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# Concise Review: Exploring Immunomodulatory Features of Mesenchymal Stromal Cells in Humanized Mouse Models

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**Key Words.** Humanized mouse • Mesenchymal stem cells • Cell therapy • Immunosuppression • Immunomodulation • Human immune system • Graft versus host disease

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## ABSTRACT

**With their immunosuppressive features, human mesenchymal stromal cells (MSCs), sometimes also termed as mesenchymal stem cells, hold great potential as a cell-based therapy for various immune-mediated diseases. Indeed, MSCs have already been approved as a treatment for graft versus host disease. However, contradictory data from clinical trials and lack of conclusive proof of efficacy hinder the progress toward wider clinical use of MSCs and highlight the need for more relevant disease models. Humanized mice are increasingly used as models to study immune-mediated disease, as they simulate human immunobiology more closely than conventional murine models. With further advances in their resemblance to human immunobiology, it is very likely that humanized mice will be used more commonly as models to investigate MSCs with regard to their therapeutic safety and their immunomodulatory effect and its underlying mechanisms. Recent studies that explore the immunosuppressive features of MSCs in humanized mouse models will be discussed in this review.** STEM CELLS *2019;37:298–305*

## SIGNIFICANCE STATEMENT

The immunosuppressive features of mesenchymal stromal cells (MSCs) have been widely demonstrated. However, a more widespread clinical use of MSCs is hampered by contradictory data from clinical trials and an incomplete understanding of the underlying mechanism by which MSCs exert this immunosuppression, resulting in inconclusive proof efficacy. The present review discusses humanized mice as a tool to develop a better understanding of the mode of action of MSCs in mitigating the immune response in an in vivo environment that closely resembles human immunobiology. The goal is that a greater understanding may enable and encourage more studies using humanized mice to investigate the immunomodulatory features of MSCs.

## INTRODUCTION

### Mesenchymal Stromal Cells

Mesenchymal stromal cells (MSCs) are of mesodermal origin and have self-renewal and multipotent differentiation capacity. They give rise to adipocytes, osteocytes, and chondrocytes and can be derived from various origins, such as the bone marrow (BM), dental pulp, umbilical cord blood (UCB), placenta, and adipose tissue [2]. MSCs have been shown to have regenerative potential and contribute to tissue repair [3]. Besides a somatic origin, MSCs can also be differentiated from pluripotent stem cells (PSCs), including embryonic stem cells and induced PSCs (iPSCs), potentially providing an unlimited source of cells for therapeutic use (reviewed in [4]). MSCs have stimulated great interest because of their immunomodulatory and anti-inflammatory properties (Fig. 1). Specifically, MSCs have been shown to inhibit effector T-cell proliferation, drive induction of regulatory T (Treg) cells [5–7], induce macrophage transformation to an M2 anti-inflammatory phenotype [8], modulate dendritic cell (DC) maturation and functional properties [9–11], directly affect B cell proliferation and maturation [12], and impair natural killer T-cell proliferation [13]. To make MSCs more effective as a cellular therapy, it is important to determine the mechanism(s) by which they exert their immunomodulatory effects. Although much remains unknown, the consensus is that MSCs act through cell-to-cell contact as well as soluble factors, either produced constitutively by MSCs or released by target cells induced by crosstalk with MSCs (reviewed in [1]). Numerous soluble factors have been

