

Editorial Reviews

Multipotent mesenchymal stromal cell therapy in renal disease and kidney transplantation

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Abstract

Cell therapies aim at differentiation of stem cells into the specific cell type required to repair damaged or destroyed cells or tissues. Over recent years, cell therapy has been introduced in a variety of application areas, including cardiovascular repair, diabetes, musculoskeletal disorders and renal repair. Multipotent mesenchymal stromal cells (MSCs), often referred to as mesenchymal stem cells, are of particular interest as a cell therapy model, as this is one of the few cell types that are on the brink of entering the clinical arena in different areas of application. MSCs can be differentiated *in vitro* and *in vivo* into various cell types of mesenchymal origin such as bone, fat and cartilage. They have important effects on the innate and adaptive immune system and possess striking anti-inflammatory properties that make them attractive for potential use in diseases characterized by autoimmunity and inflammation. In addition, MSCs have been shown to migrate to sites of tissue injury and to enhance repair by secreting anti-fibrotic and pro-angiogenic factors. In this review, evidence for the renoprotective mechanisms of MSCs as well as their therapeutic possibilities and potential hazards in acute and chronic renal disease and allograft rejection is summarized.

Keywords: mesenchymal stem cells; renal disease and transplantation; repair

Introduction

Chronic kidney disease and chronic allograft nephropathy traditionally are chronic progressive disorders where therapeutic interventions currently are aimed at slowing down loss of organ function. In recent years, it has become clear that not only fibrotic repair but also restoration of damaged kidney tissue can occur. For example, capillary repair involving both local resident cells as well as bone marrow (BM)-derived progenitor populations has been described in models varying from acute renal failure and glomeru-

lonephritis to models of chronic progressive kidney disease [1,2]. Even a classical paradigm, i.e. that podocyte loss cannot be repaired, becomes less certain with the discovery of stem cells (SCs) in the parietal epithelium of Bowman's capsule [3]. A very interesting stem cell population from a therapeutic perspective is the multipotent mesenchymal stromal cells (MSCs). These cells are present in the BM but have also been identified in the perivascular niche of many tissues including the kidney [4]. MSCs are of particular therapeutic interest because of their strong immunomodulatory effects and their capacity to enhance repair by secreting anti-fibrotic and pro-angiogenic factors. In the current review, we summarize the state-of-the-art of this intriguing cell population and describe their therapeutic potential in acute and chronic renal disease and kidney transplantation.

MSC: localization, isolation and characterization

In addition to haematopoietic stem cells, the BM stroma contains rare cells of non-haematopoietic origin (representing 0.01–0.001% of total BM cells) capable of multi-lineage differentiation into fibroblasts, osteoblasts, adipocytes and chondrocyte progenitors [5]. These BM stromal cells, known as multipotent mesenchymal stromal cells (MSCs) [6], generate the osteoblastic niche, which typically retains the haematopoietic stem cells (HSC) in a quiescent state, which is crucial for maintaining sufficient numbers of HSC (see Figure 1) [7,8]. The molecular and environmental mechanisms that control MSC differentiation are incompletely understood, and it is possible that MSCs may have a greater differential potential than was thought. Indeed, some studies have reported the capability of MSCs to differentiate into neural precursors [9], endothelial cells (ECs) [10] and hepatocyte-like cells [11]. Beside their presence in the BM, MSCs have been detected in perivascular tissues of several organs, including the kidney, and in the fat [4], and it is hypothesized that these cells may contribute to local tissue repair. At present, no unique phenotype has been identified that allows the reproducible isolation of

