Review

Immunosuppression by mesenchymal stem cells: mechanisms and clinical applications

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Abstract

Mesenchymal stem cells (MSCs) are multipotential nonhematopoietic progenitor cells that are isolated from many adult tissues, in particular from the bone marrow and adipose tissue. Along with their capacity for differentiating into cells of mesodermal lineage, such as adipocytes, osteoblasts and chondrocytes, these cells have also generated great interest for their ability to display immunomodulatory capacities. Indeed, a major breakthrough came with the finding that they are able to induce peripheral tolerance, suggesting they may be used as therapeutic tools in immune-mediated disorders. The present review aims at discussing the current knowledge on the targets and mechanisms of MSC-mediated immunosuppression as well as the potential use of MSCs as modulators of immune responses in a variety of diseases related to alloreactive immunity or autoimmunity

Introduction

Mesenchymal stem cells (MSCs), also named multipotent mesenchymal stromal cells, are largely studied as new therapeutic tools for a number of clinical applications. Indeed, these cells have been shown to have differentiation capacities as well as paracrine effects via the secretion of growth factors, cytokines, antifibrotic or angiogenic mediators [1]. A large body of studies also indicates that MSCs possess an immunosuppressive function both *in vitro* and *in vivo*. We review the present

knowledge on the mechanisms underlying the immunomodulatory characteristics of MSCs and their applications in animal models of immune suppression or in clinics.

Definition of mesenchymal stem cells

MSCs were initially isolated from bone marrow but are now shown to reside in almost every type of connective tissue [2]. MSCs are characterized as a heterogeneous population of cells that proliferate *in vitro* as plastic-adherent cells able to develop as fibroblast colony forming-units [3]. MSCs are distinguished from hematopoietic cells by being negative for the cell surface markers CD11b, CD14, CD34, CD45 and human leukocyte antigen (HLA)-DR but expressing CD73, CD90 and CD105. Importantly, the capacity to differentiate into multiple mesenchymal lineages including bone, fat and cartilage is used as a functional criterion to define MSCs [4].

Immunosuppressive mechanisms of mesenchymal stem cells

Immunosuppressive function of mesenchymal stem cells requires preliminary activation

MSC-mediated immunosuppression requires preliminary activation of the MSCs by immune cells through the secretion of the proinflammatory cytokine IFNγ, alone or together with TNFα, IL-1α or IL-1β [5,6]. This activation step has also been shown *in vivo* in a model of graft versus host disease (GVHD) since recipients of IFNγ$^{-/-}$ T cells did not respond to MSC treatment and succumbed to GVHD [7]. Indeed, MSCs from mice deficient for the IFNγ receptor 1 do not have immunosuppressive activity, highlighting the important role of IFNγ in this process [6].

Mesenchymal stem cell immunosuppression is mediated by soluble factors

Although target cell-MSC interactions may play a role, the MSC-mediated immunosuppression mainly acts through the secretion of soluble molecules that are induced or upregulated following cross-talk with target cells. Among these factors, indoleamine 2,3-dioxygenase (IDO) has consistently been reported [8,9]. On stimulation with IFNγ, this enzyme metabolizes tryptophan to kynurenin, causing depletion of local tryptophan and accumulation of toxic breakdown products. IDO, however, exerts its effects mainly through the local accumulation of tryptophan metabolites rather than through tryptophan depletion [10]. Whereas the majority of studies indicate a potentially important function for IDO, human MSCs lacking both IFNγ receptor 1 and IDO still exerted important immunomodulatory activity [11]. This observation may be explained at least in part by a recent study reporting that Toll-like receptors expressed on MSCs augment their immunosuppressive activity in the absence of IFNγ through an autocrine IFNβ signaling loop, which was dependent on protein

kinase R and able to induce IDO [12]. Contrary to human MSCs, lack of IDO activity was constantly reported for murine MSCs [13,14].

Induction of inducible nitric-oxide synthase (iNOS) by murine MSCs and production of nitric oxide was suggested to play a major role in T-cell proliferation inhibition [15]. Nitric oxide is a gaseous bioactive compound affecting macrophage and T-cell functions. iNOS is induced in mouse MSCs after activation by IFNγ and TNFα, IL-1α or IL-1β, and MSCs from iNOS$^{-/-}$ mice had a reduced ability to suppress T-cell proliferation [6] (Bouffi C, Bony C, Courties G, Jorgensen C, Noël D, submitted). The expression level of iNOS mRNA in human MSCs was minimal [14], however, and secretion of nitric oxide by human MSCs was undetectable (Bouffi C, Bony C, Courties G, Jorgensen C, Noël D, unpublished results). Indeed, different mechanisms of immunosuppression exist in different species since human MSCs employ IDO as a major effector molecule whereas nitric oxide plays a critical role in mouse MSCs [14].