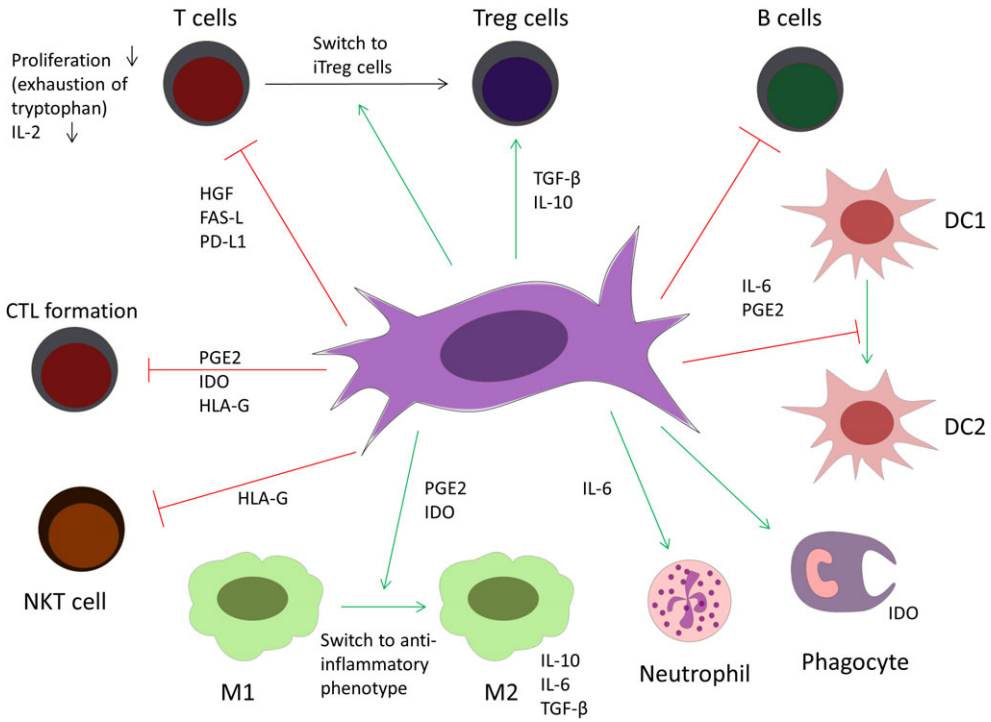



**Figure 1.** Current understanding of immunomodulatory mechanisms of mesenchymal stromal cells (MSCs). MSCs exert their immunomodulatory function through cell-to-cell contact as well as soluble factors, either produced constitutively by MSCs or released by target cells induced by crosstalk with MSCs. MSCs can inhibit the proliferation and function of T cells, NKT cells, B cells, and DCs. MSCs also preserve neutrophils viability, drive Treg cell expansion, inhibit the formation of CTL, induce IDO production in phagocytes, and induce differentiation toward macrophage M2 anti-inflammatory phenotype. Several soluble factors have been shown to play a role in the immunomodulatory effects of MSCs, including PGE2, TGF-β, IDO, nitric oxide, HGF, FAS-L, PD-L1, HLA-G, IL-6, and IL-10. Green arrow depicts stimulatory effect and red flat ended arrow depicts inhibitory effect (adapted from [1]). Abbreviations: CTL, cytotoxic T lymphocytes; DC, dendritic cell; FAS-L, FAS ligand; HGF, hepatocyte growth factor; HLA-G, human leucocyte antigen-G; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; PD-L1, programmed death-ligand 1; PGE2, prostaglandin E2; NKT, natural killer T; TGF-β, transforming growth factor-β; Treg, regulatory T.