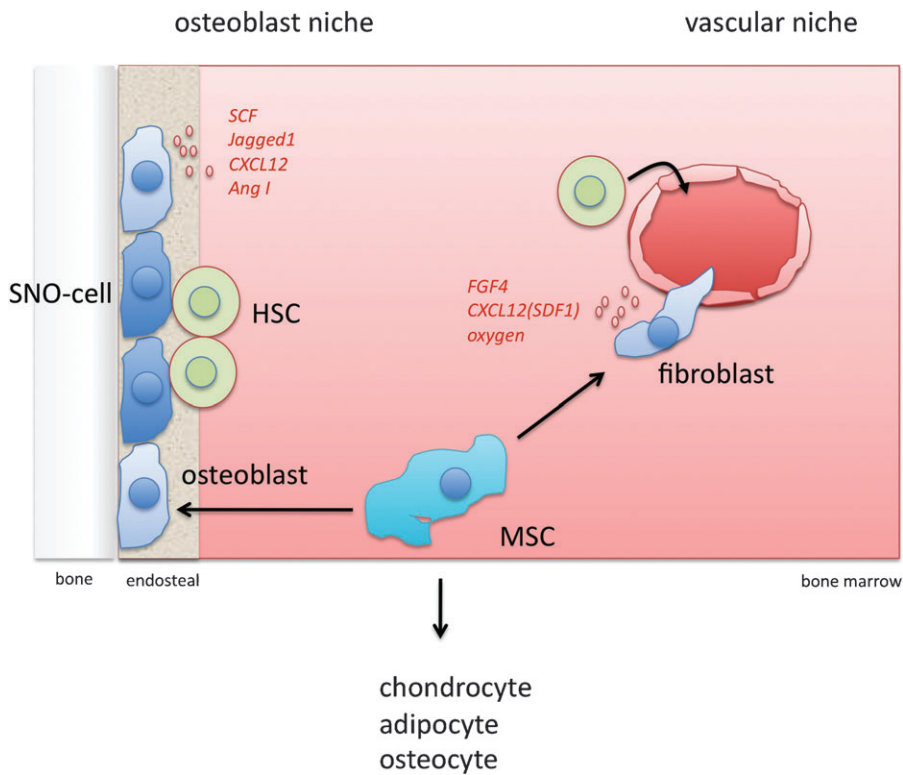


Fig. 1. Function of MSCs in the bone marrow niche. MSCs in the BM supply progenitor cells that develop into osteoblasts and fibroblasts. A subpopulation of the osteoblasts, characterized by the presence of N-cadherin [spindle-shaped N-cadherin+CD7//Osteoblastic (SNO) cell], line the bone surface and function as a key component of the niche to support survival of haematopoietic stem cells (HSCs). MSCs isolated from the BM produce several growth factors and chemokines that maintain HSC in quiescence or promote their self-renewal rather than differentiation, such as SCF, Jagged 1 (Notch ligand), CXCL12 and Ang1. MSCs can also differentiate in fibroblasts, which in turn may modulate the vascular niche, i.e. the area where HSC are recruited to the circulation and proliferate.

MSC precursors with predictable differentiation potential. Functional characterization still relies primarily on their ability to adhere to plastic and their differentiation potential (Figure 2). The International Society of Cellular Therapy recently stated that MSCs should bear at least the stromal markers CD73 and CD105 and be negative for haematopoietic markers CD14, CD34 and CD45 [12]. Important for their possible clinical application is that MSCs are easily isolated as they adhere to plastic and are capable of substantial proliferation and expansion in culture [5]. Additionally, MSCs can be cryopreserved with no loss of phenotype or differentiation potential [13].

MSCs and immunity

Several studies suggest that MSCs may play a role in modulation of immune responses [14,15]. MSCs are poor antigen presenting cells themselves and do not express MHC class II or co-stimulatory molecules, such as B7-1, B7-2, CD40 and CD40L. In accordance, expanded MSCs do not stimulate T-cell proliferation in mixed lymphocyte reactions (MLR) and are in fact able to down-regulate allo-reactive T cell responses when added to mixed lymphocyte cultures (MLC) [16]. Recently, it has been demonstrated that MSCs alter cytokine secretion profiles of naïve and effector T cells [15], dendritic cell (DC) and natural killer (NK) cells to induce a more anti-inflammatory or tolerant pheno-

type [14,17]. In addition, when present in an inflammatory microenvironment, MSCs inhibited interferon (IFN)- γ secretion from Th1 and NK cells and increased interleukin (IL)-4 secretion from Th2 cells, thereby promoting a Th1 to Th2 shift. Furthermore, MSCs have been reported to induce T-cell division arrest [18], to inhibit the differentiation and maturation of DCs [19], to inhibit B-cell proliferation [20] and to decrease the production of inflammatory cytokines by various immune cell populations [14]. These immunomodulatory properties make MSCs especially attractive for potential use in treating disease characterized by autoimmunity and inflammation, including rheumatoid arthritis, systemic lupus erythematosus (SLE), acute and chronic kidney injury and allograft rejection [14–16].

