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TOPIC HIGHLIGHT

WJG 20th Anniversary Special Issues (7): Liver transplant

Rationale for the potential use of mesenchymal stromal cells in liver transplantation

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Abstract

Mesenchymal stromal cells (MSCs) are multipotent and self-renewing cells that reside essentially in the bone marrow as a non-hematopoietic cell population, but may also be isolated from the connective tissues of most organs. MSCs represent a heterogeneous population of adult, fibroblast-like cells characterized by their ability to differentiate into tissues of mesodermal lineages including adipocytes, chondrocytes and osteocytes. For several years now, MSCs have been evaluated for their *in vivo* and *in vitro* immunomodulatory and 'tissue reconstruction' properties, which could make them interesting in various clinical settings, and particularly in organ transplantation. This paper aims to review current knowledge on the properties of MSCs and their use in pre-clinical and clinical studies in solid organ transplantation, and particularly in the field of liver transplantation. The first available clinical data seem to show that MSCs are safe to use, at least in the medium-term, but more time is needed to evaluate the potential adverse effects of long-term use. Many issues must be resolved on the correct use of MSCs. Intensive *in vitro* and pre-clinical research are the keys to a better understanding of the way that MSCs act, and to eventually lead to clinical success.

Key words: Mesenchymal stem cells; Organ transplantation; Complication; Immunosuppression; Tolerance

Core tip: For several years now, mesenchymal stromal cells (MSC) have been evaluated for their *in vivo* and *in vitro* immunomodulatory and 'tissue reconstruction' properties which could make them interesting in various clinical settings, and particularly in organ transplantation. This paper aims to review current knowledge on the properties of MSCs and their use in pre-clinical and clinical studies, and particularly in the field of liver transplantation.

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INTRODUCTION

Mesenchymal stromal cells (MSCs) are multipotent and self-renewing cells that reside essentially in the bone marrow as a non-hematopoietic cell population. MSCs represent a heterogeneous population of adult, fibroblast-like cells characterized by their ability to differentiate into tissues of mesodermal lineages including adipocytes, chondrocytes and osteocytes. In addition to the bone marrow, MSCs have been isolated from various other tissues such as adipose tissue[1], skin[2], heart and spleen[3], placenta[4], umbilical cord blood[5] as well as lung and liver[6,7], and it appears that MSCs reside in the connective tissues of most organs[8].

No specific marker for MSCs has yet been found. Presently, MSCs are identified using a number of features defined by the International Society for Cellular Therapy which states three minimal criteria[9]: (1) adhesion to plastic in standard culture conditions; (2) expression of CD105, CD73 and CD90, and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19 and Human Leukocyte Antigen (HLA)-DR surface molecules; and (3) *in vitro* differentiation into osteoblasts, adipocytes and chondroblasts.

For several years now, MSCs have been evaluated for their *in vivo* and *in vitro* immunomodulatory and "tissue reconstruction" properties that could make them interesting in various clinical settings such as organ transplantation. This paper aims to review current knowledge on the properties of MSCs and their use in pre-clinical and clinical studies in solid organ transplantation, and particularly in the field of liver transplantation.

IMMUNOMODULATORY EFFECTS OF MSCS

A large number of *in vitro* and *in vivo* studies have documented the anti-inflammatory and immunoregulatory properties of MSCs on both the adaptive and innate immune system. However, there is strong evidence that MSCs are not constitutively immunosuppressive, they have to be "activated" or primed by local inflammatory conditions. Tumor necrosis factor (TNF)-α, interleukin (IL)-1β and interferon (IFN)-γ are the key cytokines to allow MSC immunomodulation by regulating their immunophenotype[10,11]. The high dependence on environment settings could also explain conflicting data in some *in vitro* and *in vivo* studies. These settings must be further studied and considered in clinical trials.

MSC immunogenicity

Both human MSCs (hMSCs) and murine MSCs (mMSCs) show low immunogenicity and do not lead to alloreactive T lymphocyte-mediated immune response *in vitro*. Indeed, under normal conditions, MSC membranes express low levels of human leukocyte antigen (HLA) class I molecules and do not express HLA class Ⅱ (major histocompatibility complex (MHC)-Ⅱ) nor co-stimulatory molecules[12,13]. MSCs were thus considered as immune privileged cells. However, more recent data with mMSCs has suggested that MHC-I on MSCs could present antigen to CD8+ T cells[14]. In addition, a narrow window of IFN-γ could induce MSCs to upregulate MHC-I and MHC-Ⅱ and thus, induces an "antigen presenting cell-like" function. This finding has been observed with both mMSCs and hMSCs[10,15-17]. Furthermore, it has been demonstrated in an animal model of bone marrow[18] and skin transplantation[19] that donor-derived MSCs could be immunogenic and could promote graft rejection.

MSC interaction with immune cells

It is important to highlight that, in some experimental conditions, effects of mMSCs and hMSCs have been evaluated on murine immune cells. Results are not always transposable to human clinical conditions, especially as it is well known that tolerance is more easily achieved in animal models than in humans.