Prostaglandin E_2 (PGE_2) has also been involved in the immunosuppressive activity of MSCs. PGE_2 is a product of arachidonic acid metabolism that acts as a powerful immune suppressant, inhibiting T-cell mitogenesis and IL-2 production, and is a cofactor for the induction of T-helper (Th) type 2 lymphocyte activity. Production of PGE_2 by MSCs is enhanced following TNFα or IFNγ stimulation, and its inhibition using specific inhibitors resulted in restoration of T-lymphocyte proliferation [16]. MSC-derived PGE_2 was shown to act on macrophages by stimulating the production of IL-10 and on monocytes by blocking their differentiation toward dendritic cells (DCs) [17,18].

Another MSC-secreted factor, IL-6, has been reported to be involved in the inhibition of monocyte differentiation toward DCs, decreasing their stimulation ability on T cells [13,19]. In parallel, the secretion of IL-6 by MSCs has also been reported to delay apoptosis of lymphocytes and neutrophils [20,21].

Other mediators - such as transforming growth factor beta 1, hepatocyte growth factor, heme oxygenase 1 and leukemia inhibitory factor - were shown to be produced by MSCs upon activation [16,22-24]. The production of HLA-G5 by MSCs has more recently been shown to suppress T-cell proliferation, as well as natural killer cell cytotoxicity and T-cell cytotoxicity, and to promote the generation of regulatory T (T_{REG}) cells [25,26]. Cell contact between MSCs and activated T cells induced IL-10 production, which was essential to stimulate the release of soluble HLA-G5.

Any of these molecules alone does not lead to a complete abrogation of T-cell proliferation, indicating their nonexclusive role. Instead, MSC-mediated immunoregulation is the result of the cumulative action displayed by several molecules.

Suppressive effects on immune cells

Both CD4^{+} and CD8^{+} T-lymphocyte proliferation stimulated with mitogens or specific antigens is suppressed by MSCs. Suppression occurred with MSCs from autologous or allogeneic sources, indicating that it was not restricted by major histocompatibility complex (MHC) [27,28]. Inhibition of proliferation depends on the arrest of T cells in the G_0/G_1 phase of the cell cycle, independently of apoptosis, but instead MSCs promote the survival of stimulated T cells [29,30]. MSCs alter other T-cell functions, such as the decrease of production of IFNγ, IL-2, and TNFα and the increase of IL-4 secretion [16]. MSCs are not targets of CD8^{+} cytotoxic T cells but they can suppress the cytotoxic effects of cytotoxic T cells [31]. Finally, MSCs have been reported to promote, both *in vitro* and *in vivo*, the generation of CD4^{+}CD25^{+} or CD8^{+} T_{REG} cells with functional properties [32]. *In vivo* data, however, are contradictory [33,34]. Recent studies suggest that MSCs may induce a cytokine profile shift in the Th1/Th2 balance toward the anti-inflammatory phenotype Th2 [35,36] (Bouffi C, Bony C, Courties G, Jorgensen C, Noël D, personal communication). Indeed, MSCs can suppress antigen-specific T-cell proliferation and cytotoxicity as well as inducing anti-inflammatory or T_{REG} cells.

Most studies have reported that MSCs inhibit the proliferation of B cells that are activated with anti-immunoglobulin antibodies, soluble CD40 ligand or cytokines [37]. Nevertheless, activated B cells became susceptible to the suppressive activity of MSCs in the presence of exogenously added IFNγ [5]. The suppressive effect of IFNγ was possibly related to its ability to stimulate the production of IDO by MSCs, which in turn suppressed the proliferative response of effector T cells. MSCs exert their suppressive effect on B-cell terminal differentiation through the release of humoral factor(s); they also increase B-cell viability while inhibiting proliferation, arresting B lymphocytes in the G_0/G_1 phase of the cell cycle [38,39]. Another study, however, reported that MSCs promoted proliferation and differentiation of transitional and naive B cells into immunoglobulin-secreting cells, and strongly enhanced proliferation and differentiation of memory B-cell populations into plasma cells [40]. Again, differences in cell purification procedures, experimental conditions and timing of analysis may explain discrepancies between studies.