Licensing of MSCs

Studies have shown that the therapeutic use of the immunomodulatory and regenerative properties of MSCs may largely depend on the timing of their infusion [21,22]. The need for the appropriate timing is probably related to the necessity for the appropriate microenvironment to allow MSCs to acquire their immunosuppressive properties. Of interest to these observations, recent studies indicate that MSCs need to be 'licensed' in an appropriate cytokine environment before they exert their actions. Only supernatants obtained from cultures in which MSCs were incubated with activated T cells displayed an immunosuppressive effect

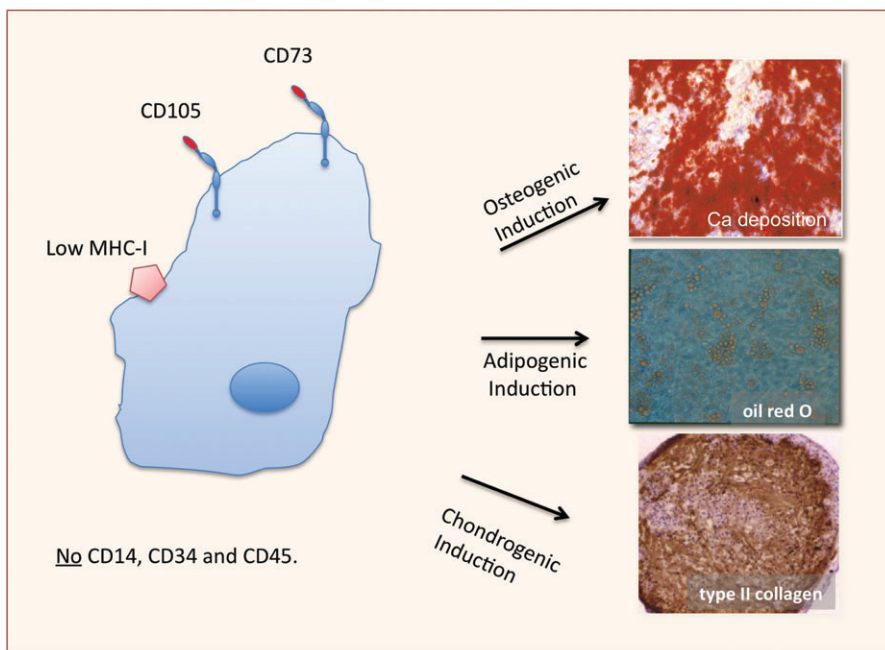


Fig. 2. Characterization of MSCs. The International Society of Cellular Therapy stated that MSCs should bear at least the stromal markers CD73 (SH3 and SH4) and CD105 (SH2 or endoglin) and be negative for the haematopoietic markers CD14, CD34 and CD45. MSCs are functionally characterized by differentiation into osteogenesis (alizarine red staining), adipogenesis (oil red O, formation of lipid vesicles) and chondrogenesis (type II collagen staining).

[23]. There was no effect detectable using supernatants from cultures of MSC alone [23]. In particular, IFN- γ was a powerful inducer of the immunosuppressive activity [24], probably via the up regulation of IDO [25]. Like IFN- γ , TNF- α has also been observed to induce immunosuppressive activity by MSCs through the production of COX-2 and PGE2 [26]. Collectively, these different studies suggest the requirement of MSC activation by T-cell- and DC-derived cytokines to be able to exert its immunomodulatory actions.

MSCs and tissue repair

Anti-inflammatory actions

Inflammation and activation of the immune system are key players in the response to injury and set the stage for repair processes by clearing debris and initiating tissue remodelling. Sustained inflammation, however, may lead to epigenetic phenotypic switches of immune cells and fibroblasts [27]. In this respect, it is of great interest that circulating fibroblasts initially share the immunomodulatory and regenerative properties of MSCs from where they are derived, while when exposed to sustained inflammatory signalling they start to contribute to scarring [28]. One mechanism by which administration of MSCs may thus contribute to tissue repair is by polarizing the immune system into its more tolerant form, as described above. Apart from its direct effects on cells of the immune system, MSCs may also modulate the microenvironment, allowing the transition of the inflammatory response to injury into one of repair [29]. In accordance, expression of pro-inflammatory cytokines IL-1 β , TNF- α , IFN- γ and inducible nitric oxide synthase have been found to be reduced, and that of anti-

inflammatory IL-10 and bFGF, TGF- α and Bcl-2 were up-regulated in MSC-treated ischaemic kidneys [30]. MSCs can also directly release hepatocyte growth factor (HGF) and bone-morphogenic protein-7 (BMP-7), which are important inhibitors of fibrosis, at least in part by reversing epithelial and endothelial to mesenchymal transformation [31]. In addition, MSCs have been shown to exert antifibrotic effects in animal models, and recently they were shown to promote matrix metalloproteinase secretion and reduce cardiac ventricular fibrosis after myocardial infarction [32]. MSCs have also been shown to enhance tubular survival [33]. As tubular cell apoptosis is an important component of acute kidney injury as well as progression of renal failure, this could have important therapeutic implications. Knock-down experiments of IGF-1 production by MSCs abrogated these cytoprotective effects, underscoring again the importance of paracrine effector mechanisms [34].