It has been demonstrated *in vitro* and *in vivo*, that MSCs may exert their immunomodulatory effects by acting on many types of immune cells including T cells, B cells and natural killer (NK) cells. The ability of MSCs to inhibit T cell proliferation has been shown in various experimental settings both with mMSCs and hMSCs. *In vitro*, hMSCs highly inhibit proliferation and cytokine production[20] as well as the development of human cytotoxic CD8+ T cells in mixed-lymphocyte reactions (MLRs)[21,22]. Moreover, it has been observed that MSCs promote human T cell anergy and inhibit alloreactive T cells through a T_H2 pathway[23]. Nevertheless, it appears that the effect of MSCs on T cells is dependent on the dose used. While a high MSC/T cell ratio exert strong inhibitory effects, low MSC/T cell ratios enhance T cell proliferation[24].

MSC-induced T-regulatory (T-reg) cell recruitment and generation probably play an important role in MSC-mediated immunomodulatory effects. This has been observed both *in vitro*[25,26] and *in vivo*[27,28] both on murine and human immune cells. Additionally, previous studies have shown that T-reg induced production requires cell contact and some MSC released factors such as prostaglandin (PG)-E2 and tumor growth factor (TGF)-β1[29] or HLA-G[30,31]. It has been suggested that this effect could also be partially mediated by an interaction between MSC chemokine (c-c motif) ligand 1 (CCL1) and its receptor on T cells, chemokine (c-c motif) receptor 8 (CCR8)[23]. More recently, it has been demonstrated that mMSCs could promote T-reg expansion by their effects on immature dendritic cells[32].

Published results on the effects of hMSCs on B cells and NK cells are contradictory. Some studies have demonstrated that MSCs could inhibit the proliferation and immunoglobulin secretion of B cells[33-35] while others have found no effect of MSCs on human B cell proliferation[11,21]. Some researchers have even found that MSCs could stimulate human B cell proliferation and antibody secretion[36,37]. MSCs have shown an ability to inhibit the proliferation of IL-2 or IL-15 stimulated human NK

cells[38,39] and their IFN-γ production[38]. The effects of MSCs on the cytotoxic activity of NK cells are even more controverted. While some studies failed to find such an effect[40] (especially in freshly isolated NK cells[41]), others have demonstrated that MSCs could inhibit NK-cell cytotoxicity[30,39]. As MSCs express HLA-1 antigens, even at a low level, it appears that they may be vulnerable to activated NK-cell lysis[42].

Many studies have shown that MSCs can prevent the differentiation, maturation and functions of antigen-presenting cells (APCs), such as human or murine dendritic cells (DC)[17,43,44], and thus indirectly modulate T and B cell functions. In addition, it was shown that mMSCs may induce murine mature DC into a Jagged-2-dependent regulatory DC population[45]. MSCs may also exert effects on innate immune cells, for example through increased IL-10 secretion by macrophages in mice[46].

Mechanisms

The mechanisms of immunosuppression by MSCs remain unclear. Whereas MSCs exert their effect by direct cell contact *via* the expression of adhesion molecules, it has also been shown that the immunomodulatory and anti-inflammatory properties of MSCs mainly involve the production of secreted soluble factors. It has been observed that MSCs are still immunosuppressive without cell contact[22]. It should be noted that the mechanisms of MSC-mediated immunosuppression seems to vary from one species to another[47].

Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catalyses the degradation of tryptophan. The resulting depletion of tryptophan and the accumulation of its metabolites have shown strong inhibitory properties on immune cells, including human T cells[48], activated B cells[11] and NK cells[39]. MSCs do not constitutively express IDO, but IDO can be upregulated under inflammatory conditions, for example after exposure to IFN-γ, TNF-α and IL-1[47,48]. IDO could play an important role regarding transplantation given that it has been shown to partially inhibit allo-responses of T cells *in vitro*, and to enhance tolerance towards the graft and allogeneic T cell transfer *in vivo*[49,50]. IDO seems to be predominant in human MSC-mediated immunomodulatory properties[47]. However IDO does not seem to be the only mechanism implicated as in some conditions where MSCs do not express IDO they keep their immunomodulatory properties[51]. A high concentration of nitric oxide (NO) is known to inhibit the immune response in both *in vitro* and *in vivo* studies. It has been shown to inhibit the proliferation of T cells in murine models. NO is synthetized by the inducible NO synthase (iNOS) that is induced in murine MSCs by interaction with CD4+ or CD8+ lymphocytes in inflammatory conditions involving IFN-γ and TNF-α or IL-1[52,53]. As in the case of IDO for human MSCs, iNOS appears to play a major role in murine MSC-mediated immunomodulation[47,52]. Both tryptophan depletion and NO are expected to have an exclusively local action[54,55].

