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REVIEW

Mesenchymal stromal cells for tissue-engineered tissue and organ replacements

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Summary

Mesenchymal stromal cells (MSCs), a rare heterogeneous subset of pluripotent stromal cells that can be easily isolated from different adult tissues, *in vitro* expanded and differentiated into multiple lineages, are immune privileged and, more important, display immunomodulatory capacities. Because of this, they are the preferred cell source in tissue-engineered replacements, not only in autogeneic conditions, where they do not evoke any immune response, but especially in the setting of allogeneic organ and tissue replacements. However, more preclinical and clinical studies are requested to completely understand MSC's immune biology and possible clinical applications. We herein review the immunogenicity and immunomodulatory properties of MSCs, their possible mechanisms and potential clinical use for tissue-engineered organ and tissue replacement.

Introduction

The ongoing shortage of donor organs and need of life-long immunosuppression for the thousands of patients suffering from end-stage diseases worldwide claim a therapeutical shift. Tissue engineering (TE) is increasingly regarded as a potential solution to allotransplantation. It focuses on the repair, replacement, and regeneration of cells, tissues or organs to restore impaired function resulting from any cause, including congenital defects, disease, trauma, and aging. By using a combination of several approaches that moves beyond traditional replacement therapies, TE has already provided functional tissue [1,2] and organ [3] human replacement. TE involves the replacement of tissues and organs by using engineered matrices or scaffolds and target cells that can be seeded on or within the matrices [4] and cells represent one of the primary ''raw material'' required for building tissues and organs in the TE approach. The aim of this review is to discuss the immunogenicity and immunomodulatory properties of the mesenchymal stromal cells (MSCs), their possible mechanisms and their potential clinical use in the field of TE.

Stem cell and TE

A renewable and expandable cell source as well as the availability of a sufficient number of cells that maintain the appropriate phenotype and perform the required biological functions is a clearly desirable focus for TE strategies. Cells must produce extracellular matrix in the correct organization, secrete cytokines, and other signaling molecules, and interact with neighboring cells/tissues. Immediately, this raises a number of potential problems, the first of which is the selection of cell type and source.

Cells used in TE may be allogeneic, xenogeneic, syngeneic, or autologous. Ideally, the cells should be nonimmunogenic, highly proliferative, easy to harvest, and have the

Table 1. Concerns and advantages of different cell types to be used in tissue engineering approaches.

Cell type	Concerns	Advantages	References
Autologous differentiated cells	Harvesting not always possible Limited expansion capacity Low risk of teratoma	No immunological response No risk of teratoma	[121–123]
Allogenic differentiated cells	MHC I/II dependent immunological response Harvesting not always possible Limited expansion capacity Low risk of teratoma	No risk of teratoma	[121,123]
Adult stem/progenitor cells	Immunogenicity cell type dependent (different MHC I/II expression) Low to moderate risk of dedifferentiation Long lasting culture increases risk of de-differentiation	Immunomodulatory capacity cell type associated High expansion capacity	[124–126]
Amniotic fluid/placenta/umbilical-cord blood (UCB) derived cells	No to low ethical consideration Tumorgenicity so-far unknown Variable immunogenicity ascribable to cell dependent MHC I/II expression Low risk of teratoma	No to low ethical consideration Stable karyotype (UCB) No teratoma risk (UCB) High source for stem and progenitor cells Easy isolation Multipotency Expression of HLA-G (immunomodulatory functions) (amniotic cells)	[126–130]
Embryonic and fetal stem/progenitor cells	Significant ethical consideration Variable immunogenicity ascribable to cell dependent MHC I/II expression Possible infection risks Potential teratoma development De-differentiation risk	Pluri- to Omni-potency High to unlimited self-renewing capacity	[125,126,131,132,133]
Induced pluripotent stem cells	Epigenetic memory of the tissue of origin, with possibility to revert into original cell source phenotype Teratoma risk Potential immunogenicity in syngeneic recipients	Pluripotency Unlimited isolation	[14,128,134–138]

MHC, major histocompatibility complex.

ability to differentiate into a variety of cell types with specialized functions. Although autologous cells are the most desirable compared with allogeneic and xenogeneic cells, with regard to immunological compatibility and pathogen transmission, in general, they are differentiated and post-mitotic. Primary cells are still used in TE approaches [5–7], however, their proliferation rates tend to be low, harvesting from a patient or donor could be related to disadvantages (*e.g.* cartilage harvesting is related to donor site pain) or could not be an option (*e.g.* brain, heart and pancreas do not provide a readily available cell source) and, depending on the extent of end-organ/tissue damage, there may not always be a sufficient pool of viable tissue or cells available for biopsy and subsequent expansion. These limitations have stimulated studies to find and develop alternative cell sources for TE strategies and stem cells, having the ability to continuously renew themselves, maintaining the ability to differentiate into various cell types, are already providing promising solutions for TE applications [8,9].