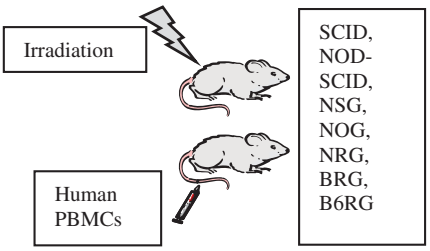
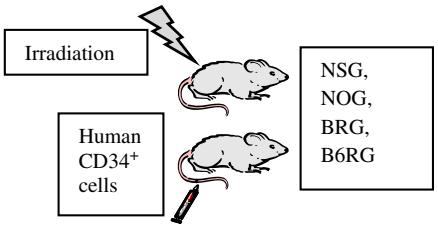
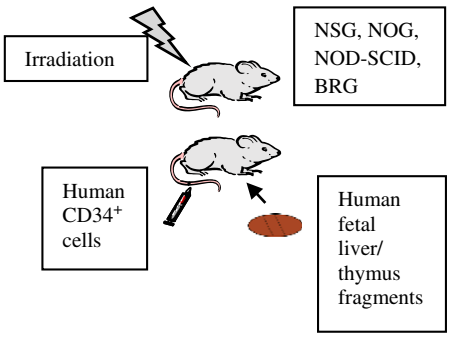
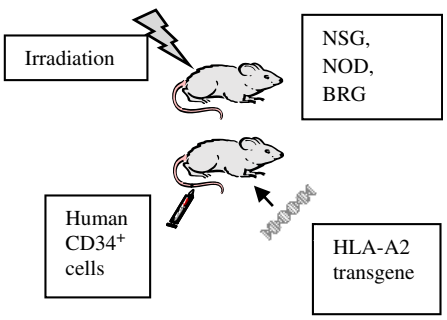
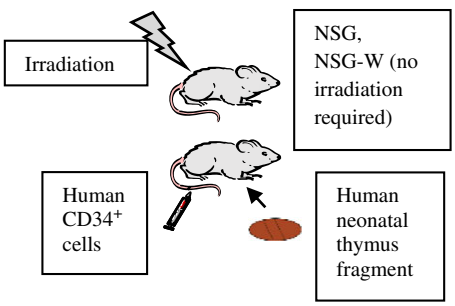
associated with MSC-mediated immunomodulation, such as indoleamine 2,3-dioxygenase [14, 15], hepatocyte growth factor, transforming growth factor-β [5], prostaglandin E-2 [16–18], interleukin (IL)-10 [19, 20], IL-6 [11], and monocyte chemoattractant protein-1 [6]. In addition, it has also been suggested that cell-to-cell contact between MSCs and T cells is necessary for MSCs to display their inhibitory effect on T-cell proliferation, cytotoxicity, and the number of antigen-specific T cells [21].

Although the mechanism by which MSCs mediate immunosuppression is still incompletely understood, multiple clinical trials have been initiated, in which adult MSCs have been used as a therapy to treat immune-mediated diseases such as graft versus host disease (GvHD), aplastic anemia, multiple sclerosis, rheumatoid arthritis, and Crohn's disease (www.clinicaltrials.gov) [1]. In Canada and New Zealand, clinical trials have resulted in the conditional approval of MSCs for the treatment of steroid-resistant and/or immunosuppressant-resistant acute GvHD (aGvHD) in pediatric patients [22–27]. However, it is not only somatic MSCs that are the subject of current clinical trials. Intriguingly, Cynata Therapeutics Limited is running a first-of-its-kind clinical trial using iPSC-derived MSCs for the treatment of GvHD (http://www.prnewswire.com/news-releases/uk-regulatory-authority-approves-cynata-gvhd-clinical-trial-300329939.html). Despite this interest in MSCs for clinical application, both in vivo studies and potentially contradictory data from clinical trials, have failed to show conclusive proof of efficacy [24, 25], and this may yet hinder the progression of this cell therapy to later clinical phases. There remains both a requirement for better MSC potency assay methods and more comprehensive immune monitoring of treated patients to further understand the mode of action of these cells in vivo [24, 28]. Better models to investigate the mechanisms underlying the immunosuppressive function of MSCs may facilitate the clinical application of MSCs, and humanized mice represent one such improved model. In this review, we introduce and summarize the findings of recent studies that have used the humanized mouse model to explore the immunomodulatory properties of MSCs.

## Humanized Mouse Models

In general, a humanized mouse is a murine model with a human component. However, most of the models relevant to the study of human immune responses use immunocompromised mice in which the immune system has been reconstituted with human immune cells/immune system (Table 1). Over recent years, increasingly elaborate immunocompromised strains have been developed to achieve higher engraftment rates of human cells. A detailed description of the development of humanized mouse models is beyond the scope of this review