The HLA-G protein is a non-classical human MHC-I molecule. Initially found in trophoblasts, where it plays a crucial role in maternal-fetal tolerance[56], HLA-G has recently been involved in immunomodulation by MSCs[57]. HLA-G has shown tolerogenic properties *inter alia* due to its interactions with inhibitory receptors on dendritic cells, NK, and T cells. Selmani *et al*[30] have demonstrated that hMSCs, by secreting the soluble isoform HLA-G5, are capable of inhibiting human allo-activated T lymphocytes, NK-cell cytolysis and IFN-gamma secretion, and of promoting the expansion of $CD4^+CD25^{high}FoxP3^+$ regulatory T cells. Likewise, HLA-G can promote $CD3^+CD4^{low}$ and $CD3^+$ $CD8^{low}$ immunosuppressive T cells. It seems that HLA-G expression is IL-10-dependent and needs close cell contact with alloreactive T cells[30]. It has been suggested that co-injection of HLA-G and MSCs could be used to prevent rejection in organ transplantation.

Another candidate mechanism involves the role of PGE2 (Prostaglandin E2) secreted by MSCs. It appears that MSC-derived PGE2 is involved in MSC-mediated immunomodulation by acting on murine and human T cells (in both T_H1 and T_H2 responses), NK cells and macrophages[46,58]. Prostaglandins have a short half-life. This suggests that they play their role using a paracrine or autocrine action mechanism. Furthermore, it has been observed in human MSCs that IDO and PGE2 have a synergistic inhibitory effect on T cell proliferation, and on the proliferation and cytotoxicity of NK cells[39,59]. However, other studies suggest that PGE2 could in fact have an immunostimulatory role by facilitating T_H1 cell differentiation and T_H17 cell expansion[60].

IL-10 plays an important role in MSC-mediated immunosuppression through the induction of IL-10 production in APCs[61]. Nevertheless, no direct secretion of IL-10 by MSCs has yet been proven.

Blocking each of these factors alone does not restore immune cell function and proliferation, indicating that multiple factors are involved.

Other factors are also secreted: TGF-β and Hepatocyte growth factor (HGF)[20] (inhibition T-lymphocyte proliferation), IL-1 receptor Antagonist[62] (anti-inflammatory), Peptide LL-37[63] (anti-inflammatory and anti-bacterial), Matrix Metalloproteinase (MMP) 3, MMP9[64] (acting on neoangiogenesis), angiopoietin-1[65] (acting on protein permeability). TNF-α and insulin-like growth factor-binding proteins[51] also seem to be implicated.

On the other hand, MSCs also have the ability to secrete pro-inflammatory chemokines and cytokines, such as monocyte chemo-attractant protein 1 (MCP-1 or CCL2)[66], IL-6, IL-8, soluble ICAM-1, Interferon gamma-induced protein 10 (IP-10 or CXCL10) and MCP-2 (or CCL8). The secretion of these factors is dependent on inflammatory conditions and could enhance immune response *via* immune cell attraction[67]. Therefore, MSCs appear to have a dual immunomodulatory capacity depending on the above-identified secreted factors.

The mechanisms involved in the immunomodula-

tory capacity of MSCs are complex and remain largely unknown. Their properties seem to be highly dependent on many parameters in which local immunologic conditions seem to play a crucial role. Finally, it is important to know that there is currently no single standard method to isolate MSCs. It is thus conceivable that changes in the culture medium used to increase and select MSC population may influence their properties.

TISSUE REPAIR/"ORGAN RECONSTRUCTION" EFFECT

In addition to their ability to differentiate into cells of the mesenchymal lineage, it has been demonstrated that MSCs can also differentiate *in vitro* into other cells such as neurons[68], cardiomyocytes[69], tubular epithelial cells in kidneys and hepatocytes[70-72]. They are also capable of differentiating and engrafting into many tissues, especially if an inflammatory signal is present[73]. These data have motivated further research in the field of MSCs as potential "tissue repairers". Cultured MSCs have shown strong evidence of "tissue repair" properties in response to tissue injury or disease in many animal models with myocardial infarction[74], kidney disease[75,76], lung injury or some neurological disorders[64]. In clinical trials, MSCs have been used successfully to treat bone and cartilage diseases[77] (*e.g.*, osteogenesis imperfecta), as well as acute and chronic myocardial infarction[78-80].

MSCs have shown the ability to home in on injured tissue after intravenous infusion. It has been demonstrated that MSCs can express several chemokine receptors such as CCR1, CCR7, CXCR4, CXCR6, CX3CR1[81], CCR4, CCR10, CXCR5[82], c-Kit, c-Met[83], VEGF receptors[84] and PDGF receptors[85]. This variety of receptors and the chemotactic migration they have shown in response to the stimulating chemokines and cytokines could partially explain their ability to migrate to sites of inflammation. This hypothesis assumes that the injured tissue also expresses specific receptors facilitating the adhesion and migration of MSCs. However, the exact mechanism of homing in on injured tissue remains largely unknown.