The different stem cell types are described below, and the concerns and advantages related to their use in TE approaches are reported in Table 1. Stem cells may be embryonic (ESCs), perinatal (cord blood, amniotic fluid) or adult. ESCs, isolated from the inner cell mass of the blastocyst, are highly pluripotent and have the potential to differentiate into almost any cell in the body, providing a chance to obtain a renewable source of healthy cells and tissues to treat a wide array of diseases. Amniotic stem cells, which can be induced to differentiate into different

cell types representing each embryonic germ layer (including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal, and hepatic lineages) [10], have intermediate characteristics between embryonic and adult stem cells, are not tumorigenic [11], and there are no ethical issues concerning their use, suggesting that they might be promising candidates for TE approaches [12]. Recently, induced-pluripotent stem cells (iPS) resulted to be a unique, nonimmunogenic, autologous alternative to ESCs, with similarly high differentiation potential; however, preclinical studies have reported iPS possible immune recognition and consequent rejection, especially in syngeneic recipients [13].

Multipotent adult stem cells have been described from a wide range of adult tissues (including the brain, heart, lungs, kidney, and spleen), and are commonly called mesenchymal stem cells or, more commonly now MSCs. MSCs hold great promise as tools for further development of TE technologies and, are at the moment highly considered as a cell-based therapeutic tool for a diverse range of clinical purposes (http://clinicaltrials.gov/). MSCs can be easily isolated from various tissue sources, *in vitro* readily expanded and differentiated. Moreover, recent studies have highlighted that MSCs possess potent anti-inflammatory and immunomodulatory effects, and through either direct cell–cell interaction or factor secretion, can exert strong effect on local tissue repair and regeneration and could then provide a preferred tool for TE approaches.

MSC immunomodulatory properties

MSCs have been identified within specific niches in a variety of human tissue/organs [such as bone marrow (BM), umbilical cord, adipose tissue, heart, brain, muscle] and have a key role in tissue and organ maintenance, regeneration, and repair [14]. MSCs are characterized by a continuous cell cycle progression for self-renewal and a potential to differentiate into highly specialized cell types of the mesodermal, endodermal, and neuroectodermal lineage [15–20]. Once implanted, MSCs are able to interact with the surrounding microenvironment, to promote tissue healing and regeneration, renew biologic function and to support and rejuvenate host cells [21–23]. MSC *in vivo* effects are mainly based on supportive and trophic functions and on crosstalk with other cells present within diseased tissues [24–26]. In addition, MSCs showed to possess immunomodulatory properties [27,28], and their immune phenotype (widely described as major histocompatibility complex (MHC) $MHCI^+$, $MHCII^-$, $CD40^-$, $CD80^-$, $CD86^-$) is regarded as non, hypoimmunogenic and allow MSCs to evade the host immune system [29,30]. It has been demonstrated that MSCs have the ability to modify and influence almost all the cells of the innate and adaptive immune system, to interfere and affect cellular proliferation, differentiation, maturation, and function to induce an anti-inflammatory/tolerant phenotype and to modulate the immune response [31–34]. In particular, allogeneic MSCs (allo-MSCs), having the ability to promote active immunological tolerance to donor MHC, could be considered as a suitable therapy for allogeneic transplantation [22,35,36].

Even if not completely understood, it has been reported that not only cell–cell interaction (direct effects) but also soluble factors (indirect effects) are involved in the mechanisms that convey these properties to MSCs [37–39] (Fig. 1). The major mechanisms responsible for MSC immuno modulatory properties are briefly reported below and summarized in Table 2.

