REVIEW

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Immunological properties of mesenchymal stem cells and clinical implications

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Abstract

The rapid evolution of experimental data has acknowledged the critical relevance of immune biology in stem cell research. It appears that efficient transfer of stem cells to patients requires robust analyses of the immune properties as well as the responses of the stem cells to immune mediators. This review discusses the biology of adult human mesenchymal stem cells (MSCs) in the context of immunology. MSCs are pluripotent, self-renewing cells with the potential for tissue regeneration, for example the repair of bone, cartilage, tendon, ligament, skeletal muscle, and cardiac muscle. MSCs have also been shown to transdifferentiate into cells of ectodermal origin, such as neurons. MSCs are located in perfused areas of adult bone marrow, whereas hematopoietic stem cells are located in poorly perfused areas of the same organ. MSCs show bimodal, i.e. anti--inflammatory and immune-enhancing, immune responses. MSCs also regulate immune responses such as the regulation of antibody production by B cells, alterations in T cell subtypes, and immune tolerance of allogeneic transplants. MSCs also have the potential for gene delivery. This review explores the diverse clinical potential for MSCs and discusses the limitations and advantages of their immunomodulatory properties.

Key words: mesenchymal stem cells, bone marrow, interferon γ, hematopoiesis, cytokines, immune stimulator.

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INTRODUCTION

Mesenchymal stem cells (MSCs) are pluripotent cells that can be found in several adult and fetal tissues. The adult bone marrow (BM) represents the major region of MSCs [5]. MSCs are also found in umbilical cord blood, although at lower frequency [31]. MSCs are morphologically symmetrical fibroblastoid type cells. They express CD44, CD29, CD105, CD73, and CD166 and lack markers that are consistent with hematopoietic cells, in particular CD45 and CD34 [28, 41]. MSCs are linked to bimodal immune functions, indicating their ability to exert both immunosuppressive and immunostimulatory effects. The type of immune challenge dictates the outcome of MSC-mediated effects, immune enhancing vs. immune suppressing. In addition, the effect of MSCs appears to be influenced by the magnitude of the stimulus [43, 44]. Regardless of the immune effects, an understanding of MSCs and other immune cells would be critical as these stem cells move into clinical trials.

IMMUNOSUPPRESSION

The implications for therapy by MSCs as immunosuppressors are broad. MSCs could be applied as inhibitors of chronic inflammatory stress and promote tolerance in an allogeneic setting [23]. Although the mechanisms of suppression have not been completely determined, their effects are partly mediated through secondary effects on immune cells [17]. This does not imply that MSCs are not designated the status of immune cells. Historically, immune cells are considered to be those that are derived from hematopoietic stem cells (HSCs). However, the immune properties of MSCs provide them with the designation of canonical immune cells. MSCs might perhaps initiate the placing of stem cells in a different category of the immune cell family and challenge the concept of a hematopoietic origin of stem cells.

Immunosuppression occurs most effectively under conditions in which MSCs make physical contact with allogeneic tissue and release soluble factors. Mediators

produced by MSCs could inhibit B cell proliferation and differentiation [9]. In fact, this paracrine property of MSCs has been emphasized since it may preclude the need for matching MHC molecules of donor and host [54]. In addition, MSCs have been shown to downregulate chemokine receptors on B cells, suggesting blunting effects on B cell migration to sites of inflammation [9]. Nonetheless, T cells have been shown to activate the release of soluble factors from MSCs in a contact-independent manner, suggesting that physical contact between MSCs and donor tissue is not an absolute requirement for immunomodulation [17].

Several studies thus far have addressed the roles of cytokines in combination with eicosanoids as mediators of immune suppression. Intravenous infusion of MSCs can lead to decreased production of T-helper 1 cytokines, in particular interferon (IFN)-γ and tumor necrosis factor (TNF)-α [1]. MSCs have also been shown to affect the production of TNF-α from dendritic cells 1 (DC1s), increase interleukin (IL)-10 production from DC2s, decrease IFN-γ release from T-helper 1 and NK cells, and increase IL-4 release from T-helper 2 cells [1]. Cytokines such as IL-2 have been shown to reverse the immune-suppressive effects of infused MSCs in mice and restore T cell responsiveness in experimental autoimmune encephalomyelitis (EAE), which is an experimental model of multiple sclerosis [57]. The discussed studies are intriguing as they explore clinical correlates.