Nevertheless, many studies have observed that MSCs are significantly trapped in the lung after intravenous infusion[86,87]. Despite their ability to migrate to inflammation sites and to differentiate into many tissues, MSCs exhibit very low and transient levels of engraftment *in vivo*[86,88]. For example, in a mouse model of acute myocardial infarction, a significant improvement of myocardial function was observed after human MSC injection, while no donor cell could be detected 3 wk after infusion. In a rat model, no MSC could be found in the liver within 7 d after injection of syngeneic rat MSCs in recipient livers through the portal vein[89]. Contradictorily, in a clinical trial treating myocardial infarction with intracoronary injection of MSCs, the MSCs were still viable 3 mo after transplantation[90]. In another study, MSCs were detected in various tissues of baboons 19 mo after intravenous injection[88].

In fact, it is thought that MSCs are likely to act through the secretion of soluble factors and change of the tissue microenvironment with paracrine interactions, rather than through their transdifferentiation capacity[91,92]. However, current *in vivo* data are not sufficient to define the exact mechanism. It has been demonstrated that MSCs could facilitate tissue repair by stimulating angiogenesis[93] and inhibiting apoptosis, as well as fibrosis, in the site of injury[94].

Furthermore, there is much evidence supporting the protective effect of MSCs in acute kidney injury models[95]. It appears that MSCs could increase the proliferation of tubular cells and reduce apoptosis[96,97]. There is a lack of data on the treatment of liver injury with MSCs, but their properties and regenerative potential mentioned above have encouraged researchers and clinicians to investigate further in this field. They could play a therapeutic role in the replacement of diseased hepatocytes, and the stimulation of their regeneration through the action of trophic molecules[98].

In a study on acute liver injury, rats were successfully treated with MSC infusion, with a decrease of biochemical markers of liver injury and an improved survival rate. Hepatocyte replication was enhanced while apoptosis decreased by 90%[98]. Similarly, it has been demonstrated that MSCs are efficient in treating fulminant hepatic failure in rats[99]. Otherwise, it has been suggested that MSCs could only be efficient in a therapeutic window, indicating that higher doses could paradoxically be inefficient or even induce liver fibrosis[98].

Although it is hoped that MSCs could potentially be an alternative to liver transplantation in end-stage liver disease, or a potential temporary solution to maintaining liver conditions of patients waiting for a graft, MSCs have been tried in only a small number of clinical trials to treat cirrhosis.

In a phase Ⅰ-Ⅱ trial, 8 patients with end-stage liver cirrhosis were treated with the infusion of autologous MSCs *via* a peripheral or portal vein. The treatment was well tolerated, with no significant adverse effects and the liver function was significantly improved[100]. A randomized placebo-controlled trial using MSCs to treat decompensated cirrhosis has recently been published[101]. Out of 27 patients, 15 received autologous bone marrow MSCs *via* a peripheral vein and 12 received a placebo. The results were evaluated using the Model for End-Stage Liver Disease (MELD) score, Child-Pugh score, liver function tests and liver volume. In this study, there was no beneficial effect of MSC infusion in cirrhotic patients. It is clear that other studies with larger cohorts are necessary to clarify the therapeutic potential of MSCs in cirrhosis.

ANTI-OXIDATIVE EFFECT/TREATMENT OF ISCHEMIA REPERFUSION INJURY

Ischemia reperfusion injury (IRI) is caused by the blood supply returning into a tissue after an ischemic period.

This sudden reperfusion and oxygenation paradoxically impairs the endothelium with a dilatation in arterioles, increased fluid filtration and plasma protein extravasation from post-capillary venules, as well as an increased production of oxygen radicals and a reduction of nitric oxide generation. This imbalance leads to the release of inflammatory mediators (*e.g.*, TNF, platelet activating factor) and the expression of adhesion molecules that cause leukocyte adhesion to the endothelium[102]. This results in the stimulation of both innate and adaptive immune responses with an accumulation of immune cells, followed by organ damage. The release of danger-associated molecular patterns (DAMPs) and the complement system are also implicated[103].

Solid organ transplantation is impacted by IRI, which contributes to acute graft rejection, delayed graft function and enhanced immunogenicity. IRI represents a major concern in liver transplantation, and use of MSCs in IRI has been studied for solid organ transplantation in animal models and in clinical trials.

MSCs seem to be recruited by hypoxic and injured tissues that express adhesion molecules and a SDF-1 gradient stimulating CXCR4 and CXCR7 on these cells[104]. Furthermore, it has been demonstrated that MSCs can transmigrate through TNF-alpha activated endothelium to join the inflamed tissue[105]. Lately, Pan *et al*[106] found that the inactivation of the MEK/ERK signalling pathway by MSCs plays a major role in the improvement of hepatic IRI in rats.

Prevention and treatment of liver IRI in animal models

MSCs have shown therapeutic effects for the treatment of IRI in the kidney, heart and lung in a significant number of studies[107]. Only a few studies have been published for IRI in the liver, and the exact role of MSCs has not yet been defined.