MSCs and innate immune system

Dendritic cells

It has been demonstrated that MSCs modulate different aspects of dendritic cell (DC) function *in vitro* (such as differentiation, maturation, and activation) [40–42], and *in vivo* [43–46]. MSCs influence DC development, impairing *in vitro* differentiation of monocytes and $CD34^+$ to DC [47–49], and maturation, inducing a decreased DC cell expression of specific markers, such as CD40, CD83, and CD86 costimulatory molecules [40,41,48,50]. By doing so, MSCs cause alteration of DC cytokine secretion profile, inducing a decreased secretion of pro-inflammatory cytokines [such as tumor necrosis factor α (TNFα), interferon-γ (IFNγ), interleukin-12 (IL-12)], and an increased production of IL-10, which is a suppressive and tolerogenic cytokine and a potent inducer of regulatory T-cells (Treg) [49,51,52]. Moreover, it has been demonstrated that DC cells, generated in the presence of MSCs, are strongly hampered in their ability to induce T-cell activation [48,50]. Thus, MSCs disrupt the three major functions that characterize DC maturation: the up-regulation of antigen presentation/co-stimulatory molecule expression, the ability to present defined antigens, and the capacity to respond to chemotactic signals (such as CCL19) [41]. *In vitro* experiments have indicated that the suppressive effects of MSCs on DC is mediated by both MSC soluble factors [such as IL-6, monocyte-colony stimulating factor (M-CSF) and prostaglandin E2 (PGE2)], which influencing DC maturation, lead to T-cell suppression [48] and by cell–cell contact that drives mature DC to differentiate into a novel Jagged-2-dependent regulatory DC population capable of suppressing lymphocyte proliferation [42]. Cell–cell contact seems to play a crucial role in mediating the MSC effect on DC function and it has been recently demonstrated that

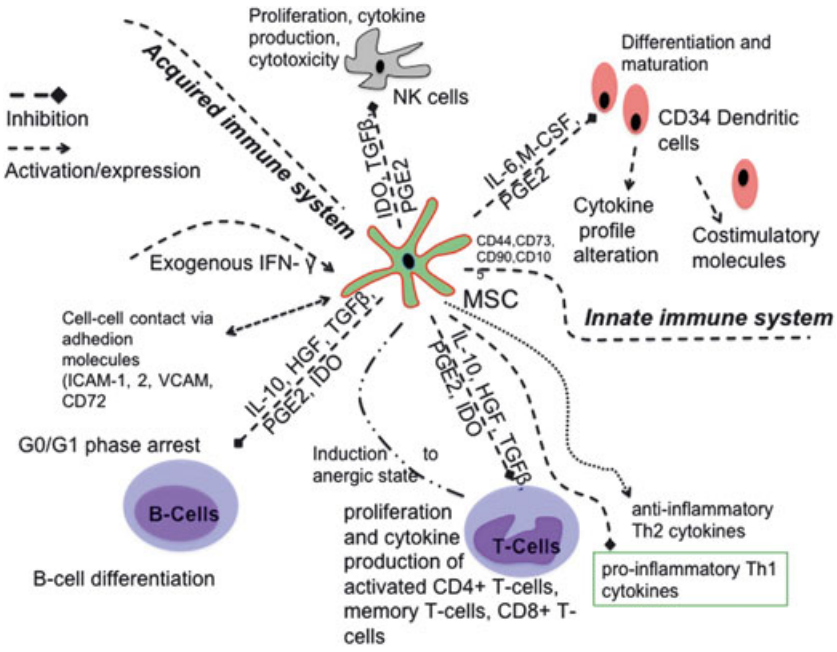

Figure 1 Immunomodulatory effects of MSCs. CD, cluster of differentiation; HGF, hepatocyte growth factor; ICAM, inter-cellular adhesion molecule; IDO, indoleamine2,3-dioxy-genase; IL, interleukin; M-CSF, monocyte-colony stimulaying factor; PGE2, prostaglandin E2; TGFβ, transforming growth factor β; Th, helper T-cells; VCAM, vascular cell adhesion molecule.

MSCs shift DCs from immunogenic to being capable of producing immunological tolerance through contact induced cytoskeleton modifications [53]. Overall, MSCs, modulating DC functionality, are indirectly able to regulate T- and B-cell activity.

Using animal model, it has been *in vivo* demonstrated that MSCs can delay the development of acute graft-versus-host disease (GVHD) altering DC migratory properties [43], and can suppress DC function during allogeneic islet transplant [54], suggesting a major role of DC modulation in immune modulation properties of MSCs.