MSCs have been linked to decreased expression of CD40 and CD86 on mature DCs [12]. In addition, MSC-derived IL-6 and vascular endothelial growth factor (VEGF) mediated the inhibitory effects of MSCs on T cell proliferation [12]. In addition to this property of MSC-derived IL-6, this cytokine has also been reported to inhibit the differentiation of BM progenitors into DCs [12]. In addition, neutralization of IL-6 was able to restore T cell proliferation in the mixed lymphocyte reaction [12]. Other relevant immune mediators affected by MSCs are Fas ligand and transforming growth factor (TGF)-β [33]. Despite the numerous reports on the effects of MSCs on cytokine production, the mechanisms remain elusive.

The antigen-presenting DCs are among the several immune cells that are influenced by MSCs [21]. Following studies of the direct effects of MSCs on *in vitro* T cell proliferation, the differentiation and maturation of DCs have become topics of interest to scientists. Co-incubation of MSCs with DCs resulted in a blunting effect of DC-derived $CD14^+$ monocytes and reduced expression of the mature DC marker CD83 [21, 27]. MSCs have also been shown to suppress monocytic differentiation into antigen-presenting cells [21]. MSCs regulate the activity of lymphocytic functions. These effects include inhibition of the proliferation of $CD4^+$ and $CD8^+$ T cells with reduced expression of activation markers [57, 29]. An important mediator in the lymphocyte suppression is nitric oxide (NO), which inhibits STAT5 phosphorylation and T cell activation. These findings were confirmed by reversal of effects upon inducible NO synthase inhibition [47]. MSCs also inhibit both T cell receptor-dependent and -independent proliferation of T cells [57]. The effects of MSCs on T cell functions could be attributed to mechanisms involving adhesion molecules [55]. At present there is no clear evidence to indicate that MSCs induce T cell apoptosis [57]. In fact, the suppression of T cell responses appears to be reversible since T cells are reactivated after the removal of MSCs [55]. Despite the vast amount of data on the immunosuppressive effects of MSCs, the mechanisms of suppression remain unclear.

The significance of MSCs in settings of allogeneic transplantation led to focused studies on MHC-II expression, which is expressed on subsets of MSCs [42]. After differentiation of MSCs to specialized cells, MHC-II molecules are expected to be decreased. However, if the MSCs are transplanted across allogeneic barriers, the re-expression of MHC-II on specialized MSC-derived cells could pose a clinical dilemma. Thus, an understanding of MHC-II regulation on MSCs would be relevant as these stem cells are transferred to patients. At low IFN-γ levels, MHC-II expression is maintained on MSCs, but is down-regulated at high levels [8]. This suggests that the degree of inflammation within an anatomical region would determine whether MHC-II is expressed on MSCs or its differentiated progeny. Future studies on MHC-II expression on MSCs are required as the use of these stem cells gets closer to translational studies.

Although much information has been accumulated on the immunosuppressive effects of MSCs, there are several areas of "black boxes". The role of IL-10 is a classic example of the disparity between the immune function of a cytokine and its confounding role in the biology of MSCs. Although IL-10 has been associated with immunosuppression, blunting of its production fails to completely reverse the immunosuppressive effects of MSCs [1, 46]. This raises the possibility that combinations of cytokines and perhaps other soluble factors could be involved in MSC-mediated immunosuppression. While IFN-γ-induced production of indoeamine 2,3-dioxygenase has been linked to the immunosuppressive effects of MSCs, others have shown no effect, but instead have implicated insulin-like growth factor binding proteins as mediators of immune suppression [16].

IMMUNOSTIMULATION

Unlike studies on the immunosuppressive effects of MSCs, information on immunostimulation by MSCs is not as well described. In early studies, baboon MSCs failed to show significant allogeneic responses [4]. In humans, MSCs can induce allogeneic responses [42]. As third-party cells, MSCs can inhibit the proliferation of B and T cells in mixed lymphocyte cultures [55]. Evidence has indicated that low numbers of MSCs stim-

ulate the immune response, whereas excess MSCs have an inhibitory effect. A possible explanation is that the stimulatory path becomes overloaded in the presence of excess MSCs, accounting for the idea that the direction of the effect (synergism versus antagonism) is influenced by the magnitude of the stimuli [30]. A similar phenomenon was observed in another study in which the level of IgG stimulation by lipopolysaccharide (LPS) or viral antigens determined the effect by MSCs. Strong LPS-mediated stimulation of IgG was associated with weak MSC-mediated IgG secretion, whereas weak LPS--mediated IgG stimulation showed opposite effects [43]. The contact formed between MSCs and immune cells appears to be important in the enhanced production of IgG [43].

