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Mesenchymal Stem Cells Modulate the Immune System in Developing Therapeutic Interventions

Sonali Rawat, Suchi Gupta and Sujata Mohanty

Abstract

Mesenchymal stem cells (MSCs) are emerging as key players in regenerative medicine for the treatment of various diseases associated with the inflammation and degeneration, thereby aiding in therapeutic advancements. Several tissues have been identified as potential sources of MSCs including the bone marrow, cord blood, dental pulp, umbilical cord, adipose tissue, peripheral blood, and fetal liver, of which some are clinically recognized. MSCs are capable of differentiating into cells of multiple lineages and therefore established as suitable candidates for transplantation in damaged organs. They have added advantage of higher proliferation, easy expansion, and, more importantly, the absence of HLA class II receptors, with potential applications extending toward allogenic settings. MSCs are actively involved in different mechanisms related to repair and regeneration of tissues via immunomodulation, transdifferentiation, paracrine factors, etc. They are known to exhibit profound immunomodulatory effect on T and B cells and natural killer (NK) cells mediated via soluble factors and direct cell-cell contact. The MSCs activate the immune responses and inhibit proliferation, maturation, and differentiation of T and B cells. The MSC-activated immune responses induce the expression of regulatory T cells (Tregs). A plethora of studies have established that MSCs suppress immune responses via immunomodulation that makes them a preferred cell source for the use in clinical trials.

Keywords: MSCs, therapeutic, immunomodulatory, GvHD

1. Introduction

Stem cells are undifferentiated cells possessing greater capacity of self-renewal and multilineage differentiation potential. This makes them unique candidates for curing a diverse variety of human degenerative diseases. Based on their potency, stem cells are classified into three broad groups: embryonic stem cells (ESCs), fetal stem cells (FSCs), and adult stem cells (ASC). ESCs are pluripotent stem cells isolated from inner cell mass of blastocysts of human embryos. The uniqueness of ESCs lies in the fact that they are capable of differentiation into all three primary germ layers, i.e., ectoderm, endoderm, and mesoderm. However, due to high tendency of teratoma formation and ethical issues regarding the destruction of human embryos, the clinical applications of ESCs are restricted. Alternatively, fetal and adult tissue-derived stem cells are gaining popularity with little ethical

concerns. Fetal stem cells can be isolated from extraembryonic tissues like cord blood, amniotic fluid, Wharton’s jelly, the placenta, and amniotic membrane [1–3]. However, adult stem cells (ASCs) are multipotent cells and are usually harvested from the bone marrow, adipose tissue, dental pulp, etc. All together, these cells possess clonogenic and self-renewing potential and plasticity to differentiate and often transdifferentiate into different tissue types.

Isolated stem cells from both adult and fetal tissues are multipotent and are recognized as MSCs. Notably, despite similar morphology and phenotypic properties, these tissue-specific MSCs have subtle differences in their regenerative potential due to the impact of stem cell niche on cell fate, known as stem cell niche theory, genetic variability, and/or epigenetic alterations [4]. Several studies have been carried out to show that there are differences in regenerative capacity of MSCs populations of

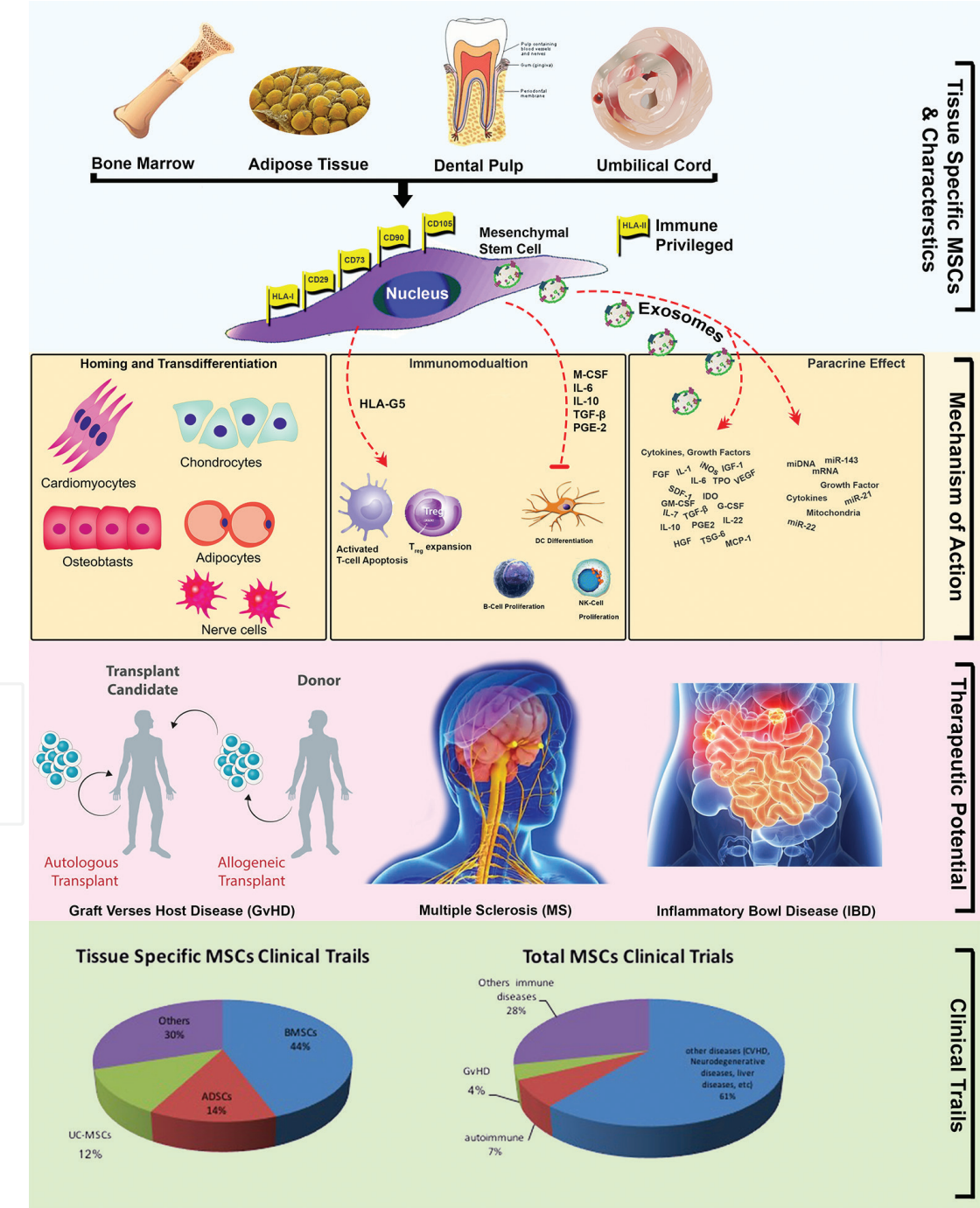


Figure 1. A diagrammatic representation of cellular characteristics, mode of action, and their therapeutic potential of mesenchymal stem cells with current status of clinical trial.

the same passage number that have been isolated from different pockets of the body [5]. A recent comprehensive report also supports the hypothesis that tissue-specific MSCs express certain source-specific markers [6]. Dominic et al. established criteria to define MSCs on the basis of the following characteristics: (1) plastic-adherent cells; (2) expression of surface markers, CD73, CD90, CD105, and HLA-ABC, but negative expression from hematopoietic lineage-specific markers, CD34, CD45, CD14, CD11b, CD19, or HLA-DR; and (3) potential to differentiate into trilineage, i.e., osteoblast, adipocytes, and chondrocytes [7].