Natural killer cells

The MSCs mediate natural killer (NK)-suppression by different mechanisms: the proliferation and cytokine production of stimulated NK cells resulted suppressed via soluble factors [such as indoleamine2,3–dioxy-genase (IDO), PGE2 and transforming growth factor β (TGFβ)] [55,56]; although the inhibition of NK-cell cytotoxicity required cell–cell contact [57]. It has been shown that MSCs exert an inhibitory effect on the NK-cell cytotoxicity against HLA class I positive targets that are less susceptible to NK-mediated lysis than HLA class I-negative cells [57]. However, to date little is known on the interaction of MSCs with NK cells, especially in *in vivo* environment.

Very few experimental evidences have been till now reported regarding the interaction of MSCs and other elements of the innate immune system (such as neutrophils, monocytes, and macrophages) and *in vivo* mechanisms are almost completely not understood. Using an animal model of sepsis, it has been reported that auto- and allo-MSCs reduced animal mortalities enhancing IL-10 production by means of a direct interaction with macrophages (mediated by monocytes) [58]. Recent reports indicate that MSCs may express membrane-associated proteins, such as toll-like receptors (TLR), which play a critical role in clinically established immunomodulation [59–61]. Indeed the ligation of TLR-3 (to double-stranded RNA) and of TLR-4 (to lipopolysaccharide and innate self antigens) block the MSC ability to inhibit T-cell responses, downregulating MSC immune modulation [60], whereas the galectins resulted were able to modulate the release of cytokines involved in GVHD and autoimmunity [62]. This suggests that MSCs have multiple effects depending on the local microenvironment and could be more effective in suppressing chronic inflammation (not driven by pathogens) without impairing inflammatory responses essential to antimicrobial defense (where TLR would be abundant) [26].

Taken together these findings suggest that the MSCs can modify the innate immune mediator functions to protect themselves and suppress different destructive inflammatory pathways.

Table 2. The multiple effects of MSCs on immune cells.

Immune cells	Effect	Mediated by	Reference
Innate immune system			
Dendritic cells (DC)	Inhibition of differentiation and maturation of $CD34^+$ DC	MSC soluble factors (IL-6, M-CSF, PGE2)	[49]
	Decrease of DC cell expression of costimulatory molecules		[49]
	Alteration of DC cytokine secretion profile		[49,51,52]
	Influence in DC maturation mechanisms		[41,48,50]
Natural killer (NK) cells	Suppression of proliferation and cytokine production of stimulate NK cells	MSC soluble factors (IDO, TGFβ, PGE2)	[55,56]
	Inhibition of NK-cell cytotoxicity	Cell–cell contact	[57]
Acquired immune system			
T-cells	Inhibition of proliferation and cytokine production of activated $CD4^+$ T-cells	MSC soluble factors (IL-10, HGF, TGFβ, PGE2, IDO)	[32,35,66,136]
	Inhibition of proliferation and cytokine production of memory T-cells	induced by exogenous factors (IFN-γ and TNFα)	[65,66]
	Suppression of the formation of cytotoxic $CD8^+$ T-cells	direct cell–cell contact: by adhesion molecules	[49,51]
	Downregulation of pro-inflammatory Th1 cytokines	(ICAM-1, 2, VCAM, CD72)	[64]
	Upregulation of anti-inflammatory Th2 cytokines		[64]
	Induction to enter an anergic state		[74]
	Production of selective Treg	MSC soluble factor (PGE2)	[32,49,76,77]
B-cells	Inhibitory effect (arresting cell cycle in G0/G1 phase) (MSCs in high doses)	MSC soluble factors (IL-10, HGF, TGFβ, PGE2, IDO)	[26,89]
	Stimulatory effects (induction of B-cell differentiation) (MSCs in low doses)	induced by exogenous factors (IFN-γ)	[90,91]

CD, cluster of differentiation; HGF, hepatocyte growth factor; ICAM, inter-cellular adhesion molecule; IDO, indoleamine2,3-dioxy-genase; IFN-γ, interferon-γ; IL, interleukin; M-CSF, monocyte-colony stimulating factor; PGE2, prostaglandin E2; TGFβ, transforming growth factor β; Th, helper T-cells; TNFα, tumor necrosis factor α; Treg = regulatory T-cells; VCAM = vascular cell adhesion molecule.