Jin *et al*[108] recently evaluated the effect of allogeneic bone marrow (BM)-derived MSCs to attenuate IRI in rats during the first 24 h after liver reperfusion. In their model partial ischemia was obtained by vascular clamping during 60 min. BM-MSCs were injected through the portal vein. Injury severity, oxidative stress response and apoptosis of the liver was regularly evaluated during the first 24 h and compared to a sham-transplanted control group. The conclusion of this study is that allogeneic BM-MSCs partially protect the liver from IRI when injected *via* the portal vein due to their ability to suppress oxidative stress and to inhibit apoptosis. Another related model using adipose-derived MSC injections *via* a peripheral vein in mice also showed a significant protective effect against liver IRI[109].

In addition to liver IRI, research has also focused on the potential beneficial effect of MSCs in partial liver transplantation. In a recent study 50% reduced-size liver transplantations in rats were used to examine whether MSC-conditioned medium (MSC-CM) could protect hepatocytes and sinusoidal endothelial cells (SEC) and enhance their regeneration[110]. MSC-CM was injected in rats *via* a peripheral vein directly after orthotopic partial liver transplantation. Compared with the control group, the MSC-CM group showed a significantly lower release of liver injury biomarkers and a clear survival benefit. More proliferating hepatocytes and SECs, and less apoptosis were observed. Many inflammatory cytokine levels and the infiltration by neutrophils and Kupffer cell activation were decreased. VEGF and MMP-9 expression was increased in the graft. All these facts suggest that MSC-CM could have potential in prevention of liver injury, and to enhance its regeneration in partial liver transplant. Kanazawa *et al*[111] also found in a model of IRI with major hepatectomy that MSCs protected the liver from IRI and that liver regeneration was enhanced.

However, it has been demonstrated in a liver IRI model that intravenously injected MSCs are short-lived, that viable MSCs do not go beyond the lungs, and that they remain in the circulation for a very limited period[112]. It has thus been suggested that other cells should be implicated to mediate the powerful immunomodulatory and regenerative properties of MSCs on target organs.

POTENTIAL USE OF MSCS IN LIVER TRANSPLANTATION

Liver transplantation represents the unavoidable treatment of end-stage liver diseases. Despite satisfactory long-term results, transplantation success mostly relies on immunotolerance, *via* acceptable graft-host immune matches and immunosuppressive measures. The latter unfortunately exposes the patient to the classical consequences of a down-regulated immune system, such as opportunistic infections and the typical outbreak of neoplasms. Due to their immunomodulatory properties, MSCs could prove highly effective in obtaining sufficient immunotolerance to reach even higher success rates while avoiding excessive immunosuppression, and thus severe and life-threatening side effects.

MSCs as immunomodulation therapy in transplantation

MSCs for graft-*vs*-host disease after hematopoietic cell transplantation: A clinical success? Graft-*vs*-host disease (GVHD) is a major complication frequently observed after hematopoietic cell transplantation (HCT), resulting from the attack of recipient organs by donor lymphocytes. MSCs might play a role in the treatment of GVHD through their immunomodulatory effects rather than their regenerative properties. Although pre-clinical studies for the prevention or treatment of GVHD by MSCs gave rise to conflicting results, MSCs have shown a clear efficacy in clinical trials, especially in steroid-resistant GVHD[113]. In a phase Ⅱ study, 68% of patients with acute steroid-resistant GVHD showed a complete response to MSC infusion with a significant decrease in mortality[114]. A series of other studies have shown similar results with varying degrees of GVHD, suggesting that MSCs have a serious potential future in GVHD management[115-117].

MSCs in solid-organ transplantation

Animal models: MSC infusion has shown the ability to prolong graft survival in heart[118-120], skin[121] and kidney[122-124] animal transplantation models. However, one group found no effect of MSCs alone on heart allograft survival in a mouse model[125], and another group found that MSCs infused after kidney transplantation could cause premature graft dysfunction[122].

Only a few studies have been published in liver transplantation models. In one such study, it was demonstrated that adipose-derived MSCs significantly decreased acute rejection after orthotopic liver transplantation in rats[126], based on serum rejection markers and on hepatocyte apoptosis. Serum levels of IL-2 were reduced and those of IL-10 were increased. In this model, MSC were infused intravenously 7 d before and 3 d after liver transplantation as well as during the operation *via* the portal vein. MSCs also played a role in a discordant liver xenotransplant model by alleviating acute rejection[127].

Another group studied the ability of BM-MSC infusion to inhibit acute graft rejection after allogeneic liver transplantation in rats[128]. MSCs were derived from the recipient, the liver donor or a third party, and infused intravenously at the time of surgery as well as once daily for 3 d thereafter. MSC-treated recipients survived significantly longer compared with the control group. Furthermore, there was no significant difference between the 3 groups receiving MSCs from various origins. Histological analysis showed severe acute graft rejection at day 7 in rats without MSC infusion, while acute graft rejection was significantly decreased in the other groups. These observations were associated with a marked increase in the number of T-reg cells in recipients receiving MSCs. This suggests an important role of T-reg cells in MSC-mediated immunosuppression.