Due to their enhanced regenerative potential, the use of MSCs has become an emerging strategy for the treatment of injured or degenerated tissues. It was observed that in *in vivo* scenario, MSCs showed profound immunomodulatory effect [8]. The most important characteristic of MSCs is its immunomodulatory property which augments and modulates both adaptive and innate immune responses as it initiates the wound healing paradigm.

MSCs are also known for their immune-privileged property due to their low immunogenicity. Human MSCs show low levels of human leukocyte antigen (HLA) class I, and they do not express HLA-DR which is necessary to escape immune surveillance. The presence of HLA class I is important as low levels of HLA class I protect cells from the natural killer (NK) cell-mediated cytotoxicity. On the contrary, cells which do not express HLA class I are targeted and destroyed easily. Another essential characteristic is that they home and migrate to the site of damage where there is secretion of inflammatory chemokines. These events are mediated by several chemokine receptors which aid in their migration and homing potential to the sites of inflammation [9]. Owing to the immune tolerance property of MSCs, they possess several clinical advantages due to which these cells are also referred as “universal donors” [10, 11]. However, as true with any other cell-based therapy, the evaluation of safety and efficacy of these MSCs in allogeneic strategies for clinical use is of utmost importance (**Figure 1**).

The initial reports of immunoregulatory properties were started with bone marrow-derived MSCs (BM-MSCs) [12]. Later, other sources of MSCs such as adipose tissue-derived MSCs (AD-MSCs) and Wharton’s jelly-derived MSCs (WJ-MSCs) were also explored for their immunomodulatory properties [1].

However, several challenges need to be overcome prior to the clinical applications of MSCs. Hence, a thorough insight of the various biological properties of MSCs will elucidate the mechanisms of MSC-based transplantation for immunomodulation.

2. Immunoregulatory function of MSCs in the inflammatory microenvironment

A key factor of survival in multicellular organisms is the maintenance and balance of homeostatic state. In the absence of inflammation, phagocytic cell is recruited to remove the apoptotic cells, whereas during acute injury, it is accompanied by inflammation, and the cell components that are released from necrotic cells result in microvascular damage due to increased vasopermeability and infiltration of macrophages and neutrophils [13]. During the process of phagocytosis of necrotic cells, there is secretion of pro-inflammatory mediators such as interleukin-1 (IL-1), interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α), various chemokines which further initiate downstream signaling pathways [14]. Adaptive immune response actively participates in the repair of damaged tissues in close association with CD4⁺, CD8⁺, T, and B cells [13]. Recently, MSCs have been recognized to be actively involved in damaged tissue repair processes. As a functional unit for

development and regeneration in various tissues, they hold utmost importance in maintaining proper functioning of tissues [15, 16]. In their undifferentiated and self-renewable state, the balance among interaction and protection of MSCs appears to be achieved by maintaining the stem cells in a specialized microenvironment called “niche.” This niche provides accommodation to different molecules to brace and coordinate stem cell activities pertaining to growth, proliferation, differentiation, and functionality. In particular, cell-cell interaction in the niche provides structural support, regulates adhesive interaction, and activates signals by secretion of certain molecules that can control stem cell functions. Stem cell immunoregulatory responses occur due to its close association with vasculature which provides metabolic cues and a conduit due to which inflammatory cytokines and immune cells, as well as humoral factors, can be delivered to the niche. In addition, the niche also provides biochemical and biomechanical parameters such as temperature, shear force, and chemical signals, which also influence stem cell behavior and fate in response to the external environment. In the process of tissue repair, MSCs are able to affect the inflamed microenvironment by secreting a cascade of various adhesion molecules, growth factors, and pro- and anti-inflammatory cytokines [17]. Since MSCs display notable immunomodulatory properties and they are able to dodge the immune system recognition mechanisms, they can potentially modulate the defense mechanisms of the host. In inflammatory condition, MSCs located in immediate location or originating from the bone marrow region start migrating to the site of injury. At the site, these MSCs associate themselves closely with numerous types of immune cells in order to initiate regeneration process of damaged tissue, which is typically accompanied with cytokine storm. The combinational sensitization of MSCs by IFN- γ and TNF- α induces the release of chemokines where they participate in chemotaxis and are able to inhibit proliferation of inflammatory effector cells. Several molecules participate in the activation, homing, and functionality of MSCs [18] (**Figure 2**). The commencement of homing process is led by selectins present on the endothelium. Specifically, for bone marrow homing, the expression of hematopoietic cell E-/L-selectin ligand (HCELL) is very important which is a functional glycoform of CD44 present on the migrating cells, while MSCs do not express HCELL but express CD44. The subsequent molecules participated in the activation of MSCs are mainly chemokine receptors [19, 20].

Chemokines are defined as positively charged short peptides (7–13 kDa). Broadly, four families of chemokines have been recognized: CCL family with adjacent cysteine residues, CXCL family with cysteine residues separated by a single amino acid, CXCL family with two instead of four cysteines, and CX3CL family with cysteines separated by three amino acids. For MSCs to home to injury site, these cells bear chemokine receptors and are identified at site of injury due to production of the chemokines. Hence a thorough understanding of the functioning of chemokine receptor profile is important for optimizing the process of both internal and external homing processes of MSCs to wound site. The site of injury produces abundant chemokines which may provide signal to MSCs or may function as chemoattractants [21]. During the process of regeneration or repair, the injury site tightly regulates the process of chemokine expression profile or expression pattern of each chemokine, which plays a unique role in directed migration of cells toward the site of injury. The subsequent expression of chemokines to attract specific immune cell types is conducted by immune response of injury.