Some additional, although preliminary, studies have pointed to the role of MSCs in immunostimulation. In lymphocytes of the blood and spleen, MSCs have been shown to mildly increase IgG and IFN-γ production [43]. Also, large increases in IL-6 levels were demonstrated in co-cultures of MSCs and spleen mononuclear cells [43]. The production of IL-6 by MSCs has been thought to act as a mediator of IgG production [43]. In summary, although the evidence for immune stimulation is still in its infancy compared with that for immune suppression, the ability of MSCs to upregulate the immune response has applications in disease states.

ALLOGENEIC VERSUS AUTOLOGOUS STEM CELL TRANSPLANT

Hematopoietic stem cell transplants have been relatively successful in several disorders, including those that are autoimmune-mediated. Both types of stem-cell transplantations are modulated by the balance between host NK cells and MSCs. NK cells can lyse MSCs, while MSCs prevent the proliferation of NK cells [51]. The expression of MHC-I on MSCs has been shown to prevent NK-mediated destruction of stem cell transplants, thereby promoting tolerance of foreign tissue. The proposed mechanism involves IFN-γ, which confers resistance to MSCs [46, 51]. Despite these successes, the problem of MHC incompatibility poses a problem during stem-cell transplantation, promoting the onset of graft-versus-host disease (GVHD) in allogeneic transplants. Autologous transplants tend to bypass this potential dilemma.

The roles of IL-10 and indoleamine 2,3-dioxygenase in transplant tolerance have received some attention. IL-10 has been known to inhibit the production of proinflammatory cytokines such as IFN-γ and to suppress the function of antigen-presenting cells. In addition to IFN-γ, TGF-β is also produced and could account for the suppression of the local immune response [34]. In a recent study, allogeneic stem-cell transplant recipients who were given IL-10-transduced MSCs showed lower mortality than controls [34]. Proinflammatory cytokine levels were lower in these patients, and the severity of GVHD was reduced [34]. IL-10 has also been studied in arthritis, in which MSCs that stably expressed IL-10 showed a two-fold reduction in alloreactive T cell proliferation [22]. Indoleamine--2,3-dioxygenase (IDO), an enzyme that converts tryptophan to kynurenine, has also been implicated in the induction of tolerance by inhibiting T cell responses [10].

ALLOGENEIC TRANSPLANTS

Two major distinguishing features of allogeneic stem-cell transplants are MHC mismatch between donor and host and decreased risk for autoimmunity. Patients who receive allogeneic transplants rather than autologous transplants typically recover slowly and exhibit more adverse effects. However, using an EAE model in mice, scientists showed lower risk of EAE recurrence with an allogeneic transplant, suggesting that allogeneic transplants pose a lower risk of autoimmunity [56]. Myeloablation of the host stem-cell compartment may assist in the treatment of autoimmune disorders [56]. This graft-versus-autoimmunity effect illustrates the importance of stem cells in potentially resetting the immune system to prevent self-destruction.

The immunomodulatory properties of MSCs could be important for future use in allogeneic transplants due to the potential to prevent graft-versus-host responses. In diseases such as Hurler's syndrome, metachromatic leukodystrophy, and severe combined immune deficiency, MSCs have been shown to significantly favor tissue transplantation [38]. The success of skin grafts, for example, increases by co-transplantation of MSCs and HSCs. The premise is that MSC-mediated immunoregulation can promote tolerance to HSCs [33]. It has been proposed that cytokine production by MSCs causes quiescence and self-renewal of HSCs [10]. In autoimmune diseases, MSCs have been shown to exert a ninety percent reduction in lymphocyte proliferation following transplantation [6]. In addition to these findings, MSCs suppressed the proliferation of transformed B cells and the ongoing lymphocyte reaction, rather than merely suppressing new proliferation [6].