Available data in humans (kidney transplantation)

Results of a phase I clinical trial studying the treatment of allograft rejection after kidney transplantation by autologous BM-MSCs, have recently been published[129]. The MSC-based treatment was well-tolerated and no related serious adverse effects were reported. Two MSC infusions were performed after a biopsy-proven rejection or interstitial fibrosis/tubular atrophy (IF/TA). In this study, MSCs showed their ability to reduce IF/TA. In addition, a donor-specific down-regulation of the peripheral blood mononuclear cell proliferation was shown. However, a potentially increased susceptibility to opportunistic infections was observed, with the development of viral infections in 3 out of 6 MSC-treated patients.

In a randomized controlled trial in living donor kidney transplantation, Tan *et al*[130] demonstrated that, in comparison with antibody induction therapy, induction by autologous MSCs significantly correlated with fewer acute rejections, a lower risk of opportunistic infections and a better renal function at 1 mo. Furthermore, fewer adverse effects were seen in both autologous MSC groups compared to the control group. This study was conducted on 156 patients recruited from February 2008 to May 2009 and divided into 3 groups (group 1 and 2 received MSCs at kidney reperfusion and two weeks later, plus a standard dose or low dose of calcineurin inhibitors (CNIs), respectively. The control group received anti-IL-2 receptor antibody plus standard-dose CNIs.

In a pilot study, Perico *et al*[131] injected autologous BM-MSC in 2 living-related kidney transplant recipients at day 7 post-transplant, after induction therapy with basiliximab/low-dose thymoglobulin. The peripheral blood showed a progressive increase of the T-reg population and a strong inhibition of memory/effector CD8 T cell function/expansion, promoting a long-term tolerogenic environment compared with the control group. However, a few days after MSC infusion transient renal dysfunction was observed. A biopsy excluded graft rejection but revealed a focal inflammatory infiltrate with neutrophil and MSC recruitment as well as a complement-C3 deposition.

The same group also investigated pre-transplant infusion of autologous BM-MSCs in 2 living-related kidney transplant recipients[132]. No renal dysfunction was observed while MSC immunomodulatory properties were preserved. In addition, it was observed that the avoidance of basiliximab in induction therapy did not facilitate further T-reg expansion.

In another recent pilot study, six patients transplanted with living-donor related kidneys received 2 donor-derived BM-MSC infusions (the first at the time of transplantation, the second one month later) in combination with sparing doses of tacrolimus[133]. Six other patients were used as a control group and received standard doses of tacrolimus and no MSCs. The MSC-treated group had stable renal function 12 mo post-transplant despite reduced tacrolimus compared with the control group. No acute rejection occurred, except for one in the control group. Significantly increased B cell levels were observed in the MSC-treated group 3 mo after transplantation. No toxic side effects were associated with MSC infusion.

Ongoing clinical trials in liver transplantation

MSC Liege study: Taking advantage of our expertise and experience concerning the use of MSCs in the HCT context[115], and using an already functioning good manufacturing practice (GMP)-compliant laboratory able to produce clinical-grade MSCs, we initiated a first trial in 2011 exploring the safety and tolerability of third-party MSC infusions after kidney or liver transplantation in a prospective phase I - II study (NCT01429038).

In this study, after successful transplantation, 10 liver and 10 kidney transplant recipients under standard immunosuppressive treatment (tacrolimus, mycophenolate mofetil (MMF) and steroids) receive an intravenous infusion of 1.5×10^6/kg-3×10^6/kg of third-party MSCs on post-operative day 3 ± 2. These patients are prospectively compared to the same number of liver or kidney transplant recipients who meet inclusion criteria but have not not received MSC infusion. Safety is assessed by recording side effects, including opportunistic infections and

cancers. The immunosuppressive potential of MSCs will be evaluated by the rate of rejection episodes, graft/patient survivals, immunohistology of 3-mo (kidney) and 6-mo (liver) graft biopsies and *in vitro* evaluation of patient immune functions. In a second step, reduction (kidney) and progressive weaning (liver) of immunosuppression will be attempted in recipients who received MSCs. Final results are expected by the end of 2014. The next step will be to assert the immunosuppressive potential of MSCs after organ transplantation, and the opportunity to develop larger, randomised and controlled phase Ⅲ trials.

"Mesenchymal stem cells in solid organ transplantation"-1 study: In a mesenchymal stem cells in solid organ transplantation phase I study (MiSOT-I) started in April 2013, the safety of MultiStem® infusion for immunomodulation after liver transplantation has been evaluated (NCT01841632). MultiStem is a new biological product derived from multipotent adult progenitor cells (MAPCs) which belong to the family of MSCs. Patients, divided into four cohorts, will receive 2 doses of MultiStem (first intraportal at liver transplantation, second at day 3 post-transplant) in addition to immunosuppression (calcineurin-inhibitor-free 'bottom-up' immunosuppressive regimen with basiliximab, mycophenolic acid, and steroids). From cohort 1 to 4, an increasing dose escalation is performed (3-6 patients in each group). The primary outcome will be infusional and acute toxicity (intraportal, pulmonary and systemic). The secondary outcomes will be biopsy-proven acute rejection, whether MultiStem promotes malignant transformationnor tumor growth, and the long-term safety of MultiStem administration (up to 6 years). Final results are expected in 2016.