Myeloid DCs are the most potent antigen-presenting cells, essential in the induction of immunity and tolerance. During maturation, immature DCs acquire the expression of co-stimulatory molecules and upregulate the expression of MHC class I and class II molecules together with other cell surface

markers such as CD11c, CD80, CD83 and CD86. MSCs inhibit *in vitro* the maturation of monocytes and CD34^{+} hematopoietic progenitor cells into DCs, as shown by a decreased cell-surface expression of MHC class II and co-stimulatory molecules, as well as a decreased production of IL-12 and TNFα [16,19,41]. This effect is at least partially mediated via the secretion of IL-6 by activated MSCs [13,19] or PGE_2, which was directly responsible for blocking DC maturation [18]. These studies suggest that MSCs might direct DC maturation toward an anti-inflammatory or regulatory phenotype responsible for an attenuated T-cell response.

Natural killer cells exhibit cytolytic activity that mainly targets cells which lack expression of HLA class I molecules. Killing by natural killer cells is regulated by a balance of signals transmitted by activating and inhibitory receptors interacting with HLA molecules on target cells. MSCs have been shown to suppress IL-2-driven or IL-15-driven natural killer cell proliferation, IFNγ production and cytotoxicity against HLA class I-expressing targets [42]. Some of these effects seem to depend on cell-to-cell contact and on the release of soluble factors, including transforming growth factor beta 1 and PGE_2, suggesting the existence of diverse mechanisms for MSC-mediated natural killer cell suppression.

Neutrophils are also important mediators of innate immunity responsible for microorganism killing via the production of reactive oxygen species. MSCs were shown to delay apoptosis of neutrophils through an IL-6-mediated mechanism that was associated with the downregulation of reactive oxygen species [20]. Delayed apoptosis was thought to preserve the pool of neutrophils that will be rapidly available in response to infections. Recently, Nemeth and colleagues suggested that LPS and TNFα stimulated MSCs during sepsis to secrete high levels of PGE_2, which in turn reprogrammed monocytes and macrophages to produce large amounts of IL-10. The released IL-10 seemed to prevent neutrophils from migrating into tissues and causing oxidative damage, thus mitigating multiorgan damage [17]. The results therefore suggest that MSCs may modulate the host innate response and improve survival by preventing sepsis.

The various studies indicate that MSCs suppress the function of several immune cells; notably the proliferation of T lymphocytes, the DC maturation and the induction of anti-inflammatory or T_{REG} cells. Some mechanisms of immunomodulation have been reproduced by several groups, in particular the secretion of IDO, PGE_2, nitric oxide and HLA-G5 (Figure 1). Differences in the secretome profile - particularly for IDO and nitric oxide - exist between humans and mice, however, suggesting that several mechanisms are likely to be responsible for the various effects reported to date. Our recent data suggest that MSCs exert two levels of action (Bouffi C, Bony C, Courties G, Jorgensen C, Noël

D, personal communication). One level occurs locally via the secretion of mediators that inhibit the proliferation of immune cells at the vicinity of MSCs. The second induces a systemic response, either an anti-inflammatory Th2 immune profile or, in some instances, the generation of T_{REG} cells.
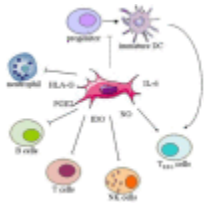

Figure 1. Schematic representation of the interactions between mesenchymal stem cells and immune cells. After activation, mesenchymal stem cells secrete soluble mediators - such as nitric oxide (NO), prostaglandin (PGE_2), indoleamine 2,3-dioxygenase (IDO), IL-6, and human leukocyte antigen (HLA)-G. Production of these mediators regulates the proliferation and function of a variety of immune cells as well as the induction of regulatory T (T_{REG}) cells either directly or indirectly through the generation of immature dendritic cells (DC). NK, natural killer.

Homing capacities

The trafficking and homing properties of MSCs are of particular interest for clinical applications aiming at using non-invasive systemic cell administration to treat inflammation. MSCs have been shown to express a variety of chemokines and chemokine receptors and can home to sites of inflammation by migrating towards inflammatory chemokines and cytokines [43,44]. Depending on the studies, heterogeneity in surface receptor expression is observed - which is probably due to differences in culture conditions and limitations in detection techniques. Homing of cultured MSCs, however, is inefficient compared with leukocytes. This inefficiency has been attributed to a lack of cell adhesion and chemokine receptors but also to the size of MSCs that promote passive cell entrapment and reduce trafficking [45]. Together with the evidence that host MSCs can mobilize in response to inflammation or injury, systemically infused MSCs are also frequently observed within the bone marrow or in injured tissues. Indeed, although the understanding of the underlying mechanisms is still required, accumulating evidence suggests that systemic infusion of MSCs may be used for immunosuppressive treatments of various disorders.