**Table 1.** Summary of current humanized mouse models with schematic presentation of the generation

| Model | Generation/mice | Advantages | Disadvantages |
|---|---|---|---|
| Hu-PBMC |  | • Easy to generate<br>• Engraftment of T cells | • No multilineage hematopoiesis<br>• No primary immune response<br>• Development of GvHD within a few weeks<br>• Allows only for short-time experiments |
| Hu-CD34$^{+}$ |  | • Easy to generate<br>• Multilineage hematopoiesis<br>• Primary immune response | • T-cell education on murine MHC molecules<br>• Murine MHC-restricted T cells may enter into complex immune interactions with human APCs |
| BLT |  | • Multilineage hematopoiesis<br>• Primary immune response<br>• T-cell education in autologous human thymus<br>• Maintenance of naïve, central memory, and effector memory T cells | • Challenging to generate<br>• Requires human fetal tissue<br>• Development of late-onset GvHD<br>• Inadequate reconstitution of the innate immune system |
| Hu-HLA-A2*tg*-CD34$^{+}$ |  | • Multilineage hematopoiesis<br>• Primary immune response<br>• T-cell education on human MHC because of transgenic expression of HLA-A2 molecules<br>• Development of HLA-A2-restricted and antigen-specific cytotoxic T cells<br>• Production of all human Ig classes | • No T-cell education on human MHC class II molecules<br>• No transgenic expression of other human antigens<br>• Inefficient production of antigen-specific IgG |
| NeoThy |  | • Advantages of the BLT also apply to NeoThy<br>• More thymus tissue available allows for ~50-fold more mice per donor compared to BLT<br>• Neonatal tissue is developmentally more mature<br>• Does not require human fetal tissue | • Challenging to generate<br>• Requires human neonatal tissue<br>• Potentially susceptible to GvHD |

Abbreviations: APC, antigen-presenting cells; BLT, bone marrow liver thymus; BRG, BALB/c-*Rag2*$^{null}$*IL2rγ*$^{null}$; B6RG, C57BL/6-*Rag2*$^{null}$*IL2rγ*$^{null}$; GvHD, graft versus host disease; HLA, human leucocyte antigen; Hu, humanized; Ig, immunoglobulin; MHC, major histocompatibility complex; NBSGW (referred to as NSG-W), NOD,B6.SCID *IL2rγ*$^{null}$*Kit*$^{W41/W41}$; NOD, nonobese diabetic; NOG, NODShi.Cg-*Prkdc*$^{scid}$*IL2rγ*$^{tm1Sug}$; NRG, NOD-*Rag1*$^{null}$*IL2rγ*$^{null}$; NSG, NOD/SCID/*IL2rγ*$^{null}$; PBMCs, peripheral blood mononuclear cells; SCID, severe combined immunodeficiency.

and has been reviewed elsewhere (reviewed in [29–32]). In brief, one of the first immunodeficient strains to be developed used the severe combined immunodeficiency (SCID) mouse, showing deficiency for immune functions mediated by T and B lymphocytes [33]. Similarly, models of immunodeficiency were also generated based on the use of *recombination activating*

*gene* (*Rag*) *1* or *2* knockouts [34]. The next generation of immunocompromised mice, including, for example, the nonobese diabetic (NOD)/SCID/*IL2rγ*$^{null}$ (NSG), NODShi.Cg-*Prkdc*$^{scid}$*IL2rγ*$^{tm1Sug}$, BALB/c-*Rag2*$^{null}$*IL2rγ*$^{null}$, C57BL/6-*Rag2*$^{null}$*IL2rγ*$^{null}$, and NOD-*Rag1*$^{null}$*IL2rγ*$^{null}$ models, were generated by introducing multiple genetic manipulations resulting in a multidysfunctional immune system [29, 32, 35]. To subsequently "humanize" immunocompromised mice, human immune cells, such as peripheral blood mononuclear cells (PBMCs) are injected, resulting in engraftment of human T cells [36]. Immunodeficient mice may also be reconstituted with human CD34$^+$ cells, resulting in development of human B cells, T cells, monocytes, and DCs [37]. However, both these models are limited by the education and selection of human T cells in the murine host thymus. The BM liver thymus (BLT) mouse, conversely, allows for T-cell selection on human major histocompatibility complex (MHC). To generate a BLT mouse, human fetal thymus and liver fragments are implanted under the murine kidney capsule, followed by injection of CD34$^+$ cells, derived from the same fetal liver, which allows for T-cell selection in the implanted autologous human thymus [38]. To circumvent the need to use fetal human tissue, Brown et al. recently developed the NeoThy humanized mouse, in which human neonatal thymus and human CD34$^+$ cells are engrafted into immunocompromised mice [39]. Another strategy for T-cell education on human MHC molecules is based on the use of genetically modified humanized mice that express human leucocyte antigen (HLA) molecules. After reconstitution with human CD34$^+$, these mice show development of HLA-A2-restricted and antigen-specific cytotoxic T cells [40].