Integrins are key players associated with the balance activation-dependent arrest of MSCs in the second last step of homing. They are known for cell-cell mediated matrix and adhesion, and they belong to the largest family of receptors. Mammals contain 18α and 18β subunits of integrin that combine to form at least 24 different heterodimers, each of which corresponds to a specific set of cell surface,

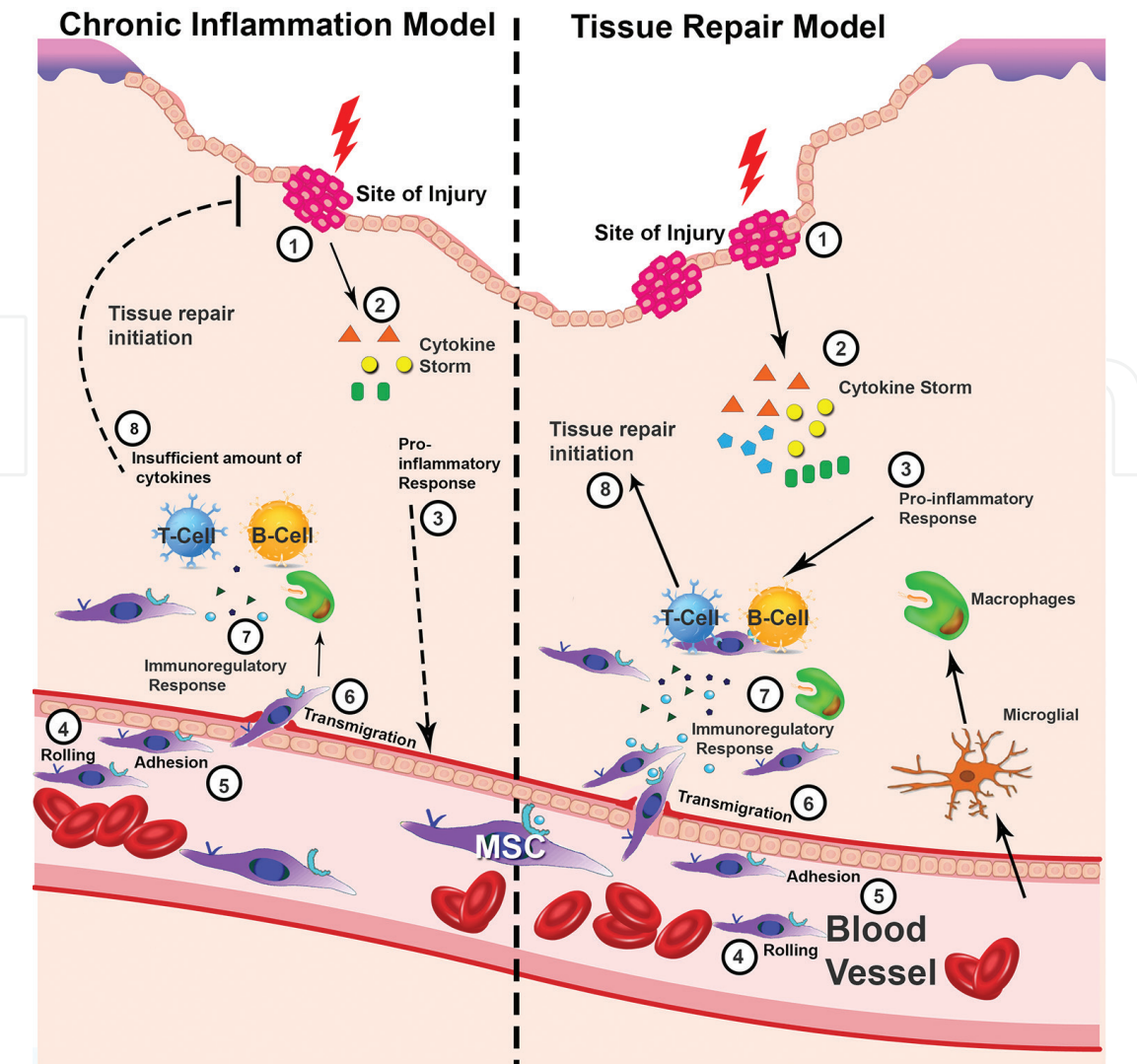


Figure 2. Immunoregulatory action of MSCs at chronic inflammation (left panel) versus acute inflammation (right panel). MSCs home to the injury site due to local cytokine storm secreted by activated immune cells. Activation and migration of MSCs lead to secretion of multiple immunomodulatory and growth factors. Depending on the cytokine signal (acute versus chronic inflammation), MSCs initiate the immunoregulatory response and repair the injury site or are unable to inhibit the persisting chronic inflammatory signals resulting in cellular fibrosis.

extracellular matrix (ECM) or soluble protein ligands. They are multifaceted receptors, transmitting bidirectional signals across the cell membrane, which is crucial for building a suitable interaction between the exterior and interior of the cell. Numerous cell processes like morphology, migration, proliferation, differentiation, and apoptosis are unexpected on this recurrent discussion. According to few reports, it was suggested that MSC homing will be affected if integrin- β 1 is inhibited. Also, interaction of vascular cell adhesion molecule-1-very late antigen-4 (VCAM1-VL4) is involved functionally in MSC homing [22].

During the last stage of transmigration across the endothelial cell layer and below the basement membrane, various lytic enzymes are essential to cleave components of the basement membrane, such as the matrix metalloproteinases (MMPs) [23]. Specifically, gelatinases, MMP-2 and MMP-9, preferentially degrade collagen and gelatin, two of the major components of the basement membrane which facilitate MSC migration. The MSCs reportedly help migrate MMP-2 and tissue inhibitor of metalloproteinases 3 (TIMP-3) [23].

The initial reports pointing to MSC homing were toward investigating the origin of BM-MSCs after allogeneic bone marrow transplantation. Those studies also concluded that the hematopoietic cell population was provided by the donor, but

the stem cells were provided by the recipient [24]. Therefore, a number of trials at both the preclinical and clinical levels have been carried out, and MSCs are seen to help migrate in a variety of tissues. Initial studies in animal models also confirmed that the presence of MSCs transplanted to donor was present in the bone marrow, thymus, spleen, and liver [12, 24, 25].

To elucidate the dynamics of MSCs migration, a systemic infusion of MSCs was studied by using varied techniques, i.e., after infusion of MSCs, they were first trapped in the lungs, and eventually, the cells disappear from the lungs and are distributed to other organs. Other aspects of MSCs homing was also studied by few groups under which they studied the factors such as early cell passage, irradiation and younger animals and observed that they influence the short-term bone marrow homing and condition which in result increases the homing [26–30].

Once MSCs are activated and recruited to the site of injury, there is onset of T-cell activation because of the presence of various pro-inflammatory cytokines such as IFN- γ , TNF- α , IL-1 β , etc. IFN- γ is a critical player in providing stimulatory signals for activation and expansion of T cells and its subsets, such as it begins to suppress the T cell proliferation, differentiation, and inhibition of various biological functions. Other than IFN- γ , TNF- α and IL-1 β also activate MSCs, either in synergy or alone. After stimulation with pro-inflammatory cytokines, MSCs also release other significant immunomodulatory factors. These stimulated cells modulate many immune effecters in vitro as well as in an animal model [31, 32].