Further evidence for the immunosuppressive role of MSCs comes from studies involving mixed lymphocyte reactions, in which T cell proliferation fails to occur upon introduction of mismatched MSCs from a third party [4]. These effects have been termed veto-like, indicating that MSCs would facilitate engraftment while minimizing graft-versus-host disease [22].

MSCs are not thought to be intrinsically immunoprivileged, and their origin might influence the outcome of allogeneic transplants. The effects of MSC infusion of donor MSCs and host MSCs in allogeneic transplant are not similar. Though much evidence shows that host MSCs can promote tolerance of allogeneic tissue, donor MSCs seem to have contradictory effects. Infusion of MSCs from the donor has been reported to cause

a memory T cell response and subsequent graft rejection [37]. Since co-transplantation of allogeneic MSCs and allogeneic tissue led to immune responses, this indicates that MSCs could not have intrinsic immunoprivileged properties, but the source of MSCs determines the immune effects [37]. As discussed above, the outcome of the immune effects by MSCs depends on the nature of the stimulus. Given the above argument, it is clear that the context in which MSCs function, rather than their inherent nature, determines their effects.

Allogeneic transplantation of HSCs is the preferred choice for treatments of leukemia and non-Hodgkin's lymphoma and it aids in replacing the immune microenvironment [56]. A drawback of this procedure is the uncertainty of susceptibility to autoimmune disease by the stem-cell donor. The recipient's age also plays a role in the transplant of choice, as GVHD poses a greater problem with increasing age [56].

AUTOLOGOUS TRANSPLANTS

The outcome of autologous transplants is more challenging to study because of the difficulty in discriminating between residual stem cells and re-introduced stem cells [55]. Nonetheless, autologous transplants offer much hope with respect to targeting tumors; autoaggression elicited by these transplants can serve as anti-cancer therapy [36]. In autologous transplants, cyclosporin A represses the effects of autoreactive T cells and inhibits the reconstitution of the immune system [32, 36]. Furthermore, it has been shown that autoreactive $CD8^+$ T cells from autologous transplants can target breast cancer cells, myelomas, and lymphomas [36]. The benefit of such transplants is a decrease in the relapse rate of hematological malignancies in patients with GVHD, suggesting the therapeutic benefits of such treatments [36].

CLINICAL CORRELATIONS I

A model disease that clearly illustrates the regulatory properties of MSCs on the immune response is GVHD, a complication that typically arises from the introduction of allogeneic stem cells to hosts. Cells of the donor tissue mount an immune response against the recipient's tissue, including the liver, gastrointestinal tract, and skin. The standard treatment of GVHD consists of steroids, but some patients develop resistance to these immunosuppressive agents. Furthermore, steroids increase the probability of fatal infection during treatment [34]. In a recent clinical study of the role of MSCs in GVHD, 8 patients with grades III and IV GVHD were given MSCs from family and non-family members. GVHD was resolved completely in 6 of the patients and no acute side effects were observed. Among 16 patients with steroid-resistant GVHD, MSC administration showed an increase in the survival rate [45].

The immunosuppressive effects of MSCs were also demonstrated in mice with EAE, which serves as a model for human autoimmune disease. In a recent study, EAE mice were subjected to transplantation with MSCs at the onset and at the peak of the disease [57]. Pathology of the central nervous system showed lower levels of demyelination and inflammation in the MSC-treated mice than in controls [6]. In another study, injection of MSCs into EAE mice resulted in milder phenotypes than controls. Furthermore, MSCs affected the pathogenic responses to EAE induction by reducing proliferation of T cells from the spleen and lymph nodes, and also reduced the production of the proinflammatory TNF-α and IFN-γ [15].

In addition to the effects of MSCs on GVHD, MSCs can also exert immunosuppressive effects in arthritis. In a recent study of the effects of MSCs in type II collagen-induced arthritis, allogeneic MSC administration prevented irreversible immune destruction of cartilage and bone. The mechanism may involve diminished responsiveness to T cell proliferation. Serum levels of various cytokines, including IL-4, IL-10, and IFN-γ, were decreased also. Such findings suggest a possible therapeutic avenue for autoimmune diseases [3]. A recent report on the exacerbation of collagen-induced arthritis by DC-derived IL-6 production opens a role for MSCs in arthritis [49]. Since MSCs are also linked to bone and cartilage replacement, MSCs might perhaps have multiple functions in the treatment of arthritis [2, 11, 13].