MSCs and acquired immune system

T-cells

The MSCs modulate the activation, proliferation, and function of both effector and Treg: MSCs inhibit proliferation and cytokine production of activated $CD4^+$ T-cells [37,63–65] and of memory T-cells [66,67] and suppress the formation of cytotoxic $CD8^+$ T-cells [51,52]. It has been reported that MSCs actively attenuate T-cell activation, up-regulating anti-inflammatory helper T-cell (Th)2 cytokines (such as IL-3, IL-5, IL-10, and IL-13) and down-regulating pro-inflammatory Th1 cytokines (such as IL-1α and β, IFNγ, and TNFα) [68]. On the contrary, even if relatively resistant to cytotoxic T-cells, MSCs resulted not to be able to inhibit their cytolytic activity [66]. Furthermore, MSCs exert an anti-inflammatory effect, by inducing Treg phenotype in Th cells [69]. It has been demonstrated that MSCs down-regulate T-cell response both through secretion of anti-inflammatory and tolerogenic cytokines (which may involve also the recruitment of Tregs) and direct cell–cell contact [68].

Several MSC-derived soluble factors seems to be involved in this inhibitory effect, such as IL-10, hepatocyte growth factor, TGFβ, and PGE2 [32,67,70]. T-cell proliferation resulted also to be inhibited by up-regulation of IDO expression, which is responsible of the inhibition of cell proliferation [71,72]. Moreover, it has been recently demonstrated that IDO effect could be also exerted via the local accumulation of tryptophan metabolites [73]. *In vitro* studies, evaluating the effect of supernatants from MSC cultures, suggested that these suppressive factors are not constitutively expressed/secreted by MSCs but require a dynamic crosstalk between MSCs and T-cells and are induced by exogenous factors (such as IFN-γ and TNFα) [35].

The MSCs express adhesion molecules (such as ICAM-1, 2, VCAM, CD72), up-regulated under inflammatory conditions, which having high affinity for T-cell, keep T-cells in close proximity increasing the inhibitory effects of released cytokines [74].

In addition, MSCs have been reported to induce T-cells to enter an anergic state (arrest in G0-G1 phase of the cell cycle, associated with inhibition of cyclin D2 expression),only partly reversed by exogenous IL-2, [75] and to promote the survival of resting T-cells, by protecting them from apoptosis [76]. Moreover, even if not completely clear, it seems that MSCs modulate immune response also in an indirect way: increasing the production

only of Treg with suppressive properties and stimulating Treg proliferation in a selective way [32,49,77,78].

Preclinical studies demonstrated that pretransplant infused MSCs, inducting Treg cells, prolong the survival of allogeneic transplants [44,79,80] and prevent diabetes mellitus [81,82]. Moreover, recent human clinical studies have demonstrated that, *via* the same mechanisms, MSCs are able to suppress the immune responses to allo-antigen, preventing allograft rejection, GVHD and autoimmunity [26,36,83–88].

Even if these results confirm *in vivo* the clinical relevance of MSC induction of Treg cells, several questions (*e.g.* the efficacy of regulatory effect and the possible translation of animal studies on human) still remained to be clarified.

B-cells

The concentration of MSCs is determinant to the effect on B-cells. It has, indeed, been reported that in high doses, MSCs exert inhibitory effect on B-cells, while in low doses have stimulatory effects. The inhibition is mediated not by the activation of apoptosis pathways, but arresting cell cycle in G0/G1 phase [89,90]. On the other hand, the stimulatory effect seems to be activated because MSCs, acting like DC, induce B-cell differentiation and rescue them from apoptosis [91]. Furthermore, a recent study demonstrated that MSCs can support survival, proliferation and differentiation of B-cells to antibody secretion cells [92]. Moreover, the release of soluble cytokines (such as IFN-γ) by activated T-cells resulted to play a role in mediating the effects of MSCs on B-cells [93].

Preclinical studies produced conflicting results: some groups reported MSC inhibition of antigen-specific antibody production, although others did not reveal an auto-antibody MSC suppression, suggesting a complex interaction, involving inhibitory pathways and potential for stimulatory effects.

Overall, MSCs seem to regulate immune responses by reducing the generation/differentiation of DC, down-regulating NK-cell cytotoxicity and proliferation, suppressing effector T-cells, and increasing the number of Tregs.