3. Secretion of anti-inflammatory factors by MSCs is regulated by pro-inflammatory cytokines

To participate in tissue repair, MSCs must be in close association with several stromal and immune cells. The mode of action of MSC tissue repair is complex wherein MSC-derived immunoregulatory factors play a critical role.

MSCs are reported to release an array of growth factors and immunomodulatory molecules (**Table 1**).

To investigate how the inflammatory microenvironment modulated secretion of anti-inflammatory factors at the sites of tissue damage and it was concluded that MSC-mediated immunosuppression occurs in the microenvironment surrounding the MSCs: the inflammatory factors produced during the immune response act to turn on the immunosuppressive capacity of MSCs. For example, during pregnancy, the developed immunological tolerance along with the fetus development highlights the key role of fetus-derived MSCs. These pleotropic cells inhibit a group of cells involved in innate and adaptive immunity such as B cells, dendritic cells (DC), macrophages, and various effector cells such as NK, CD4+ T, CD8+ T, regulatory T (Treg), and NKT cells [33, 34]. The contribution of MSC-derived molecules toward the immunoregulation has been discussed (**Figure 3**).

3.1 Indoleamine-2,3-dioxygenase (IDO)

Indoleamine-2,3-dioxygenase is a mammalian cytosolic enzyme responsible for catalyzing the initial step in tryptophan catabolism via the kynurenine degradation pathways. IDO is comprised of two alpha helical domains with a heme group located between them and is an essential amino acid which catalyzes the rate-limiting step in the degradation of tryptophan, with the kynurenine pathway [35]. Any reduction in the concentration of local tryptophan or its metabolite results in immunomodulatory effect by IDO expressing cells.

MSCs Secretome Profile	Functions
(a) MSCs Soluble factors	
HGF	T-cell inhibition
HLA-G5	T-cell inhibition/T reg expansion-NK
IDO	T-cell inhibition-NK inhibition
IGF	T-cell inhibition
IL1RA	T-cell inhibition-MF inhibition
IL-6	T-cell inhibition-DC inhibition
LIF	T-cell inhibition/T reg expansion
PGE-2	T-cell inhibition-NK inhibition
(b) MSCs membrane bound factors	
Jagged 1	T-cell inhibition
HLA-G1	T-cell inhibition/T reg expansion
PDL-1/PDL-2	T-cell inhibition
CD54	T reg expansion
CD58	T reg expansion
(c) Chemokines Factors	
CCR2, CCR3, CCR4, CCR5,CCR6,CCR7	Attractor; adhesion; transmigration
CXCR1, CXCR2, CXCR3, CXCR4, CXCR5	Recruitment, attraction, activation
Selectins	Tethering; rolling
VCAM	Arrest, adhesion, transmigration
SDF-1	regulates progenitor cell mobilization
(d) Growth Factors	
FGF	Induce angiogenesis (together with VEGF), has anti-apoptotic and anti-fibrotic effects,
VEGF	Induce angiogenesis, stimulate proliferation of peritubular capillaries, anti-apoptotic effects
TPO	Supports maintenance and proliferation of HSCs
SCF	Supports maintenance and proliferation of HSCs
HGF	Associated with mobilization of progenitor cells, induction of angiogenesis, improves cell growth, anti-apoptotic and anti-fibrotic effects
BDNF	Promotes survival and differentiation of neuronal tissue
MMP	Matrix and Growth Factor Degradation, Facilitate Cell Migration
PDGF	Tissue Repair
IGF-1	Wound Healing, Neurogenesis

Table 1.
Secretome profile of hMSCs and their functions.

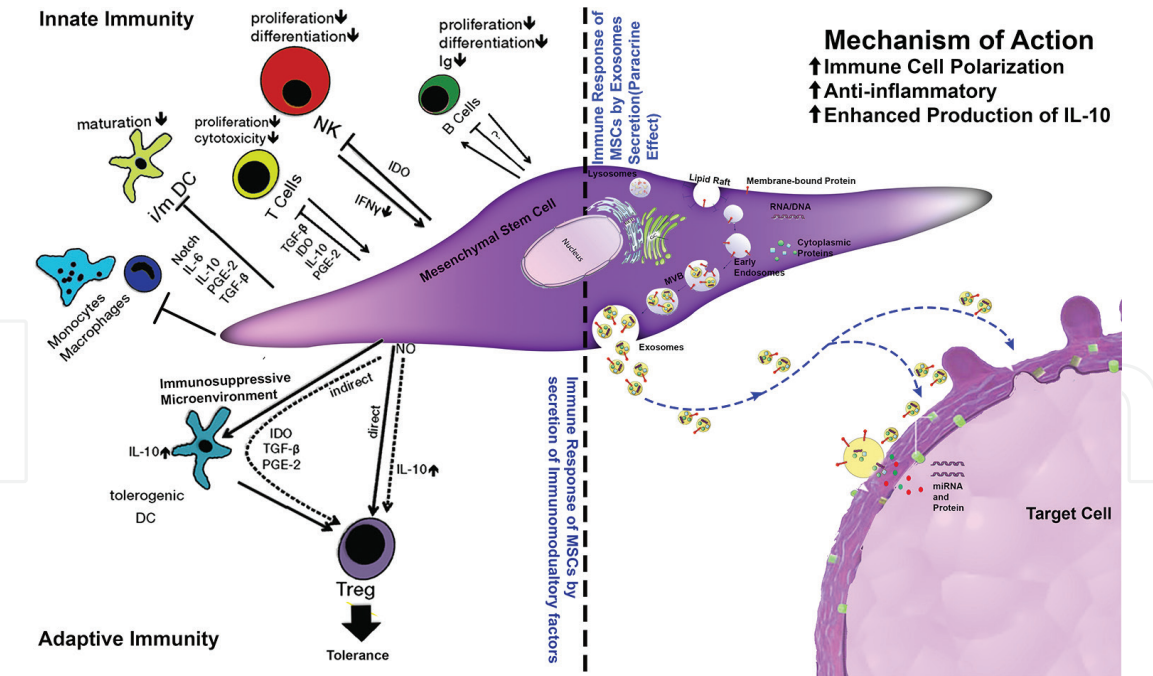


Figure 3. Representative image shows the immune response of MSCs by secretion of IM factors (left panel). Immunoregulatory function of MSCs on different cell types of the innate and adaptive immune cells. (Right panel) paracrine effect of MSCs through secretion of exosomes and their fusion with the target cell membrane and release of the biological active content for immunomodulatory effect.

The studies carried out with placental cells showed that they are capable of preventing maternal T-cell destruction of the fetus during pregnancy, which happens due to the expression of IDO in placental cells. During pregnancy, the fetus expresses paternal antigens that do not provoke rejection by the mother like other semi-allogeneic grafts [36, 37]. Dendritic cells can also express IDO and thus induce a tolerogenic response. Su et al. suggest that MSCs do not have the innate ability to express IDO but gain this ability following stimulation by the pro-inflammatory cytokines IFN- γ and TNF- α in combination with IL-1 β [38]. Recently, the role of IDO in MSC-mediated immunoregulation has been demonstrated in the suppression of various immune cell populations, including T cells and NK cells [39, 40].