Humanized mice have been used in the investigation of many clinical indications, including human-specific viral infections, tumor immunology, transplantation, and autoimmunity (reviewed in [30, 41–43]). In the fields of transplantation and immune-mediated conditions, despite the availability of various animal models, results do not always translate to clinical efficacy [43]. This is highlighted by the incomplete mimicry of disease phenotypes by conventional mouse models. For instance, classic mouse models that have been transplanted with thymus fragments from myasthenia gravis (MG) patients do not reproduce clinical weakness, whereas a humanized mouse model transplanted with MG thymus resulted in MG-like symptoms [44]. Equally, a humanized mouse model of pulmonary fibrosis exhibited a more severe disease phenotype than nonhumanized murine models, as a direct result of human immune cells being present in the lungs [45]. The availability of humanized mouse models has enabled a mechanistic investigation of human immune responses and also a more relevant testing approach to therapeutic intervention.

## Results and Discussion

### Humanized Mice as Models to Study the Immunomodulatory Effects of MSCs

Although in vitro studies have successfully provided important insight into the immunomodulatory features of MSCs [5, 8–13], the in vitro environment cannot fully reflect the complexity of a human immune response. In addition, although murine models have been used for investigating the immunosuppressive properties of MSCs in vivo [46, 47], there remain fundamental differences between the human and murine immune systems. For example, humans and mice differ in their expression of MHC, cytokines, and costimulatory molecules [48], and more specifically, human MSCs differ in their immunomodulatory mechanisms compared with murine MSCs [49]. These differences highlight the urgent need for models that resemble the human milieu more closely, particularly in the field of immune-mediated diseases. To meet this need, humanized mice are increasingly being used as a tool with which to test the safety and efficacy of a range of therapeutic strategies [30]. We present here the application of these models to the evaluation of the therapeutic potential of MSCs.

### Clinical Safety and Efficacy of MSCs

Prior to the acceptance of MSCs as a viable therapeutic option, there is a requirement to demonstrate whether these cells show efficacy and are safe for clinical use. This safety evaluation would comprise the usual regulatory requirements for any cell therapy, provided by National Regulatory Authorities (https://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/; http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000405.jsp&mid=WC0b01ac058002958a). One of the key considerations is the immune response of the recipient to the graft. MSCs that have been used in the clinic are often of allogeneic origin and therefore retain the potential to elicit an immune response in the recipient. Although MSCs have been suggested to be an immune-privileged cell population, there are some reports that indicate that allogeneic MSCs are immunogenic [50]. In this context, humanized mice are a useful tool for assessing the immunogenicity and safety of MSCs. Furthermore, MSCs derived from different donors/origins have also been shown to vary in their efficacy [25, 51, 52]. If this is true, then it is clearly important to establish the most appropriate source of MSCs for clinical application, and humanized mice represent an attractive model in which the efficacy of different MSC types can be compared.

Lee et al. have used the NSG mouse model reconstituted with human CD34$^+$ cells (NSG-CD34$^+$) to investigate the immunological safety of allogeneic human MSCs [53]. As MHC molecules are the main mediators of an allogeneic immune response, the expression levels of MHC represent a key component in the potential immunogenicity of a cell. The authors demonstrated that MSCs derived from UCB did not express MHC class II in vitro. Furthermore, coculture with PBMCs induced expression of HLA-G in UCB-MSCs, which is associated with immune tolerance. To confirm these results in vivo, T-cell proliferation and proinflammatory cytokines were quantified in response to the injection of UCB-MSCs versus PBMCs into NSG-CD34$^+$ mice. UCB-MSCs resulted in lower T-cell proliferation and reduced interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and immunoglobulin G (IgG) production, suggesting that allogeneic human UCB-MSCs exhibit reduced immunogenicity.