CLINICAL CORRELATIONS II

The prospect of using MSCs to target cancer cells has received some attention, but this area is understudied when one considers its therapeutic potential. MSCs can invade the stroma created by cancer cells, an effect that may be mediated by the tumor microenvironment [52]. The basis of this concept is that MSCs aid in the process of wound healing, which is analogous to tumor stroma formation [52]. Based on this homing property of MSCs, inducing MSCs to produce large amounts of tumor-targeting proteins may be used to restrict tumor growth [18]. The use of MSCs as vehicles to deliver anti-cancer gene therapy to tumors offers hope for cancer treatment.

In a study that assessed the invasion of MSCs into metastatic tumors, MSCs facilitated the formation of tumor stroma [20]. This concept was further addressed by transducing IFN-β into MSCs, which inhibited the growth of A375SM melanoma cells, independent of the host immune system [52]. The same group later showed that MSCs transfected with IFN-γ inhibited pulmonary metastasis [53]. In addition, breast carcinoma cells co-cultured with IFN-γ-expressing MSCs showed reduction in growth. In a recent study, MSCs have been shown to promote breast cancer metastasis in an experimental model [24]. The cancer cells induce the production of the chemokine CCL5 from MSCs. CCL5 mediates can-

cer-cell motility, invasion, and metastasis. Based on these findings, functions linked to MSCs could be targets of breast cancer metastasis, and perhaps other cancers.

Some of the molecular mechanisms of the anti-cancer activity of MSCs have been elucidated in experiments involving infusion of MSCs in an *in vivo* model of Kaposi's sarcoma. Such experiments have shown that MSCs home to sites of tumorigenesis and inhibit tumor growth [26]. The mechanism may involve MSC-dependent inhibition of Akt protein kinase activity in a contact-dependent manner [26]. Therefore, cancerous cells demonstrating dysregulation of the PI3K/Akt pathway are a potential target of MSC activity. However, the approach has its limits, since co-incubation of MSCs with the prostate tumor line PC-3 or breast tumor line MCF-7 showed no inhibition of Akt within the tumor cells [26]. Such findings call for further investigation on this topic.

TUMORIGENIC PROPERTIES OF MSCs

The bimodal nature of MSCs is not exclusive to immunoregulation, for MSCs have bimodal function with regard to cancer as well. Although MSCs can potentially be used as anti-cancer vehicles, they might also show oncogenic functions. In a recent study of the transformation properties of MSCs, repetitive passaging of MSCs caused immortality and *in vivo* formation of fibrosarcomas. These findings were associated with chromosomal instability, including double mutants and *c-myc* amplification [35]. MSCs have also been shown to have angiogenic characteristics, secreting VEGF to facilitate endothelial cell proliferation [25]. A paracrine angiogenic response that is independent of VEGF may be induced by physical stimulation of MSCs. Furthermore, MSC expression of matrix metalloproteinases may also contribute to tumorigenicity [25].

MSCs not only possess intrinsic tumorigenic properties, but can be induced to possess tumorigenic properties. The neoplastic potential of MSCs was demonstrated in an experiment in which MSCs were transduced with *hTERT*, a human telomerase gene, which led to loss of contact inhibition followed by tumorigenicity in the mice [48]. Another study underscoring the idea of MSCs favoring tumor growth showed that the presence of MSCs facilitated osteolytic activity of neuroblastoma, a common neural crest tumor of childhood [50]. This contrasts with the typical osteolytic pathway, in which only osteoclast-activating factor (not MSCs) mediates cancerous invasion of bone [50].

The overall gap between stem cells and cancer has been decreasing throughout the past few decades. One major requirement for tumorigenicity is angiogenesis, which depends on recruitment of endothelial and mesenchymal cells. These progenitor cells are derived from BM, which is also the source of stem cells. Further evidence of the bridge between stem cells and cancer comes from the identification of cancer stem cells, which may be the driving force behind tumor growth for multiple reasons: the requirement of a large number of cancer cells to induce tumorigenicity, the self-renewal properties of tumor cells, and the presence of molecular pathways that are common to both stem cells and cancer cells [35].