3.2 Human leukocyte antigen-G (HLA-G)

HLA-G is a major histocompatibility complex class I antigen encoded by a gene on chromosome 6p21. It differs from classical HLA class I molecules by its restricted tissue distribution and limited polymorphism in the coding region. HLA-G can be expressed as seven distinct protein isoforms, each encoded by a specific, alternatively spliced transcript. Four isoforms are membrane-bound proteins (HLA-G1, HLA-G2, HLA-G3, and HLA-G4), and the other three isoforms are soluble proteins (HLA-G5, HLA-G6, and HLA-G7) [41]. It exerts its immunomodulatory functions by interacting with multiple receptors such as LILRB1 (ILT2/CD85j), LILRB2 (ILT4/CD85d), and KIR2DL4 (CD158d) which are differentially expressed by immune cells. Besides these receptors, HLA-G can also bind to CD8 without T-cell receptor (TCR) interaction, provoking NK cells and activated CD8 + T cell-induced apoptosis as well as FASL upregulation and secretion [42, 43]. HLA-G plays a fundamental role in maternal tolerance and transplantation. HLA-G expression by MSCs can be positively modulated by IL-10 and leukemia inhibitory factor (LIF). Other molecules such as glucocorticoid and interferon- β (IFN- β) are found to regulate HLA-G expression in immune cells. HLA-G has been

investigated for allogeneic solid organ transplantation and has been well associated with reduced number of immune rejection cases in kidney and liver allogeneic transplantations [44, 45].

3.3 Prostaglandin E2 (PGE-2)

Prostaglandins are small molecule derivatives of arachidonic acid (AA), produced by cyclooxygenase (COX, constitutively active cyclooxygenase COX1 and inducible COX2) and PG synthases. It can be produced by all cell types of the body, with epithelia, fibroblast, and infiltrating inflammatory cells representing the major sources of PGE-2 in the course of an immune response. The receptors of PGE2 (EP1–EP4) are present on multiple cell types, reflecting the ubiquitous function of PGE-2. It is relatively stable in vitro although its decay is accelerated by albumin [46]. In contrast, PGE-2 has a very rapid throughput rate in in vivo conditions and is quickly eliminated from tissues and circulation. This property of PGE-2 is most likely to contribute toward immune pathology and constitutes a potential target for immunomodulation. It is worth noting that the effect of PGE-2 in MSC-mediated immunoregulation in most cases is exerted in combination with other immunosuppressive molecules. With human MSCs, PGE-2 has been found to act with IDO to alter T-cell proliferation, during proliferation, cytotoxicity, and cytokine production by NK cells [47].

3.4 Inducible nitric oxide synthase (iNOs)

Nitric oxide synthases are family of enzymes catalyzing the production of nitric oxide from L-arginine. The enzymes convert arginine into citrulline and produce NO in the process. NO activity is independent of the level of calcium in the cell. However, its activity as other NO isoforms is dependent upon the binding of calmodulin (CaM). NO in high concentration is known to inhibit immune responses through mechanisms that remain largely unidentified. In addition, upon induction cytokines such as TNF- α and IFN- γ , alone or in combination, stimulate NO. This has a significant impact on both primary and secondary immune responses. For example, NO targets dendritic cells (DCs) that have a crucial role in making powerful immune response. It was found to prevent maturation of rat lung DCs by inhibiting granulocyte-macrophage colony-stimulating factors. Similarly, NO inhibits TNF- α and prevents DC maturation in humans [48]. MSCs produce large amounts of chemokines and adhesion molecules; immune cells accumulate in close proximity to the MSCs, where the high concentration of secreted NO can suppress the immune cells [49, 50].

3.5 Interleukin 10 (IL-10)

IL-10 is produced by both myeloid and lymphoid cells. While it is good immune suppressor, it has some immune stimulatory effects. IL-10 is recognized by its effect on T cells, macrophages, and monocytes which ultimately prohibit inflammatory responses. Thus, it regulates growth and differentiation of B cells, T cells, NK cells, and other cells of the immune system hence influencing inflammatory responses. IL-10 has the capability to inhibit the production of IL-2, TNF- α , IL-12, and IFN- γ . Furthermore, it will downregulate HLA class I. Although IL-10 has been implicated in MSC-mediated immunosuppression, direct IL-10 production by MSCs has not been demonstrated so far. Instead, contact of antigen-presenting cells such as dendritic cells or monocytes with MSCs has been found to induce IL-10 production [51–53].

3.6 Other mediators

In addition to the above molecules, several additional mediators are produced by MSCs or other adult stem/progenitor cells upon inflammatory stimulation, such as the inhibitory surface protein programmed death ligand 1 (PD-L1) [54], heme oxygenase-1 (HO-1) [55], leukemia inhibitory factor (LIF), galectins [56], and TGF- β [57]. However, their modes of action and underlying molecular mechanisms that drive MSC-mediated immunosuppression require further investigation.

3.7 Molecular and cellular interaction of MSCs with innate and acquired immune cells

3.7.1 MSC interaction with T and B cells

The antigen-specific immune system allows the development of immunological memory. It comprises of CD4⁺ T helper and CD8⁺ cytotoxic T lymphocytes that deliver a customized antigen-specific immune response following antigen processing and presentation by antigen-presenting cells (APCs). T helper cells comprise a subpopulation of cells called Tregs, which are specialized in suppression of T cell-mediated immune response [58]. The innate immune system plays an important role in the activation and subsequent course of adaptive immune response [59]. In addition, MSCs are able to suppress *in vitro* T-cell proliferation induced by cellular or non-specific mitogenic stimuli through the secretion of various soluble factors that include (transforming growth factor-beta 1) TGF- β , HGF, PGE-2, IDO, HLA-G5, and NO. The effect of these suppressive factors is upregulated by pre-sensitization of MSCs with TNF- α and IFN- γ . It is also known that MSCs polarize T cells toward a regulatory phenotype that serves as an important mechanism by which MSCs dampen inflammation [60, 61]. Tregs comprise a subpopulation of T helper cells, which are specialized in suppression of T cell-mediated immune response and characteristically express the forkhead box P3 (Fox P3) transcription factor. These are two main subsets of Tregs including a population of Fox P3⁺ natural Tregs which are thymus derived and specific for self-antigen and induced or adaptive Tregs that are derived from mature CD4⁺ + CD35-FoxP3 precursors in the periphery following inflammatory stimuli. The *in vitro* co-culturing of MSCs with PBMNCs induced the differentiation of CD4⁺ T cells into CD25⁺ + FoxP3⁺ expressing regulatory T cells [40, 62]. The possible reason of abovementioned mechanism is due to cell-cell contact of MSCs with helper T cells and secretion of PGE-2 and TGF- β . All together, these studies indicate that MSCs are able to maintain the balance between inflammatory effector T cells and anti-inflammatory Tregs.