Another related study compared levels of MHC class II expression between different MSC populations, including MSCs derived from iPSCs, fetuses (fMSCs) and adult BM [54]. No MHC class II expression was detected in any of these MSC populations in the absence of IFN-γ. IFN-γ is a proinflammatory cytokine, naturally present in sites of inflammation and therefore often used to recapitulate an in vivo inflammatory environment. When MSCs were stimulated with IFN-γ, MHC

class II molecules were expressed at minimal levels in iPSC-MSCs as opposed to higher levels in fMSCs and BM-MSCs, which suggests that iPSC-MSCs exhibit low levels of immunogenicity. To test whether iPSC-MSCs are not only safe but also show efficacy in vivo, the authors of this study compared the repair efficacy, survival-rate after transplantation, and effect on inflammation between iPSC-MSCs and BM-MSCs in a humanized NSG-PBMC model of hind limb ischemia [54]. The levels of inflammation in the ischemic limbs were determined by quantifying $CD45^+$ and $CD4^+$ cells in the muscle tissue of the ischemic limbs. In order to trace the cells post-translation, MSCs were labeled with green fluorescent protein (GFP) before being injected intramuscularly into four sites of the thigh of the ischemic hind limbs. The results suggested that human iPSC-MSCs led to less inflammation and a better recovery of hind limb ischemia compared with BM-MSCs. Moreover, quantifying $GFP^+$-MSCs revealed that iPSC-MSCs exhibited higher cell survival than BM-MSCs.

Taken together, these studies show that the humanized mouse can be used as an in vivo model to evaluate the immunological safety of MSCs [53, 54]. Moreover, when used as a specific disease model, such as a model of hind limb ischemia, it allows for an assessment of the efficacy of MSCs as a regenerative therapy [54]. It should be mentioned, however, that Lee et al. recognized the limitations of the NSG-$CD34^+$ model, as there was only a small proportion of mature T cells present after reconstitution. They contributed the prevalence of immature T cells to abnormal thymic selection and therefore emphasized the need for confirmation of their results in a more relevant humanized mouse model, such as the BLT mouse [53].

## MSC Therapy as a Treatment in GvHD

Although MSCs have received approval for the indication of aGvHD in some countries [22–27], the data from clinical trials have been ambiguous and have failed to show conclusive proof of efficacy [24, 25]. In addition, it has proven to be challenging to determine the mode of action by which MSCs exercise their immunomodulatory phenotype. To further investigate MSCs as a cellular therapy in GvHD, Tobin et al. administered human MSCs into NSG-PBMC humanized mice, which served as model of GvHD [55]. MSC treatment resulted in the reduction of liver and gut pathology and significantly increased survival of GvHD NSG mice. However, the administration of MSCs did not prevent GvHD development in the longer term, corresponding with data from clinical trials [26, 27]. Furthermore, the study suggests that MSCs exert a direct suppressive effect on donor T-cell proliferation and reduced TNF-α production as the underlying mechanism of MSC immunosuppression in GvHD [55]. Another study that used UCB-MSCs to ameliorate GvHD in NOD/SCID mice (reconstituted with human PBMCs) suggested that multiple doses of UCB-MSC were necessary to prevent the development of GvHD, but MSCs were not effective once GvHD had been fully established [56]. This is in accordance with the conclusion by the authors of the first study, proposing that MSCs mediate a more transient mitigating effect on GvHD development, rather than induction of immune tolerance [55]. Given that these findings are in good agreement with the data from some recent clinical trials, the authors reasonably concluded that the NSG-PBMC GvHD mouse serves as a suitable model to explore the underlying mechanism of MSC immunosuppression, and the potential of MSCs as cellular-based therapy in GvHD. Further reflecting the sometimes-conflicting data from clinical trials, other studies utilizing the humanized NSG-PBMC model to mimic GvHD have found that MSCs are not effective in preventing GvHD, even if MSCs were administered in multiple doses [57, 58]. Although humanized mice offer the advantage of enabling human MSCs and MSC-derived soluble factors to interact with human immune cells, GvHD remains a complex disease. Different sources of MSCs, different routes of administration and doses, as well as variability in patient responsiveness to MSC treatment means that, in addition to more relevant disease models, further efforts to standardize therapeutic approaches will be required to improve the outcomes of studies/clinical trials on GvHD.