FUTURE POTENTIAL OF MSCs

In the past, MSCs have been shown to have therapeutic potential with regard to replacement of mesodermal tissue, such as bone, cartilage, adipose, tendon, ligament, and muscle. For example, research on intervertebral disc replacement by MSC-dependent cartilage production has been recently conducted. Surprisingly, MSCs are not restricted to the formation of mesodermal tissue since the literature points to the idea of an ectoderm-generating capacity of MSCs. The future of MSCs may hold a treatment for spinal cord injury, as MSCs can likely migrate to an injured spinal cord and differentiate into neuronal-like cells that secrete neurotrophic factors [40]. Thus the potential for the use of MSCs in the recovery of neurological function should be explored in more detail. Among all types of stem cells, MSCs are particularly essential because of their reduced risk of teratoma formation, which occurs more often with the use of embryonic stem cells [54].

The past decade has shown promise for patients with autoimmune diseases and GVHD by stem-cell transplantation. A recent study underscored the importance of MSCs to patients who received unsuccessful steroid treatment for hepatic GVHD induced by HSC transplantation. Administration of MSCs derived from adipose tissue resulted in complete resolution of GVHD [14]. The underlying mechanism for stem cell-dependent remission of autoimmune diseases may involve reprogramming of the autoimmunity, not simply extended immunosuppression. The idea of reprogramming is a field that requires more attention. By gaining a better understanding of the immunoregulatory effects of MSCs, scientists can take steps towards enhancing the tolerance of highly necessary BM and organ transplants. Since the immunosuppressive effects of MSCs on GVHD have shown lower mortality rates than MSC-untreated patients with GVHD, MSCs have the potential to alleviate the adverse effects of transplantations. Other potential applications of MSCs include treatment of osteogenesis imperfecta, metachromatic leukodystrophy, and Hurler's syndrome. In children with osteogenesis imperfecta, for example, MSC infusion promoted bone remodeling after allogeneic transplants of stem cells [19].

One factor that perplexes the study of MSCs is the lack of a single immunophenotyping marker that is specific to MSCs. MSCs express a plethora of markers, including CD29, CD44, CD73, CD105, CD106, and

Table 1. Mediators of immunomodulation by MSCs

Mediators	Effects
c-kit	Assists in MSC proliferation
IL-2	Reverses immunosuppression
IL-6	Inhibits DC production; mediates IgG production
IL-10	Inhibits proinflammatory cytokine production; suppresses APC function
LPS	Stimulates IgG secretion (effects depend on signal strength)
TGF-β	Suppresses local immune response
IDO	Induces tolerance by inhibiting T cell responses
PGE_2	Stimulates VEGF and IL-6 secretion
iNOS, nitric oxide	Inhibits T cell activation via phosphorylation of STAT5

Table summarizes the mediators discussed in the review.

CD166, and a combination of these markers must be used to identify MSCs due to the absence of an exclusive marker [39, 55]. The identification of such a marker may provide a better means of exclusive isolation and characterization of these cells.

As evident in the brief summary shown in Table 1, several mediators are involved in the immune properties of MSCs. However, additional studies should focus on the *in vivo* immunomodulatory properties of MSCs pertaining to the treatment of disorders of the normal immune response. For example, MSC-mediated immunosuppression may have potential therapeutic uses in the treatment of autoimmune diseases, whereas MSC-mediated immunostimulation can potentially limit the spread of cancer. The exploitation of IL-10 and IDO in promoting transplant tolerance can offer a critical therapeutic strategy in allogeneic and autologous grafts, whereas cyclosporin A-induced GVHD in autologous transplants suggests an avenue for targeting tumor cells, including malignancies of the breast and BM [36]. The evidence that MHC-II expression on certain MSCs can stimulate a mild allogeneic response may offer potential in targeting cancer cells as well. MSC-mediated delivery of IFN-β to sites of tumor implies that other anti-cancer factors can be loaded into MSCs for use in oncology. In contrast to exogenously administered IFN-β at maximal doses, which has a short half-life and can be toxic when given systemically, MSC-mediated delivery to the tumor microenvironment may offer a better therapeutic potential [53, 52]. Human fetal MSCs can be used as vehicles for *ex vivo* gene therapy using onco-retroviral or lentiviral vectors, providing a mode of treatment for genetic disorders [7]. Clearly, the implications of MSCs with regard to their immunological characteristics are very expansive.

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