Transdifferentiation and fusion

Transdifferentiation is another mechanism that has been put forward to explain repair by SCs and progenitor cells. Although it has been reported that exogenously administered MSCs can engraft into injured tubules [33,35], there is growing evidence that the process of transdifferentiation is rare and probably does not have therapeutic relevance to renal repair from injury *in vivo* [36]. In most studies, the protective effect of injected MSCs is observed within 24–48 h, and at that time, the number of observed MSC-derived epithelial cells appears so low that a role in nephron repair by epithelial cell replacement is highly unlikely. Humphreys *et al.* found that, during repair from ischaemic injury, no dilution of the genetically labelled tubulus epithelial cells

occurred, indicating that repair was entirely derived from pre-existing tubular cells [37]. Fusion of exogenously administered MSCs with resident renal cells has been postulated as well as a potential mechanism involved in repair, as it may result in exchange of genetic material with the host cell and thus restore production of e.g. a defect protein [38]. This mechanism has been put forward as one possibility to explain the beneficial effects of BM-derived cell populations on genetic models of Alport's disease [39].

However, in general only limited potential fusion events are observed, and it may well be that some of these effects are directly related to experimental conditions such as whole body irradiation to allow for the BM transplantations in these experiments [40].

Angiogenesis and vasculogenesis

A mechanism that could be of great importance in tissue repair is the effect of MSCs on preservation of the microcirculatory bed. Indeed, a functional microvasculature has been shown of critical importance in the prevention of epithelial loss and fibrosis [41]. A large body of evidence indicates that MSCs could stabilize blood vessel formation and enhance angiogenesis *in vitro* and *in vivo* in different injury models [42–44]. Angiogenesis is a complex process controlled by a delicate balance of pro-angiogenic and antiangiogenic factors [45]. Vascular endothelial growth factor (VEGF) is a key regulator for angiogenesis and EC survival, and its interaction with VEGFR-2 results in EC activation and leukocyte recruitment. Although required as an initiating step in angiogenesis and for remodelling of the microcirculation, this phase of angiogenesis is clearly a pro-inflammatory process [46]. In contrast, Ang1-Tie2 interactions mediate neovessel maturation, reduce endothelial permeability and maintain vessel integrity through the recruitment of peri-endothelial cells and the reestablishment of the basement membrane. MSCs were shown to produce angiogenic factors that promote stabilization of the vessels and might thereby stimulate vascular integrity and physiological angiogenesis [47]. In addition, MSCs probably also participate in the control of the vascular niche in the BM, where HSC proliferation and recruitment is regulated [48]. This may explain their capacity to produce cytokines that modulate endothelial function such as VEGF and SDF1 [49].

Taken together, MSCs might be protective by creating a microenvironment that favours the stabilization of vessels and limits pathological inflammatory angiogenesis. Interestingly, by testing approaches to improving the ability of grafted MSCs to survive and secrete paracrine factors, it was shown that *ex vivo* treatment with the pineal hormone melatonin could improve survival and pro-angiogenic/mitogenic activity and efficiency of MSCs injected into ischaemic kidneys [50].

In vivo studies on MSCs in renal disease

Migration of MSCs to injured tissue

For MSC therapy to work, it is crucial that MSCs reach the site of injury. Different studies indicate that systemically