## MSCs in Transplant Rejection

Traditionally, patients who experience allograft rejection following organ transplantation receive immunosuppressive agents, which can lead to severe side effects, such as the development of opportunistic infections [59]. In light of this, alternative immunosuppressive approaches with less severe adverse effects are being investigated. The administration of MSCs in kidney-transplant patients has been promising in this regard, as it resulted in lower incidence of acute rejection, decreased risk of opportunistic infection, and better graft function [60]. Currently, BM-MSCs are the most commonly used type of MSCs in the clinic [22, 26]; however, BM aspiration is an invasive procedure and so other sources of MSCs, that can be harvested more conveniently and have comparable immunomodulatory efficacy, may represent an alternative to BM-MSCs. Roemeling-van Rhijn et al. compared the immunomodulatory efficacy of BM-MSC and adipose tissue-derived MSCs in the context of a humanized SCID-PBMC mouse, which was engrafted with a human allogeneic skin graft [61]. The skin grafts showed pronounced $CD45^+$ T-cell infiltrates consisting of $CD4^+$ and $CD8^+$ T cells and increased IFN-γ expression, reflecting rejection of the graft. To exclude rejection responses because of xenogeneic recognition, a control group was transplanted with the human skin graft but did not receive an adoptive transfer of human PBMCs. The control mice did not demonstrate leukocyte infiltration, confirming that the graft is recognized as an allotransplant rather than a xenotransplant. Alloreactivity toward the skin graft was significantly suppressed by both BM-MSCs and adipose tissue-derived MSCs, with similar efficacy. Importantly, this study demonstrated the utility of the humanized SCID-PBMC allograft model for the evaluation of MSC immunosuppressive efficacy in allograft rejection.

In a diabetic NSG-PBMC model, human BM-MSCs were used to mitigate the immune response to human islet transplants [62]. Islet transplantation as a treatment for type 1 diabetes was introduced in the late 1990s; however, its more widespread application was hampered by the availability of islet grafts and also by transplant rejection and the loss of islet viability and function [62]. Wu et al. showed that cotransplantation of human islets with BM-MSCs improved islet allograft survival and significantly prolonged the duration of insulin independence in the humanized mouse model. Addressing the underlying mechanism of transplant tolerance, the authors suggested BM-MSC-mediated activation of monocytes to produce IL-10 and the promotion of Treg cell proliferation through soluble factors.

Although MSCs have been previously shown to improve islet transplantation in vivo [63], the presence of human immune cells in the humanized mouse model led to an improved understanding of the mechanism by which BM-MSCs promote immunomodulation in allograft transplantation.

These studies demonstrate that humanized mouse models primarily offer an in vivo environment that closely represents the human immune milieu. However, they also provide a potential source of "humanized" immune cells and serve as an alternative to invasive sampling of human cells, such as BM, from a human donor. Importantly, easier access to cells of this type may facilitate research in this area. As an example of this, Chen et al. have used BM from the humanized NSG-CD34$^+$ mouse model to obtain human DCs to investigate MSCs and their effects on allograft rejection [64]. On a molecular level, transplant rejection occurs when transplant alloantigens are recognized as "foreign" by the host. There are three known pathways of allorecognition: direct, indirect, and semidirect allorecognition. Although direct allorecognition describes the presentation of donor MHC molecules by donor antigen-presenting cells (APCs) (received by the host in the donor graft), indirect allorecognition involves host APCs presenting donor MHC molecules to host T cells. The semidirect pathway of allorecognition proposes that recipient APCs acquire intact allogeneic MHC-peptide complexes through cell-to-cell contact from donor APCs [65]. All three pathways rely on APCs as key players in the response. As DCs are one of the most important types of APC, they play a crucial role in alloantigen presentation and thus transplant rejection. It has previously been suggested that MSCs have immunomodulatory effects in vitro on human DC differentiation and maturation [9, 10]. Although these reports focused on DCs derived from human PBMCs as well as from human CD34$^+$ cells, the study reported by Chen et al. investigated the effects of MSCs on BM-derived DCs, where the DCs were derived from the BM of the humanized NSG-CD34$^+$ model [64]. To explore the effect of MSCs on DC maturation and differentiation, MSCs were cocultured with the human BM-derived DCs. The study showed that MSCs inhibited DC differentiation and kept DCs in an immature or quiescent state, demonstrated by changes in phenotype and function [64]. The authors conclude by suggesting that inhibition of DC differentiation and maturation may represent one of several potential mechanisms that explain the beneficial effects of MSCs in clinical trials but also highlight the need for confirmation of these results in an in vivo model. Furthermore, this study showed that the humanized NSG-CD34$^+$ model represents an important tool to generate human BM-derived DCs.