The Beijing study

A third study is ongoing. This phase I study will include a total of 50 patients randomly assigned to two groups; in the first group, patients will receive conventional immunosuppressive agents plus umbilical cord (UC-) MSCs at the day of liver transplantation and then once every 4 wk, at a dose of 1×10^6 UC-MSCs/kg for 12 wk (NCT01690247). In the second group patients will receive conventional treatment plus a placebo. Both groups will be followed for 48 wk. The study will evaluate the incidence of acute rejection and early liver function recovery, as well as patient and graft survival rates, and the prevalence of adverse events as secondary outcomes.

VARIABLES TO BE CONSIDERED/ISSUES TO BE RESOLVED

At present many questions remain unanswered in the field of MSCs therapy in solid-organ transplantation. These issues could explain the conflicting data obtained in previous studies. Further *in vitro* investigations and pre-clinical studies could help to define the settings of future clinical trials through a better understanding of the mechanisms of action of MSCs.

Dosage and sources of MSCs

The ideal amount of MSCs necessary to achieve some clinical effect has not yet been studied, and additionally, the ideal source of MSCs in the setting of organ transplantation has not been determined. Usually isolated from the bone marrow, MSCs can now be isolated from other more easily accessible human tissues such as adipose tissue or cord-blood. Compared with BM-derived MSCs, adipose- and cord- derived MSCs have comparable phenotypical and immunomodulatory properties[134]. Nevertheless, it seems that many genes are differentially expressed in MSCs depending on their tissue origin[135]. These differences could alter the function of MSCs in clinical use.

Although not quite clear, it should be noted that MSCs derived from adipose tissue seem to be more likely to develop chromosomal abnormalities than BM-derived MSCs, after many passages in culture[136,137]. High-passage MSCs should thus be avoided for clinical applications.

Origin of MSCs- autologous vs allogeneic

MSCs can be isolated from the organ recipient (autologous) or from the organ donor, or from a third party (allogeneic).

While some have suggested that allogeneic MSCs may be more efficient as immunosuppressors[138], others have shown in animal models that donor-derived MSCs could be preferable[139]. In a recent study, it has been demonstrated that both autologous and allogeneic MSCs were able to inhibit alloreactivity and had comparable efficacy[22,127].

In terms of alloreactivity, MSCs appear to bear low immunogenicity (see above). In a clinical case of osteogenesis imperfecta, no sign of alloreactivity was observed in the recipient after infusion of fully mismatched allogeneic MSCs[140]. Yet some papers have reported the induction of memory T cell responses and immune rejection after allogeneic MSC infusion[18,141]. One cannot exclude that donor-derived MSCs could induce alloreactivity and accelerate graft rejection. Nevertheless, in the field of kidney transplantation, Crop *et al*[22] have demonstrated that donor-derived MSCs are not immune-rejected and are even able to inhibit alloreactivity in kidney transplant patients when infused before transplantation.

MSC interaction with immunosuppressive drugs

In clinical transplant studies, MSCs are used concomitantly with immunosuppressive drugs. As MSCs and immunosuppressive drugs inhibit the same targets (essentially T cells), it is reasonable to consider that interactions between them can occur. Therefore, it is essential to know which drugs can (positively or negatively) affect MSC function.

In vitro, some have shown that tacrolimus (a calcineurin inhibitor) and rapamycin (a mTOR inhibitor) decrease MSCs immunosuppressive properties[142], and conversely, that MSCs reduce the immunosuppressive capacities of tacrolimus and rapamycin. Such an effect has not been

found with mycophenolic acid (MPA). Moreover, a high dose of tacrolimus seems to be toxic for MSCs, while MPA and rapamycin at a therapeutic dose just inhibit MSC proliferation[143]. Nevertheless, others have shown that cyclosporine A (CsA) (another calcineurin inhibitor) and MSCs exert cumulative effects against alloactivated lymphocytes[138]. Furthermore, it has been demonstrated that MPA and MSCs have a synergistic immunosuppressive effect[143].

In vivo, MPA and MSCs also synergize to promote long-term allograft tolerance in rat heart transplantation[144]. In contrast to what is observed *in vitro*, rapamycin and MSCs synergize as immunomodulators to promote cardiac allograft long-term survival[119]. Moreover, in a rat renal transplantation model, it has been shown that CsA antagonizes MSC efficacy, and that this combination has no advantage in terms of allograft survival rates compared with CsA alone[122]. Nevertheless, this study has to be contrasted with other studies using various immunosuppressive drug used together with CsA in which MSC efficacy was not altered[19,110]. The choice of concomitant immunosuppressive drugs is an important matter for debate, and more studies are needed to define which are the most effective drugs to use with MSCs.