delivered MSC can indeed home to the kidney after renal injury [51]. As MSCs express a variety of chemokine receptors and adhesion molecules [52], it is most likely that chemokines are regulators of this preferential migration. Different factors have been described in controlling MSC migration to injured kidney, including CXCR4-SDF [53] and platelet-derived growth factor (PDGF) [54]. In addition, CD44 and hyaluronic acid were recently shown to recruit exogenous MSCs to injured renal tissue and to enhance renal regeneration [55]. Following these observations, in most studies MSCs are administered through a standard intravenous route. Disadvantages of systemic intravenous delivery of MSCs can be low uptake in the site of injury and their entrapment in the microcirculatory bed due to the relative large size of MSCs. For example, in mice, major amounts of intravenous-injected MSCs are trapped within the pulmonary capillaries, causing pulmonary and hemodynamic alterations and preventing the intended access to other organs [56]. One option to improve delivery and to prevent cell trapping in the microvascular bed might be to administer MSC concomitant with nitroprusside. This has been shown to increase MSC passage through the lung capillaries and potentially facilitates also cell access to injured organs [56]. Alternatively, MSCs may be administered locally, e.g. through the renal artery, or directly in the kidney capsule. The group of Behr tested direct parenchymal injection of MSCs in the kidney and gave up this approach because of bleeding and tissue destruction leading to kidney damage. Intra-renal arterial injection of autologous MSCs led to high engraftment of the targeted kidney in an ovine model of ischemia reperfusion injury [57]. More studies are needed to compare the safety and efficacy of systemic versus local routes of administration of MSCs.

Effects of MSCs on acute renal failure

Different studies have reported multiple beneficial effects of MSC infusion on acute repair in the kidney. In bilaterally ischaemic mice, MSC infusion resulted in the accelerated recovery of renal function at Days 2–3 compared to non-treated mice. Within 2 weeks, infused cells partially differentiated towards endothelial or smooth muscle cell lineage and contributed to angiogenesis, vasculogenesis and endothelial repair [42]. It is still unclear whether such beneficial effects are sustained in long-term observations after recovery of initial renal injury. One can, e.g. speculate that differentiation of MSCs towards endothelial versus myofibroblastic lineage is an important determinant of the long-term outcome of renal ischaemia.

The therapeutic potential of BM-derived MSCs of human origin was recently studied by Morigi *et al.* in immunodeficient NOD/SCID mice with cisplatin-induced acute renal failure (ARF). In this model, infused BM MSCs became localized predominantly in peritubular areas and acted to reduce renal cell apoptosis and to increase proliferation. BM-MSCs also preserved the integrity of the tubular epithelium and of peritubular vessels and also prolonged survival in ARF [43]. In the kidney, most studies on repair have concentrated on the tubulointerstitial part and only a few on glomerular repair. Rat MSCs injected into a renal artery could accelerate recovery from mesangiolytic damage

and prevent transient ARF in rat anti-Thy1.1 nephritis [58]. However, the early beneficial effects of MSCs on preserving glomeruli and maintaining renal function were offset by maldifferentiation of MSCs into adipocytes and glomerulosclerosis [59].

Effects of MSCs on chronic kidney disease

To date, few studies still have evaluated the effect of MSCs in chronic kidney disease (CKD). MSC therapy was explored in a model of chronic progressive renal fibrosis, i.e. mice that are genetically deficient of the collagen 3IV-chain (Alport mice). In this study, weekly injections of MSCs in the Alport mice prevented loss of peritubular capillaries and was associated with less fibrosis and a markedly improved renal function [39]. Different studies have analysed the effect of MSCs on the prevention of hyperglycaemia and renal damage in diabetic mice [60,61]. In diabetic NOD/SCID mice, human MSCs homed and promoted repair of pancreatic islets and renal glomeruli. The results raised the possibility that MSCs may be useful in enhancing insulin secretion and perhaps improving the renal lesions that develop in patients with diabetes mellitus [61]. A recent study demonstrated that intrauterine transplantation of human foetal MSCs improved renal glomerulopathy in a collagen type-I-deficient mouse model. The data support the feasibility of prenatal treatment for hereditary renal diseases [60].

In vivo studies on MSCs in renal transplantation

An attractive potential indication for MSC therapy in view of its potent effects on DC and T-cell biology is kidney transplantation. In a rat renal transplantation study, MSCs combined with low-dose cyclosporine A (CsA) protected graft function but could not prolong animal survival compared with CsA monotherapy [62]. This lack of beneficial effect of the MSC treated group over the CsA monotherapy may well be related to direct effects of CsA on MSC function. Therefore, the effect of lower doses of CsA in combination with MSCs are being explored to retain the beneficial effects of both [62]. MSCs may potentially not only allow for reduced doses of CsA but may also promote immune tolerance [14,63–65]. The possible role of MSCs in allograft rejection has also been studied in other transplantation models. In a study on MSCs from non-human primates, Bartholomew *et al.* [66] observed baboon MSCs to suppress the proliferative activity of allogeneic peripheral blood lymphocytes *in vitro*. They observed a modest but significant prolongation of skin graft survival with a single dose of MSCs administered intravenously. In a rat heart transplantation model, donor MSCs suppressed allogeneic T-cell responses both *in vitro* and *in vivo*, and intravenous administration of MSCs prolonged the survival of transplanted hearts, possibly by induction of allograft tolerance through changing the Th1/Th2 balance [67]. In a recent study, pre-transplant infusion of MSCs prolonged the survival of semi-allogeneic (B6C3 in B6) murine heart transplants through the generation of T regs. In this model, pre-transplant infusion of MSCs of recipient origin was as effective in inducing long-term acceptance of cardiac