B cells are also a major cell type involved in adaptive immune response, known for antigen presentation and antibody production. The balance between the different B-cell subsets has been identified as an important factor for optimal graft outcomes. To support the beneficial effect of B-cell depletion at the time of transplantation to impair T cell-mediated allo-response, the CD8 and CD4 T-cell memory is impaired when the antigen-presenting function of B cells is absent [63]. The exposure of enriched B-cell population to irradiated third party PBMNCs led to an increase in immunoglobulin (Ig) production that was abrogated by the addition of MSCs. There are diverse results among the studies to analyze the effect of MSCs upon exposure of isolated pure B cells [64]. These effects have been shown to be cell-cell contact independent or indirect through inhibition of pDC-induced B-cell maturation. On exposure, MSCs increased the viability of B cells and mediated the arrest of cell cycle at G0/G1 and inhibition of their differentiation into plasma cells and subsequent Ig formation, whereas it was observed that pre-treatment of

MSCs with IFN- γ was necessary for their suppressive effect on B cells [66]. The activated B cells and memory B-cell subsets when exposed to MSCs were seen to increase their survival and proliferation [65]. The studies carried out by Schu et al. [66] showed that when allogeneic MSCs were injected into rats, a strong humoral response was elicited as compared to injection with syngeneic cells in an immunocompetent host.

To support whether the allogeneic MSCs exert a humoral response in the recipient to prove the notion, they performed the experiment in which a rat was injected with allogeneic MSCs; on the other hand, contradictory reports were also published stating after transplantation none of them developed anti-MSC antibodies [66]. These studies indicate some disparity of humoral response directed against the injected MSCs, and possible reasons may be the source of MSCs, number of injected cells and frequency of injections, route of administration, or concurrent immunosuppression used.

3.7.2 MSC interaction with NK cells

Natural killer cells or NK cells are a type of cytotoxic lymphocytes critical to the innate immune system, evolve as progenitors in the bone marrow, and circulate as mature cells in the blood. They provide rapid responses to viral-infected cells, acting 3 days after infection, and respond to tumor formation. They play a major role in the mechanisms of rejection of graft and are central to the regulation of cytotoxicity in response to human leukocyte antigen molecule. With increasing trends in therapeutic usage of MSCs for treatment of GvHD, it is important to investigate the underlying effects of interaction of MSC and NK cells. They function in the manner that they get activated and inhibited on cell surface because of receptors transmitting the signal into the cell. Usually, NK cell possesses regulatory functions and can secrete cytokines and chemokines which modulate the host's immune response. IL-12 is the most important pro-inflammatory factor which responds to penetrating pathogens and acts through its high affinity receptors. It is released from accessory cells like monocytes, macrophages, and dendritic cells (DCs). Also, the most important cytokine released by NK cells is IFN- γ which is produced upon stimulation of IL-12. The NK cell-derived IFN- γ reinforces the expression of IL-12 and DCs via feedback mechanism. BM-MSCs directly interfere with the proliferation, cytokine production, and in some cases cytotoxicity of NK cells. MSC-NK interactions are complex and largely dependent on the microenvironment and activation status of the NK cells. Mainly, MSCs suppress the production of IL-2, IL-15, and INF- γ but not the cytotoxicity of freshly isolated NK cells. In addition, when activated NK cells come into contact with the MSCs, it interferes with NK-mediated cytotoxicity which is primarily mediated by cell-cell contact and secretion of IDO, PGE-2, TGF- β 1, and HLA-G5. Other reports mentioned that when licensed MSCs were exposed to IFN- γ , they are protected from NK-mediated cell killing, potentially due to their upregulated cell surface expression of HLA-I and downregulation of ULBP-3. This alongside an increased production of both IDO and PGE-2 offers multiple mechanisms for dampening NK responsiveness to the MSCs [59, 67, 68].

3.7.3 MSC interaction with dendritic cells

The potent antigen-presenting cells (APCs) and dendritic cells (DCs) play a pivotal role in initiating immune response. The life span of DCs can be divided into two major phases, an immature stage and a mature stage. These phases can be differentiated further on the basis of molecules expressed (CD80, CD86, OX62, HLA-II, and CD11b/c) on their surface. DCs can be immunostimulatory or immunosuppressive,

depending upon their maturation stage and specific DC subset. Immature DCs (iDCs) express low levels of HLA-II but no co-stimulatory molecules. The interaction of MSCs with DCs leads to the inhibition of maturation of monocytes and CD34⁺ precursor cells. Moreover, the direct activation of DCs leads to the release of PGE-2, IL-6, TSG-6, MCSF, and jagged-2 mediated signaling. Tolerogenic phenotype occurs when DC secretome of pro-inflammatory cytokines (TNF- α and IL-12) shifts toward anti-inflammatory IL-10 in which further downstream induces Th2 and Treg responses [69–71].

4. Paracrine interaction with in the niche (exosomes)

Advances in stem cell technology have opened interesting perspectives within the realm of regenerative medicine. As reported, MSCs participate in repair and regenerative processes via different mechanisms like homing and transdifferentiation and immunomodulation, which depends on paracrine mechanisms [72, 73]. The initial studies using MSCs were based on local engrafting of MSCs and differentiating into multiple tissue types. However, with the in-depth study of different mechanisms of MSC action, it has been reported that <1% MSCs are able to survive transiently after systemic administration [74]. This suggests that paracrine mechanisms through secretion of various molecules called secretome might be the possible mechanism for MSC regenerative potential. This has attracted significant attention for the potential use of MSC secretome in tissue repair and regeneration.

The secretome released by MSCs includes various biologically active growth factors and cytokines which aid in immunomodulatory properties of MSCs [75]. However, we cannot neglect the fact that the secretome released in the milieu of ECM of the cells is an easy target for denaturation due to the presence of proteases and other enzymes in the microenvironmental niche. Therefore, these growth factors and cytokines have been shown to be packed into small vesicles called exosomes which are secreted by MSCs in the extracellular milieu of cell along with the secretome [76]. They function through encapsulation of biological active molecules such as miRNA, proteins, and immunomodulatory molecules and protect them from degradation.

4.1 Exosome formation to secretion

Exosomes are lipid membrane-bound extracellular vesicles which possess a diameter of 30–120 nm and a density of 1.09–1.18 g/mL and are secreted by all cell types. These exosomes carry cellular components like proteins and nucleic acids and aid in cell-cell communication. The exosome was first discovered in 1984 by Johnstone in sheep reticulocytes [77]. It was initially believed that exosomes remove unwanted proteins from cells. Later on, it was demonstrated that many other cell types also secrete exosomes including immune cells, cancer cells, stem cells, and many more [78].