Most of the research, evaluating immunomodulatory properties of MSCs, have been performed using BM-derived MSCs, However, recent findings suggest that MSCs derived from different sources (adipose tissue, umbilical-cord blood, and cord Wharton's jelly) are comparable in terms of ability to suppress mitogen-induced T-cell proliferation and mechanism of action (mediated by IDO) [94], and that the antiproliferative effect of MSCs is a property shared by all stromal cells [95]. These results suggest that these cells could be suitable alternatives to BM stromal cells for allogeneic transplantation in TE [96].

Future challenges

Initial clinical trials, evaluating the potential of MSC immunomodulatory effects, have been completed or are underway [97]: donor MSCs have been reported to attenuate some aspect of the GVDH [98], and numerous patients (affected by different pathologies, e.g. GVDH, diabetes mellitus, stroke) have safely received allo-MSC therapy [26,85]. Although evidences of therapeutic benefits, recent large clinical trials reported disappointing results in term of the MSC efficacy [99] and, in particular, it remains unclear if the efficacy of allo-MSCs and auto-MSCs are equivalent and which mechanisms are *in vivo* induced by allo-MSCs. Further research and a critical analysis of the immunomodulatory properties of the MSCs, in particular allo-MSCs, are, then, needed before translating the promising early studies into clinical practice.

Several key issues have to be addressed and clarified to fully understand MSC therapeutic capacity.

One of the major questions concerning the MSC clinical application is the importance of their origin: autologous or allogeneic. Although it has been demonstrated that allo-MSCs are better immunosuppressors [78], they resulted to protect from sepsis death [58], neuronal loss [99], neurological injury [100,101], and to enhance wound closure [102] in a similar way to auto-MSCs. Moreover, both auto- and allo-MSCs were able to induce both immunogenicity and immune modulation, which could be a beneficial for the use of MSC against autoimmune disease. However, an evidence of allo-MSC immunogenicity has been often reported: in several studies the allo-response was weak, although in others, allo-MSCs demonstrated to be highly immunogenic. Table 3 reports a summary of recent preclinical animal studies in which immunogenic properties of allo-MSCs have been *in vivo* evaluated or in which allo-MSC effects have been compared with auto-MSC.

The administration route seems to exert an important role in determining cell immunogenicity: only intrarticular, intracerebral, intracranial, and direct implantation into skin wounds resulted to be correlated with none or low immunogenicity. However, Coyne *et al.* [103] reported that intracerebral-administrated MSCs were rejected as early as 14 days. A plausible hypothesis for this contradictory result could be that MSCs used underwent 10–15 cellular passages, which could have modified cell phenotype (such as a decrease in protein involved in signal transduction) and intrinsic properties [45]. As a consequence, the safety profile of MSC administration route must continue to be scrutinized, and an improved understanding of how culture conditions may affect the immunogenicity of MSCs and how they might be optimized to promote MSC functionality, is required.

Table 3. Preclinical animal studies evaluating *in vivo* immunogenic properties of allo-MSCs.

Injection environment	Animal used	Disease	*In vitro* results	Long-term survival	Immune response	Main conclusion	References
Intra-arterial	Rat	Kidney injury	Not tested	Not engrafted after 3 months	MSC administration not associated with adverse events	Allo-MSCs reduced loss of renal function. Auto-MSCs more effective	[86]
Intra-articular	Mouse	Tumor growth	Not tested	Present after 2 months; able to differentiate into bone	MSCs able to inhibit host antitumor immune response, if injected with melanoma cells	Allogeneic MSCs induced immunotolerance in short- and long-term	[137]
Intracranial	Rhesus macaque	Immunogenicity	No lytic activity upon rechallenge	Not reported	MSCs induced cell-dose- and haplotype-dependent allograft response	MSCs weakly immunogenic	[138]
Intracerebral	Rat	Immunogenicity	Not tested	Not reported	Graft rejection after 14 days	Donor inflammatory response rejected MSCs	[103]
				Present after 63 days	Absence of immune rejection	Transplanted MSCs decrease immunogenic cell activity	[45]
		Parkinson's disease	Not tested	Present after 24 days	Allo-MSCs elicit cellular immune response	Allo-MSCs did not prevent behavioral deficits	[139]
Intramyocardial	Rat	Acute myocardial infarction	Not tested	Low integration (disappear within 28 days)	MSCs did not elicit immune system	Allo-MSC transplantation could be a useful therapy for myocardial infarction	[140]
Intraperitoneal	Rabbit	Osteogenesis	Immunoprivileged status, even after differentiation	Present after 28 days (osteoblast MSC-derived)	MSCs provoked an immune response only when differentiated in osteoblast	MSCs immunosuppressive function diminish upon differentiation.	[104]
	Mouse	Immunogenicity	Not tested	Not reported	Accelerated skin graft rejection after allo-MSCs injection	MSCs elicited a complete immune response and do not induce tolerance	[141]
	Mouse	Autoimmune encephalomyelitis (EAE)	MSC conditioned medium inhibit T-cell activation	Not reported	MSCs resulted not immunogenic, only if not induced by IFN-γ	MSCs could modulate EAE biology	[101,112]
Intravenous	Mouse	Bone marrow transplantation	MSCs possess immunosuppressive properties	Not reported	Host MSCs enhanced allogeneic BM engraftment, donor MSCs increased BM rejection Infusion in naïve mice induces memory T-cell response	MSCs are capable of modulating immune responses in relation with MHC antigen matching MSCs are not intrinsically *in vivo* immunoprivileged	[48]