### MSCs in Other Immune-Mediated Diseases

The immunosuppressive features of MSCs are also being explored for clinical application in various immune-mediated diseases. An example of this is MG, which is a rare autoimmune neuromuscular disease characterized by the presence of antiacetylcholine receptor (AChR) antibodies. These autoantibodies react against proteins of the neuromuscular junction, which causes fluctuating skeletal muscle weakness and fatigability. To simulate MG in a murine model, Sudres et al. transplanted thymic fragments from MG patients into NSG mice [44]. The NSG-MG mice exhibited MG-like symptoms and displayed mouse anti-human AChR antibody levels correlating with the levels observed in the patient sera. The authors compared the therapeutic efficacy of MSCs isolated from human adipose tissues in a resting state (rMSCs) with the same population of cells in an in vitro preconditioned state (cMSCs). Preconditioning consisted of 3-day in vitro coculture of MSCs with allogeneic PBMCs. The study showed that systemic administration of cMSCs led to an improvement in disease phenotype with decreased MG occurrence and severity in treated mice, and this was much more marked than that seen with rMSCs. Consequently, the authors suggest that preconditioning of MSCs could enhance efficacy and may present a promising strategy for the treatment of MG and potentially other autoimmune diseases. Furthermore, investigation of the mode of action identified that inhibition of cellular proliferation and a reduction in the expression of several molecules of the TNF pathway and costimulatory molecules contributed to the immunosuppression mediated by cMSCs. Importantly, the correlation between each mouse experiment and the respective patient's MG phenotype suggests that the humanized NSG-MG is a suitable disease model, which can be used to investigate the efficacy and mode of action of MSCs as therapy for MG [44].

The immunomodulatory effects of MSCs have also been investigated for the treatment of pulmonary fibrosis, a condition in which immune cells and their secreted cytokines play a critical role in promoting scarring of lung tissue [66]. Ni et al. established a pulmonary fibrosis humanized mouse model, utilizing *Rag2*$^{null}$*IL2rγ*$^{null}$ mice reconstituted with human PBMCs, which then received an injection of bleomycin to induce pulmonary fibrosis [45]. Importantly, they confirmed that humanized mice exhibited a more severe disease phenotype than murine models and suggested that this is a direct result of human immune cells being present in the lungs. Furthermore, human CD8$^+$ T cells were identified to be critical for the induction of pulmonary fibrosis. Human BM-MSCs injected into these humanized mice resulted in an alleviation of pulmonary fibrosis. The improvement in symptoms was attributed to MSC-mediated modulation of bleomycin-induced abnormal T-cell activation. Furthermore, experiments revealed that the expression of programmed death-ligand 1 by MSCs played a critical role in suppressing pulmonary infiltrating T cells. This study highlights the superiority of humanized mice over alternative murine models, to both mimic pulmonary fibrosis, but also to begin to determine the underlying mechanism of MSC-mediated attenuation of symptoms.

## Conclusion

Over recent years, the development of humanized mice has led to models that can recapitulate elements of the human immune system. The rapid pace of development of these models may soon permit their use as both preclinical models for a number of immune-mediated diseases and also for the exploration of the mode of action of therapeutic intervention strategies. Although MSCs are being used in the clinic already, the underlying mechanisms of the MSC immunomodulatory effects are far from fully understood. Better understanding of MSC immunobiology is particularly important because results of clinical trials have often been controversial and conclusive proof of efficacy has been lacking [24, 25]. Advances in our understanding may lead to the discovery of new ways to modify MSCs to be therapeutically efficacious. Taken together, it is clear that further progress toward humanized mouse models that most closely mimic human immune biology may be of particular benefit in the clinical translation of the exciting therapeutic potential offered by MSCs.

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## Author Contributions

V.J.M.: conception and design, manuscript writing; C.J.B.: conception and design, manuscript writing; M.L.M.: conception and design, manuscript writing.

## Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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