigen–matched siblings undergoing bone marrow transplant demonstrate the potential utility of MSC therapy in GVHD (80).

Evidence that MSCs may be dysfunctional in disease is controversial. For example, MSCs are not abnormal in children with aplastic anemia, but they may be impaired in patients with idiopathic thrombocytopenia and in systemic lupus erythematosus (81–84). At least two reports (82, 83) suggest that abnormal osteogenic potential contributes to osteoporosis and that MSC growth, proliferative response, and osteogenic differentiation may be affected in this disease.

Provided in **Table 1** is a summary of clinical trials that are completed, active, or waiting for activation. The trials are primarily



interventional, although some aim to discern how to measure any observed effects of MSCs. Occasional trials are primarily observational and are focused on increasing knowledge of the mechanisms utilized by MSCs.

hMSCs are cultured primarily from bone marrow. However, an increasing number of publications suggest that adipose-derived hMSCs may also exert an immunoregulatory function (37, 38, 56, 61, 62, 85–87). How these MSCs differ from bone marrow–derived MSCs in terms of their pharmacokinetics, homing, and repair capacity and potency remains largely unexplored (88, 89). Also, there are a few reports of isolation of gingiva-derived MSCs whose functional effects are similar to or more potent than MSCs from bone marrow (90, 91). Immune suppression by human umbilical cord MSCs have also been reported in vivo in mouse and rat models of stroke, degenerative neurological disease, and bleomycin-induced lung disease (92–97).

## CONCLUSIONS

In this review, we highlight known mechanisms that may mediate the effects of MSCs within the immune system. We also attempt to identify gaps in our knowledge as to when cell-cell contact is necessary for hMSC-mediated effects on the immune system, and we highlight potential differences between hMSCs and mouse MSCs, as well as MSCs derived from different tissue sources. The clinical trials utilizing hMSCs (**Table 1**), although interventional, may not provide mechanistic information even if desirable effects are observed. Challenges over the coming decade will be to define appropriate disease targets for MSC-based therapy, develop potency assays for MSCs from different sources, elucidate the many actions of MSCs in vivo, and develop in vitro readouts to positively predict the effects of MSCs in vivo. hMSCs will be approved for clinical use long before we have a precise picture of the mechanisms that govern their efficacy.

### SUMMARY POINTS

1. hMSCs appear to have potent immune regulatory actions that make them attractive for use in human diseases in which tissue injury and/or inflammation occurs.
2. The precise mechanisms utilized by MSCs probably depend on the environment and the nature of the repair process required.
3. hMSCs alleviate GVHD without any observed adverse effects, making them attractive therapeutic agents in a variety of diseases.
4. The hypothesis that one important mechanism of action of hMSCs is to induce human Tregs appears plausible.
5. IDO metabolites and tryptophan depletion may play a role in hMSC suppression of T cell proliferation, but the precise mechanism(s) of how this process occurs requires further study.

### FUTURE ISSUES

1. The range of diseases in which hMSCs could be therapeutic needs to be fully defined.
2. The range of sources from which therapeutic hMSCs can be isolated remains to be fully defined.

3. In vitro correlates that reflect the in vivo effects of hMSCs must be defined in terms of both predicting the effect of hMSCs in vivo and assessing potency between hMSC isolates.
4. The best route and number of hMSC infusions for disease-specific indications remain to be defined.

## DISCLOSURE STATEMENT

A.I.C. is a founder of Osiris Therapeutics, Inc. (OTI). Case Western Reserve University receives royalties from OTI that are distributed to A.I.C. N.G.S. is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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