Table 3. continued

Injection environment	Animal used	Disease	*In vitro* results	Long-term survival	Immune response	Main conclusion	References
	Baboons	Immunogenicity	Not tested	MSC presence after 4 weeks	Host T-cell decreased, without suppressing alloantibodies production	Multiple administration of allo-MSCs affected immune responses without compromising the overall immune system	[142]
	Pig	Immunogenicity	Not tested	Not reported	MSCs resulted immunogenic after repeated injection	Immunogenicity increased after repeated doses or in inflamed damaged tissues	[111]
	Mouse	Graft-versus-host disease	Not tested	Not reported	Activated MSCs suppress GVHD and prevent mortality	Activated MSCs may represent a new strategy for preventing GVHD	[115]
		BM transplant	Immunosuppressive activity	MSCs survived more than 120 days in immunodeficient animals and only 40 days in fully immunocompetent allogeneic recipients	Allo-MSCs are able to prolong BM allograft survival in a transient way	MSCs not intrinsically immune privileged; induce rejection in allogeneic	[143]
		Sepsis	Not tested	Not reported	Allo-MSCs resulted therapeutic	Cultured MSCs may be effective in treating sepsis	[58]
		Heart transplantation	Not tested	MSC present over 100 days	MSCs combined with immunesuppresive therapy achieved long-term graft survival (>100 days) with normal histology.	MSCs attenuated acute allograft rejection and synergized with immune therapy to promote allograft tolerance.	[80]
Subcutaneous	Mouse	Erythropoietin secretion	Immunosuppressive activity	Present after 6 months in syngeneic recipients	In mismatched recipients, MSC induced strong cellular immune response	MSCs are not intrinsically *in vivo* immunoprivileged	[144]
	Pig	Infarction	MSCs possess low immunogenic profile	Not reported	Allo-MSCs induced a stronger immune response when injected intra-cardiac respect to subcutaneous	MSCs elicit a complete immune response (cellular and humoral)	[107]
Skin wound	Mouse	Wound healing	Not tested	Present after 28 days into the entire wound	No rejected and similar effects of auto-MSCs in enhancing wound closure	Allo-MSCs exhibited ignorable immunogenicity and resulted equally efficient as auto-MSCs	[102]

MSC, mesenchymal stromal cells; IFN, interferon; BM, bone marrow; GVHD, graft-versus-host disease; MHC, major histocompatibility complex.

It is not fully understood if cell differentiation alters the MSC immunogenic properties. It has been reported that osteogenic cells, differentiated from MSCs, retained their *in vitro* immunoprivilege and immunomodulatory properties, and after implantation, they do not provoke an immune response at early stage. However, *in vivo* the cells gradually expressed MHC II, with a consequent loss of their suppressive activity [104].