Therapeutic applications of mesenchymal stem cells

The hypoimmunogenicity of MSCs supports their therapeutic interest in a variety of diseases related to alloreactive immunity or autoimmunity. Indeed, the poor immunogenicity of these cells demonstrated *in vitro* and *in vivo* favors the possible use of allogeneic MSCs in acute clinical conditions where the availability of sufficient numbers of cells is rapidly needed. The use of autologous cells, however, may have therapeutic applications in autoimmune diseases or pathologies that allow enough time for isolation and *in vitro* expansion of MSCs. The few clinical applications performed to date confirm safety with a lack of major adverse side effects. Indeed, serial magnetic

resonance imaging performed in 226 patients who received MSCs for various orthopedic conditions showed no evidence of malignant transformation for a mean follow-up of 10.6 ± 7.3 months [46]. Accordingly, although some studies described the capacity of human MSCs to accumulate chromosomal instability *in vitro*, it was recently reported that, even though some aneuploidy was detected, MSCs showed progressive growth arrest and entered senescence without evidence of transformation either *in vitro* or *in vivo* [47]. Further studies are needed, however, to address the *in vivo* survival of MSCs, ectopic tissue formation and malignant transformation on a larger number of cell preparations.

Transplantation

One of the first *in vivo* studies showed that systemic infusion of MSCs isolated from bone marrow prolonged the survival of allogeneic skin grafts from 7 to 11 days in baboons receiving MSCs [48]. Using a semi-allogeneic heart transplant mouse model, infusion of donor-derived MSCs prolonged cardiac allograft survival through tolerance induction, which was due to $CD4^+CD25^+Foxp3^+ T_{REG}$ cell expansion and impaired anti-donor Th1 activity [49].

In hematopoietic stem cell (HSC) transplantation, MSCs may help reconstitution of the bone marrow stroma after chemoradiotherapy and enhance HSC engraftment. As early as 2000, autologous MSC infusion was shown to improve the outcome of HSC transplantation in advanced breast cancer patients [50]. Infusion of allogeneic MSCs, contrary to syngeneic MSCs, has since been demonstrated to result in rejection of stem cell grafts in a murine model of allogeneic bone marrow transplantation [51]. The results in animal models on the potential use of MSCs to prevent rejection of allogeneic grafts are conflicting (for a review, see [52]). In a more recent study, however, co-transplantation of donor MSCs with HLA-disparate $CD34^+$ HSCs resulted in sustained hematopoietic engraftment in 14 children without any adverse reaction, indicating that MSCs reduce the risk of graft failure in haplo-identical HSC transplant recipients [53].

MSC infusion may also be very helpful in cord blood transplantation where the limited dose of stem cells delays engraftment and favors graft failure. This cell therapy approach has also been used as GVHD prophylaxis in HSC transplantation.

Graft versus host disease

The most significant results on the immunosuppressive effects of MSCs so far have been observed in the treatment of acute GVHD after allogeneic stem cell transplantation. GVHD occurring beyond 100

days after HSC transplantation is generally called chronic GVHD, which has to be distinguished from acute GVHD that includes persistent, recurrent, or late-onset acute GVHD.

The first case of *ex vivo* expanded haplo-identical MSC injection in a patient with severe grade IV GVHD of the gut and liver resulted in a striking improvement of the disease [54]. A phase II study has since reported that 30 out of 55 patients had a complete response and nine patients showed improvement, indicating that, irrespective of the donor, MSC infusion might be an effective therapy for patients with steroid-resistant, acute GVHD [55]. Another report on patients with leukemia, however, showed effective prevention of acute GVHD but a higher incidence of relapses in patients who were co-transplanted with MSCs and MHC-identical allogeneic HSCs [56]. Co-transplantation of third-party donor HSCs with cord blood transplants has been shown to overcome the limitation posed by low cellularity of cord blood units for unrelated transplants in adults. For optimization of this therapeutic approach, the risk of GVHD still has to be reduced. The co-infusion of MSCs from the same HSC donors was therapeutically effective for severe acute GVHD but no significant differences in cord blood engraftment and incidence of GVHD were observed [57]. The results indicate the therapeutic potential of MSCs for acute GVHD control, but underline the need for better control of safety issues.