Exosomes are endosomal in origin, formed within multivesicular endosomes (MVEs). These vesicles are being released when membranes of MVEs fuse with that of the cellular plasma membrane. These exosomes express various surface markers like CD63, CD81, and CD9. They carry surface molecules that are present on the parent cell which aids in identification of exosomes and their parent cell source as well [77].

Moreover, the exosomes secreted by stem cells carry various proteins (growth factors and cytokines) and nucleic acids (mRNA and miRNA) that can influence their mode of action [79]. The content carried by these vesicles depends on the type

of cell (including source of cells) and its state of activation. Once released, these vesicles have local as well as remote effect by interacting with the neighboring cells or by circulating in the body fluids (bloodstream, saliva, serum, etc.).

4.2 Exosomes and immune cells

The exosomes were studied for their multifaceted application in antigen presentation, and vastly studied immune cell was dendritic cells. The clinical studies have been conducted to evaluate the dendritic cell derived exosomes for their therapeutic potential. However, compared to preclinical studies, only a few clinical trials have been conducted using exosomes. Reported studies were conducted where dendritic cell-derived exosomes were evaluated for their safety, tolerability, and efficacy in cancer patients. Exosomes carry parental cell surface marker expression. In this regard, DC derived exosomes are HLA-II positive as a result they can only be used in patient specific studies [80, 81]. In contrast, MSCs are immunologically naïve as they express only HLA class I molecules and lack HLA class II, CD40, CD80 and CD86 expression on their cell surface. Also, they are capable of immune escape and fail to induce an immune response by the transplanted host. Similarly, exosomes secreted by them are also immunologically naïve [82]. Considering all of the above properties, several recent studies have focused their research on evaluation of stem cell-derived exosomes in the area of immunomodulation with fewer reports.

In a recent study, exosomes derived from MSCs were specifically identified to mimic the effect of MSCs, and this paved the way to cell-free therapeutic approach using exosomes instead of the cell itself [83]. The first report using MSC exosomes were in cardiovascular diseases where Lai et al. [85] identified exosomes as the cardioprotective components in MSC paracrine secretion [84]. This was followed by several other studies where exosomes isolated from tissue-specific MSCs were studied for their therapeutic potential in various diseases.

Initial studies were performed on bone marrow-derived exosomes for evaluating their regenerative potential in cardiovascular diseases [85], acute kidney injury [86], bone defects, etc. [87]. By 2013, only researchers started exploring the regenerative potential of exosomes derived from adipose tissue and Wharton's jelly sources. These studies have explored the various mechanisms by which these exosomes mimic MSCs. The content of these exosomes was evaluated by using various techniques like RNA sequencing, mass spectrometry, etc., to identify different molecules and their target effect.

Conforti et al. reported the effect of MSC-derived vesicles on B-cell proliferation which was further confirmed by Di Trapani's group in 2016 [88, 89]. They observed that exosomes had higher levels of miRNAs compared to MSCs and induce inflammatory priming via increasing levels of miR-155 and miR-146. These are two miRNAs involved in the activation and inhibition of inflammatory reactions. Similar studies were reported where MSC-derived exosomes were shown to increase the ratio between regulatory and effector T cells along with the increase in cytokine such as IL-10 [90]. Similarly, Chen et al. [91] has also reported immunomodulatory effects of MSC-derived exosomes toward peripheral blood mononuclear cells (PBMCs) focusing specifically on T cells. It was observed that there was significant inhibition of pro-inflammatory cytokines, IL-1 β and TNF- α , but enhancement of the expression of anti-inflammatory cytokine, TGF- β 1. This cytokine profile in their study mimics the immunomodulatory effect of MSCs [91]. Zhang et al. showed that these exosomes may polarize monocytes toward M2-like phenotype, which in turn induces CD4⁺ T-cell differentiation into regulatory T cells [92].

Blazquez et al. demonstrated AD-MSC-derived exosomes as a therapeutic agent for the treatment of inflammation-related diseases. They showed that exosomes

exerted an inhibitory effect on the differentiation of activated T cells, reduced T-cell proliferation, and IFN- γ secretion in an in vitro stimulated T-cell model [93]. Favaro et al. has shown the effect of BM-MSC-derived exosomes on PBMCs isolated from type I diabetic patients. These exosomes were able to inhibit the IFN- γ production and significantly increased the production of immunomodulatory mediators such as PGE-2, TGF- β , IL-10, and IL-6 [94]. The in vitro studies were complemented by the in vivo studies which confirmed the immunosuppressive effect of exosomes in mouse allogeneic skin grafting models [95]. Bai et al 2017 have subcutaneously administered exosomes isolated from human embryonic stem cell-derived MSCs and showed that there was delayed occurrence of GvHD for 2 days, concomitant with increasing Treg polarization. In continuation, the author has also demonstrated that exosomes released from WJ-MSCs can effectively ameliorate experimental autoimmune uveoretinitis (EAU) in rats by inhibiting the migration of inflammatory cells [95]. Moreover, there is only single case study on humans in which MSC exosomes have been tested in the treatment of resistant grade IV acute GvHD patient which experienced improvement in symptoms for 5 months. There were no side effects reported, and the decrease of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IFN- γ was observed. The anti-inflammatory molecules IL-10, TGF- β 1, and HLA-G contained in the exosome preparations were believed to contribute to the immunosuppressive effect of MSC-Exo [96].

Although there are limited studies available using MSC-derived exosomes, future advancements into research and gain in in-depth knowledge of immunomodulatory properties of these MSC exosomes could be seen. These nano-vesicles can be developed as cell-free therapies. The use of these exosomes as cell-free therapies provides following key advantages:

- a. Less tumorigenicity.
- b. Easy storage without application of potentially toxic cryopreservative agents.
- c. Mass production through tailor-made cell lines.
- d. Potential to be used as ready-to-go biologic product.
- e. Off-the-shelf secretome therapies.
- f. The time and cost of expansion and maintenance could be greatly reduced.
- g. The biological product obtained could be modified to desired cell-specific effects.

Despite these advantages of MSC-derived exosomes, there has been a lack of manufacturing process that is required to generate exosomes with clinically relevant quantities. Therefore, there is an urgent need for technological advancements. Nevertheless, regulatory requirements will be necessary to establish the safety and efficacy profile of these exosome products.