allografts as donor-derived MSCs [22]. A single recipient-derived MSC infusion given peri-transplant was marginally effective, and a single MSC dose given 1 day after transplantation was not effective at all.

Although thought to be mostly immune privileged, there is also evidence that MSCs may, under certain conditions, be subject to immune rejection. In a study by Nauta *et al.*, donor-derived MSCs were immunogenic in an allogeneic host and stimulated donor graft rejection in a non-myceloablative setting [68]. In support of previous findings, it is observed that a single injection of allogeneic MSCs is sufficient to induce memory T cells that are able to eliminate subsequently injected allogeneic MSCs [17]. The possibility that allogeneic or third-part MSCs are immunogenic should be taken into account in designing clinical studies in the setting of allogeneic transplantation. Another possible source would be the use of autologous MSCs, which has been proven to be feasible and safe in different studies [69]. However, it is still unclear whether MSCs derived from patients with CKD display altered functions, and the impact of terminal renal failure and uraemia on the phenotype and functions of human MSCs is still unknown. This would be important in the clinical setting since, in that case, we could take MSCs under anaesthesia during the renal transplantation, culture these cells and keep them cryopreserved till they are needed in case of graft rejection.

Critical remarks

Due to their immunosuppressive and tissue-remodelling properties, MSCs are a promising therapeutic tool in a variety of human diseases [64,69–78]. However, today we still know little about the functional regulation of MSCs *in vivo* and in particular in disease conditions. Nonetheless, autologous and allogeneic MSC transplantation have already been used clinically for the treatment of a wide variety of diseases. Encouraging results have been demonstrated in reducing the incidence of graft-versus-host disease (GvHD), treating GvHD, promoting cardiac tissue recovery after myocardial infarction and treating fatal disorders such as Hurler syndrome [64,69–77]. Also, biotechnology companies are now developing intravenous therapy with third-part BM-derived MSCs (Provacel; Osiris Therapeutics, Baltimore, <http://www.osiristx.com>; Cellerix, <http://www.cellerix.com/>) into phase III studies for conditions varying from acute myocardial infarction, acute GvHD, impaired wound healing and Crohn's disease.

Currently, more than 1000 patients have received MSCs, and though acute toxicity appears low, little is known about long-term unwanted side effects [79]. Also, it is important not to overestimate the potential therapeutic effects of MSCs, since almost all the clinical studies reported so far are non-randomized studies. In addition, safety concerns remain over immunogenicity and the dysfunction of MSCs due to co-morbidity, as mentioned before. Moreover, potential hazards include the possibility of malignant transformation, ectopic tissue formation and the use of foetal calf serum (FCS) for their culture. Indeed, reports showed that MSCs can stimulate the growth of cancers [80] and promote metastasis in mice [81]. *In vitro* cultures of human adipose

derived MSCs have been shown to undergo spontaneous transformation after being passaged for a long time (4–5 months). However, no such abnormalities were reported by Bernardo *et al.*, who proliferated BM-derived MSCs from 10 healthy donors for up to 44 weeks until reaching either senescence or passage 25 [82]. The tendency for MSCs to undergo malignant transformation could vary according to the source (i.e. adipose tissue vs. bone marrow), species (i.e. human vs. mice) and culture conditions.

Culturing and expanding MSCs still requires the use of foetal calf serum. As the use of FCS may be associated with the xenogenic transmission of disease and antibody formation, FCS-free protocols need to be developed to overcome this problem [83].