Autoimmune diseases

Based on their ability to moderate T-cell proliferation and function, MSCs have also been proposed as a therapeutic option in the treatment of autoimmune diseases. They have therefore been tested in a variety of animal models of diabetes, experimental autoimmune encephalomyelitis, systemic lupus erythematosus or rheumatoid arthritis.

Contrasted results were reported in rheumatoid arthritis using the experimental collagen-induced arthritis model. We first showed that injection of the allogeneic C3H10T1/2 MSC line did not reverse the disease score [58]. In the same model, however, a single injection of primary MSCs was shown to prevent the occurrence of severe arthritis, which was associated with a decrease in serum proinflammatory cytokines [59,60]. The use of human adipose-derived MSCs was effective in the xenogeneic collagen-induced arthritis model. The therapeutic efficacy was associated with decreased antigen-specific Th1/Th17 cell expansion, enhanced secretion of IL-10 and generation of $CD4^+$ $CD25^+$ $Foxp3^+$ T_{REG} cells with the capacity to suppress self-reactive T-effector responses [61]. Another study reported no convincing increase of T_{REG} cells *in vivo* despite *in vitro* evidence of T-cell inhibition by MSCs [62]. Our recent data with primary syngeneic and allogeneic MSCs indicate that MSCs may have a dual effect: locally, reducing the clinical signs of inflammation in the joints,

probably via the secretion of antiproliferative mediators; and systemically, by switching the polarization of the host response towards a Th2 immune profile (Bouffi C, Bony C, Courties G, Jorgensen C, Noël D, personal communication). The divergent mechanistic results obtained from the various studies underline the complexity of the MSC-mediated immunosuppressive process and the differences that may be attributed to the various MSC species used [14] and to the different techniques of MSC isolation and culture [7,59].

In the experimental autoimmune encephalomyelitis murine model of multiple sclerosis, MSCs were shown to decrease the clinical signs associated with demyelinization when injected before or at the onset of the disease, demonstrating the therapeutic efficacy of MSCs [34]. This effect was associated with immune suppression of effector T cells leading to IL-2 reversible T-cell anergy. Subsequently, it was reported that MSCs inhibited T-cell activation with reduced IL-17 and TNFα levels via the secretion of CCL2 by MSCs [63].

MSC transplantation conferred significant therapeutic effects in the systemic lupus erythematosus mouse model of lupus by reconstructing the osteoblastic niche and restoring immune homeostasis [64]. On the basis of these promising results, four treatment-refractory patients were treated with allogeneic MSCs. The patients presented a stable 12-month to 18-month disease remission, showing improvement in serologic markers and renal function [64]. Their data showed that MSC infusion restored the Foxp3$^+$ cell levels in both mice and systemic lupus erythematosus patients.

Development of autoimmune diabetes results from immune cell dysfunction to maintain peripheral and central tolerance. MSCs may therefore be helpful in regulating T_{REG}/autoreactive T-cell balance. The first results were obtained in the NOD/SCID model of chemically induced diabetes using human MSCs. In the treated diabetic mice, an increase in pancreatic islets and beta cells producing insulin was detected with a few glomerular endothelial cells of human origin. There was also a decrease in macrophage infiltration, resulting in the prevention of pancreatic injury [65]. The role of MSCs was first suggested to induce the regeneration of endogenous insulin-secreting cells, and, second, to inhibit the T-cell-mediated immune responses against newly formed beta cells [66]. A shift of the host immune response toward Th2-like responses was proposed to occur in treated NOD mice [67].

Conclusion

Overall, the current data indicate that MSCs represent a promising alternative strategy in the treatment of various immune-mediated diseases. Encouraging results have already been obtained from the clinics. Many questions remain to be addressed, however, in order to provide better ways to control

and optimize the immune response for the benefit of the patient. This implies a better understanding of the underlying mechanisms of immunosuppression as well as satisfying safety concerns as regards the *in vivo* survival, formation of ectopic tissue and malignant transformation.

Abbreviations

DC: dendritic cell; GVHD: graft versus host disease; HLA: human leukocyte antigen; HSC: hematopoietic stem cell; IDO: indoleamine 2,3-dioxygenase; IFN: interferon; IL: interleukin; iNOS: inducible nitric-oxide synthase; MHC: major histocompatibility complex; MSC: mesenchymal stem cell; PGE_2: prostaglandin E_2; Th: T-helper cell; TNF: tumor necrosis factor; T_{REG}: regulatory T cell.

Competing interests

The authors declare that they have no competing interests.

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