The MSC dose and timing of injection (in respect to the moment of transplantation or the stage of disease) have also to be defined. The local high MSC concentration used in *in vitro* studies are indeed not achievable in clinical applications ascribable to cell distribution to other tissues/organs and to cell-loss. In a multicentre trial designed to assess safety and efficacy of MSCs for refractory acute GVHD, a range of MSC dose, not leading to adverse side effects, has been reported. Authors reported that, on one hand, clinically meaningful responses were obtained after infusing a dose as low as 0.8×10^6 cells per kg, whereas on the other, doses as high as 1.9×10^6 cells per kg were not successful in all cases. However, any conclusion to suitable dose result to be premature [105]. Concerning timing, preclinical studies reported that MSCs result to be ineffective when given several days after graft transplantation [106], whereas were effective when infused before onset of inflammatory process or at the peak of disease [100]. These results have been further demonstrated in phase II clinical trial in which clinical conditions of more than half of the patients with steroid-refractory acute GVHD, who do not respond to corticosteroids and other immunosuppressive therapies, improved after MSC treatment [35,105]. The best dose of cells in each infusion, the more suitable time of injection, and the possible interactions of cells with other drugs require further investigation. Indeed, MSC *in vivo* efficacy seems to depend also on the concomitant administration of an immunosuppressive therapy.

Without immunosuppressive therapy, MSCs resulted were able (independently of the administration route) to elicit a complete (cellular and humoral) immune response, which resulted to be attenuated in the presence of an immunosuppressive therapy [107], suggesting that MSCs act synergistically with immunosuppressive drugs [44,108]. Moreover, it has been recently reported that low dose of immunosuppressant regiment could support the therapeutical effects of allo-MSCs [109].

The inflammatory cells and factors are commonly present in injured sites, and it has been demonstrated that infused MSCs preferentially immigrated into inflammatory sites [110], suggesting that the inflammatory environment may play an important role in mediating MSC immunosuppressive properties. *In vivo* animal studies reported that IFN-γ increased MSC expression of MHC I and MHC II, leading to loss of disease suppression and to MSC rejection [111,112]; although others showed that the exposure to inflammatory signals (such as high levels of IFN-γ) or prestimulation with IFN-γ, upregulating IL-10, TGF-β1, PGE2, and IDO expression, improves MSC suppressive effects [113–116]. The role of IFN-γ seems to be more complex than just being an activating agent: its level and the contemporary presence of other inflammatory cytokines seem indeed able to change MSC functional profile. IFN-γ can enable MSC to act as antigen-presenting cells but only at low concentrations; as IFN-γ levels increase, MHC II molecule expression on MSC decreases with the loss of alloreactive-inducing activity [117,118]. Other inflammatory cytokines, such as TNF-α or IL-1β, can influence MSC immunosuppression and determine substantial changes in their immunophenotypic profile [119]. Indeed, IFN-γ alone seems to be sufficient to induce IDO and B7-H1 upregulation, although, when in combination with TNF-α, the two cytokines act synergistically in the induction of COX2 [113] and in the upregulation of the secretion of MSC anti-inflammatory enzyme [120]. These results suggest that the inflammatory environment to which MSCs are exposed is a fundamental factor influencing MSC functions, resulting in the capability of shaping their properties in completely opposite directions: MSC immune suppressive properties could be both induced and decreased and the fine kinetics of the interactions between MSCs, inflammatory and immune factors is critical for the clinical outcome. A better understanding of the interaction between MSCs and inflammation environment will be then an essential step in improving MSC clinical use for inflammatory and immune-mediated diseases.

Conclusions

The unique MSC immunomodulatory properties suggest that MSCs could have important clinical implications for their use as a potential cell source also for TE approaches. Homing and immunomodulation are important aspects for MSC function and clinical effects. In particular it has been proposed that MSC antiinflammatory and antiapoptotic effects may promote tissue regeneration by creating favorable environment supporting tissue healing by resident stem cells. To date, recipient's own cells, such as BM MSCs, are the most suitable candidates to obtain an immunological accept tissue-engineered construct. However, it is not always possible to obtain cells from the patient and the time needed to isolate, differentiate, and grow autologous stromal cells, to populate and create an engineered construct, may not be feasible for the patient who urgently requires a tissue/organ replacement. Therefore, an ''off the shelf'' product, obtained using allogeneic,

nonimmunogenic MSCs, would be a more suitable and clinically accepted strategy. The experimental and clinical results obtained till now suggest that MSCs could provide a suitable cell source for TE an off the shelf construct, which would be immune privileged: MSCs could be isolated from any donor, expanded and cryopreserved, providing a readily available ''universal'' source of cells for TE.

Although this is an attractive supposition, ongoing efforts focused on evaluating *in vivo* effectiveness, shortcomings, and adverse effects of MSCs are needed to determine if their immunomodulatory properties will evolve from theoretical to clinical benefit.

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