Clinical application and future directions

It is likely that a mix of cytokines and growth factors is required for resolution of renal repair damage in both the acute and chronic settings (Figure 3). The administration of MSCs that produce such a mix of anti-inflammatory, anti-fibrotic and angiogenic cytokines may offer a novel strategy that could act as a multidrug delivery system in acute and chronic kidney disease and allograft rejection. Recent years have shown considerable progress in bringing MSC therapy in nephrology closer to the clinics (Table 1). The first phase I trial of autologous MSCs in acute kidney injury (AKI) is currently recruiting [36]. This safety study will enrol cardiac surgery patients with pre-existing renal risk factors (such as pre-existing renal disease, diabetes, age >60 years) at high risk for developing AKI. Kidney transplantation is the other indication where the potential of MSC will be explored. The initial focus here is on its

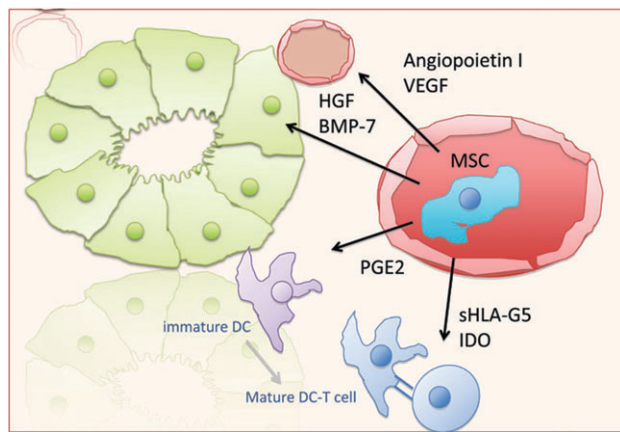


Fig. 3. Potential actions of MSCs in the kidney. MSCs have important effects on the innate and adaptive immune system. As an example, they have been demonstrated to interfere with DC differentiation, maturation and function. In addition, immature DCs in the presence of MSCs are hampered in their ability to induce activation of T cells. The suppressive effect of MSCs is mediated by a variety of soluble factors (including IDO, PGE2 and sHLA-G5). MSCs also suppress T-cell proliferation and down regulate allo-reactive T cell responses. MSCs can directly release HGF and BMP-7, which are important inhibitors of fibrosis. MSCs have direct effects on the microvasculature by enhancing angiogenesis and capillaries. All these effects by MSCs could be of major importance for repair of the injured kidney.

Table 1. Clinical trials of MSC therapy in renal disease currently registered with ClinicalTrials.gov

Trial	Cell type/dose	Delivery route/time of delivery	Primary endpoint	Design	(Estimated) patient number	Site/company
MSC and subclinical rejection	Autologous MSCs, 2 × 1–2 × 10.6/kg	Intravenous/6 weeks after Tx in the case of SCR in the renal biopsy	Safety and feasibility	Non-randomized, open label, uncontrolled	10	LUMC, single centre
MSC under basiliximab; low-dose RATG to induce renal transplant tolerance	Autologous MSCs, 2 × 10.6/kg	Intravenous/induction therapy	Safety and efficacy	Randomized, open, label, active control	6	Mario Negri Institute for Pharmacological Research, single centre
MSC transplantation in recipients of living kidney allografts	Autologous MSCs, two infusions	Intravenous/within 24 h and 2 weeks post-transplantation	Safety and efficacy	Randomized, open, label, active control	60	Fuzhou General Hospital, single centre
MSC transplantation in the treatment of CAN	MSC	?	Safety and efficacy	Treatment, open label, historical control	15	Fuzhou General Hospital, single centre
Effect of MSC transplantation in the treatment of CAN	Autologous MSCs, one infusion	?	Safety and efficacy	Treatment, open label, active control	20	Fuzhou General Hospital, single centre
MSC transplantation for refractory SLE	Allogeneic MSCs	Intravenous, 1 × 10.6/kg	Safety and efficacy	Treatment non-randomized, open label	20	Nanjing Medical University
Allogeneic MSC treatment for acute kidney injury following cardiac surgery	Allogeneic MSCs	Intra-aortic, dose-escalating	Safety	Treatment non-randomized, open label	15	Intermountain Medical Center, single centre

potential as induction treatment and to treat allograft rejection. At the same time, many critical issues for our understanding of MSC biology still need to be elucidated before we can develop MSCs into an accepted clinical treatment. Apart from the issues described in this paper such as conditioning of differentiation and immunological properties, they also include a robust regulatory and ethical framework for good manufacturing practice (GMP) production of a reproducible, well-characterized and biologically predictable MSC product.

Conflict of interest statement. None declared.

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