5. MSCs in preclinical and clinical trials

MSCs delivered alone or with a biomaterial have been used in a variety of regenerative medicine strategies. In vivo evidence supports the hypothesis that MSCs have immunosuppressive properties that include prevention of graft versus

host disease (GvHD), decreased graft rejection, prevention of experimental acute encephalomyelitis, prolonged skin graft survival, etc. [8]. However, in recent years, consistent reports on its immunomodulatory properties have opened up newer avenues for studying MSCs, other than regenerative medicine. As of May 2018, there were over 843 MSC-related clinical trials registered on the NIH Clinical Trial Database (<http://clinicaltrial.gov/>). Interestingly, half of all registered clinical trials (~45%) are being conducted for immune-mediated diseases. Of these, 69 are based on autoimmune disease, 38 on GVHD, and the rest 290 on other inflammatory diseases. However, in early phase I, phase II, phase III, and phase IV, there were 3, 76, 7, and 6 clinical trials conducted, respectively. Hematopoietic stem cells (HSCs) have been the most successful in providing therapeutic application using stem cells. MSCs represent a lifesaving treatment for patients suffering with hematopoietic malignancies and genetic diseases. HSC transplantation is performed either autologous or with matched allogeneic/third party, depending upon the clinical scenario. Furthermore, in allogeneic transplantation, immunosuppression is necessary to reduce the graft rejection in patients. But despite immunosuppressant therapy, immune rejection in the form of GvHD is still a major cause of morbidity and mortality. The clinical application of MSCs for GvHD developed more rapidly than for any other immune-mediated diseases [68]. The probable cause could be case reports in severe GvHD where BMSCs were infused and showed dramatic therapeutic effects. Clearly, MSCs have a strong potential as therapeutic agents for GvHD, but detailed tailoring of patient population and stringent MSC processing criteria are necessary to deliver consistent and reproducible results [97–100].

Apart from GvHD condition, multiple sclerosis (MS), joint diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA), inflammatory bowel diseases (IBD) and inflammatory airway, and pulmonary diseases are few examples of inflammatory diseases in which preclinical studies have established strong therapeutic effect of MSCs [101]. In multiple sclerosis, reports showed that MSC treatment increases accumulation of Th2 cytokines-IL-4 and IL-5 and generation of Treg in vivo both of which help reduce EAE symptomatology. The possible molecular mechanism by which MSCs polarize CD4 T cells in EAE is via IDO. Indeed, both small and large animal studies demonstrate that MSCs decrease inflammation in joint diseases and facilitate cartilage repair [102].

The critical part of IBD is the uncontrolled immune response to intestinal microbes, and it is progressively fatal without curative treatment, making MSCs an attractive therapeutic option for these chronic inflammatory diseases. In several experimental models of IBD, MSCs given by intraperitoneal or intravenous routes showed prevention of DSS-induced injury of the intestines. It was observed that MSCs can specifically reduce Th1 and Th17 responses as well as serum level of pro-inflammatory cytokines (IL-1b, IL-6, IL-17, TNF- α , and IFN- γ) while enhancing the numbers of Tregs and splenic (myeloid-derived suppressor cells) MDSCs. A very recent trial using allogeneic placenta-derived MSC-like cells (which were not registered) also showed favorable immune responses. Thus, MSC therapy for IBD—especially CD fistula formation—appears to be safe and a viable option [103, 104].

Pulmonary diseases like chronic obstructive lung disease (COPD) are driven by alveolar macrophages, cytotoxic T cells, and neutrophils leading to progressive limitations in airflow with small airway fibrosis and alveolar destruction. In in vivo studies documented post-MSC infusion showed downregulation of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 and upregulation of VEGF and TGF- β [105, 106]. In addition, MSCs or MSC-conditioned medium improved tissue damage and survival. This involves MSC-derived factors with microvesicles such as exosomes, which are considered as carriers.

6. Conclusion

MSCs are excellent candidates for therapeutic use as cellular therapies can potentially revolutionize the current pharmaceutical landscape. Emerging data suggests that MSCs have an immunomodulatory function, but thorough understanding of the mechanisms underlying the complex molecular interplay between MSCs and inflammatory responses will be crucial for exploiting MSC-based therapies in therapeutic applications. One important aspect is to delineate functional differences in tissue-specific MSCs isolated from different sources; current ISCT standardization does not include immune-related functional tests or more detailed molecular validation. Based on the evidence of several clinical trials, the safety of this therapy appears clear; however, the efficacy of such cell therapy is largely uncertain. The overwhelming positive results seen in preclinical animal studies have not yet been translated into clinic. In brief, there is still much to learn, explore, and optimize with regard to the interactions of MSCs in human pathological conditions. In the near future, based on current development and results, MSCs are expected to hold tremendous potential to achieve clinical relevance in regenerative therapy.

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Conflict of interest

The authors declare that they have no competing interest.

Abbreviations

ESC	embryonic stem cell
ASC	adult stem cell
MSC	mesenchymal stem cell
ISCT	International Society for Cellular Therapy
HLA	human leukocyte antigen
NK Cells	natural killer cells
BM-MSCs	bone marrow-derived MSCs
AD-MSCs	adipose tissue-derived MSCs
WJ-MSCs	Wharton’s jelly-derived MSCs
INF- γ	interferon gamma
TNF- α	tumor necrosis factor- α
IL-1	interleukin-1
HCELL	hematopoietic cell E-/L-selectin ligand
ECM	extracellular matrix
VCAM1	vascular cell adhesion molecule-1
VL4	very late antigen-4
MMP	matrix metalloproteinases
TIMP-3	tissue inhibitor of metalloproteinases 3
NKT	natural killer T cell

DC	dendritic cells
Treg	regulatory T cells
IDO	indoleamine-2,3-dioxygenase
HLA-G	human leukocyte antigen-G
LILRB1	leukocyte immunoglobulin-like receptor B1
LILRB2	leukocyte immunoglobulin-like receptor B2
KIR2DL4	killer cell immunoglobulin like receptor
TCR	T-cell receptor
IFN-beta	interferon-B
PGE-2	prostaglandin E2
AA	arachidonic acid
COX	cyclooxygenase
iNOs	inducible nitric oxide synthase
CaM	calmodulin
PD-L1	protein programmed death ligand 1
HO-1	heme oxygenase-1
LIF	leukemia inhibitory factor
APCs	antigen-presenting cells
TGF-β1	transforming growth factor-beta 1
HGF	hepatocyte growth factor
FoxP3	forkhead box P3
PBMNCs	peripheral blood mononuclear cells
Ig	immunoglobulin
GvHD	graft versus host disease
ULBP-3	UL16-binding protein 3 (ULBP3)
MVEs	multivesicular endosomes
EAU	autoimmune uveoretinitis
HSCs	hematopoietic stem cells
MS	multiple sclerosis
OA	osteoarthritis
RA	rheumatoid arthritis
IBD	inflammatory bowel diseases
EAE	experimental autoimmune encephalomyelitis
COPD	chronic obstructive lung disease
VEGF	vascular endothelial growth factor

Author details

Sonali Rawat, Suchi Gupta and Sujata Mohanty*
Stem Cell Facility, DBT-Centre of Excellence for Stem Cell Research, AIIMS,
New Delhi, India

*Address all correspondence to: drmohantysujata@gmail.com

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