Timing of administration of MSCs

MSCs can be injected before, during or after transplantation, and with single or repeated injection(s). Timing of administration is another important point for discussion. It has been shown *in vivo* that pre-transplant infusion could be more effective than peri-transplant infusion in preventing graft rejection in a murine heart transplantation model[120]. On the other hand, it has been demonstrated that MSCs are effective in the treatment of steroid-resistant GVHD[113], so at the peak of the disease. In a clinical trial, Perico *et al*[131] observed that early post-transplant infusion of MSCs could induce a transient renal dysfunction. This group is now investigating pre-transplant infusions[132].

Protocols investigating timings of administration will probably have to be defined according to expected effects and drugs used concomitantly. Regarding liver transplantation, our group infuses MSCs at day 3 post-liver transplantation, while the MiSOT group performs 2 injections of MSCs at day 0 (intra operatively) and day 3 post-transplantation. In the Beijing study, an injection is performed on the day of liver transplantation and then once every 4 wk during a 12-wk period.

Administration route

In case of liver transplantation, MSCs can be injected through a peripheral vein or through intraportal infusion during surgery, or a combination of both. Intraportal infusion could be helpful in increasing the amount of MSCs homing to the liver. On the other hand, MSC homing behaviour to the inflammation site[69] could potentially concentrate them in the liver when intravenously infused after hepatic transplantation. However, some studies have observed that MSCs could be trapped in the lung after intravenous infusion[86,87]. Whatever the case, it is clear that to define the best route of administration, it is necessary to better understand the homing capacity of MSCs, and whether MSCs really require close contact with the target organ in order to be effective.

MSC side effects and safety

To date, no major adverse effects have been reported in the mid-term in the significant number of clinical trials using MSC-based therapy, for example in the context of BMT[113-117], solid-organ transplantation[129-133] and in many completed clinical trials for various therapeutic applications[145]. Only some studies have shown mild and transient adverse effects around the time of injection[145]. More experience is needed in order to confirm the long-term safety of MSCs.

To reach a sufficient number of cells for MSC-based therapy, *in vitro* expansion is needed. In this context, one of the major concerns is the potential risk of a neoplastic transformation of MSCs[122]. The occurrence of chromosomal aberrations is not uncommon after *in vitro* culture of mMSC, especially after long-term culture. It has been shown *in vivo* that these chromosomally unstable cells could transform into malignant cells with generation of tumors *in vivo*[146-148].

Contrary to mMSCs, *in vitro* expansion of hMSC seems to be far more stable and does not seem to generate genomic instability in these cells even after long-term culture. They do not transform into malignant cells after transplantation in mice[149,150]. Nevertheless, a French study observed the occurrence *in vitro* of transient chromosomal aberrations (aneuploidy) in twenty preparations of BM-MSCs obtained under GMP with two different culture processes. However these cells showed the same senescence as "normal" MSCs and did not lead to tumoral process after injection in immunocompromised mice[151]. Another study has found a high rate of human MSCs spontaneously transformed in malignant cells *in vivo*[152] but this has been strongly controverted suggesting a cross-contamination with cancerous cells[153]. Moreover, in two recent reviews analysing numerous studies, no evidence was found to affirm the potential of human MSCs for malignant transformation and so far, no risk of malignant transformation has been found in clinical use of hMSCs[149,154].

As MSCs are used as immunosuppressors, another concern is the potential emergence of opportunistic infections and induced cancers. In the case of solid organ transplantation with MSC-based immunosuppression, no increase risk of viral opportunistic infections has been observed so far-one group having even observed a decrease[130]. Nevertheless, another group reported viral opportunistic infections in three patients[129].

Interestingly, the MiSOT study group recently established a system to objectively score the potential emerging adverse effects related to MSC infusions (intravenous or intraportal infusion) after liver transplantation[155]. This

score is calculated using three parameters (pulmonary toxicity, intraportal-infusional toxicity and systemic toxicity), each of them receiving a score of 0 (no adverse events) to 3 (severe adverse events). It has been retrospectively validated on a cohort of 187 liver-transplanted patients not receiving MSCs as a control population. It has been suggested that this new tool could be helpful in assessing the safety of MSC use in solid organ transplantation.

CONCLUSION

The accumulating evidence shows that MSCs have immunosuppressive and reparative capacities *in vivo* and *in vitro*, as well as a potential beneficial effect in ischemia-reperfusion injury. These three principal properties suggest that MSCs could be interesting in liver transplantation to prevent or treat IRI, allograft dysfunction and graft rejection by inducing a durable tolerogenic environment. Using MSCs, and thereby removing or reducing the need for immunosuppressive drugs could avoid the serious side effects associated with these drugs.

Currently available data in clinic show that MSCs are safe to use, at least in the medium-term, but more time is needed to evaluate their potential adverse effects on the long-term. Caution is therefore recommended. Even if encouraging, the results of MSC use *in vitro* and *in vivo* (animals and humans) are sometimes contradictory. Nevertheless, negative results do not necessarily mean that MSCs are not effective in solid-organ transplantation, but rather that a countless number of still unknown (or poorly known) parameters may influence their effectiveness. At the same time, many issues must be resolved to optimize their use. Intensive *in vitro* and pre-clinical research is certainly the key to a better understanding of the way that MSCs act, and to eventually lead to clinical success.

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