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REVIEW

Mesenchymal stem cells as a therapeutic tool to treat sepsis

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Abstract

Sepsis is a clinical syndrome caused by a deregulated host response to an infection. Sepsis is the most frequent cause of death in hospitalized patients. Although knowledge of the pathogenesis of sepsis has increased substantially during the last decades, attempts to design effective and specific therapies targeting components of the derailed host response have failed. Therefore, there is a dramatic need for new and mechanistically alternative therapies to treat this syndrome. Based on their immunomodulatory properties, adult mesenchymal stem or stromal cells (MSCs) can be a novel therapeutic tool to treat sepsis. Indeed, MSCs reduce mortality in experimental models of sepsis by modulating the deregulated inflammatory response against bacteria through the regulation of multiple inflammatory networks, the reprogramming of macrophages and neutrophils towards a more anti-inflammatory phenotype and the release of anti-microbial peptides. This report will review the current knowledge on the effects of MSC treatment in preclinical experimental small animal models of sepsis.

Key words: Adult mesenchymal stem cells; Therapy; Sepsis

Core tip: Sepsis remains as the most frequent cause of death in hospitalized patients and, therefore, new therapeutic alternatives are needed. Adult mesenchymal stem cells reduce mortality in experimental models of sepsis by modulating the deregulated inflammatory response against bacteria through the regulation of multiple inflammatory networks, the reprogramming of macrophages and neutrophils towards a more anti-inflammatory phenotype and the release of anti-microbial peptides. In this report we aim to provide a comprehensive snapshot of the potential clinical use of cell therapy with mesenchymal stem cells for sepsis.

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INTRODUCTION

Sepsis is a clinical syndrome caused by a deregulated host response to an infection. Sepsis is the most frequent cause of death in hospitalized patients. Although knowledge of the pathogenesis of sepsis has increased substantially during the last decades, attempts to design effective and specific therapies targeting components of the derailed host response have failed. Sepsis will remain an important clinical problem in the future, especially in light of the ageing population and emerging antibiotic resistance. Therefore, there is a dramatic need for new and mechanistically alternative therapies to treat this syndrome. Based on their immunomodulatory properties, adult mesenchymal stem or stromal cells (MSCs) can be a novel therapeutic tool to treat sepsis. This report will review the current knowledge on the effects of MSC treatment in preclinical experimental small animal models of sepsis.

SEPSIS

Epidemiology

The incidence of sepsis varies between different reports, largely due to the use of different case definitions and diagnosis codes[1,2]. Nevertheless, sepsis clearly is a leading cause of death, and the most frequent cause of death in non-coronary intensive care units (ICUs) in the developed world[2]. In the United States the yearly incidence of severe sepsis is estimated at 300 cases per 100000 person-years population, which accounts for 10% of all ICU admissions[3]. The incidence of severe sepsis was recently reported to increase[4], although it is uncertain whether this signifies a true increase or altered coding and registration practices[2,5]. Mayr *et al*[2] have recently reported that the mortality of severe sepsis and septic shock lies between 25%-50%, with the extent and number of organ failures as the strongest predictors of an adverse outcome. Notably, the case fatality rate for sepsis has declined in the past decade, most likely due to improved general care in the ICU[6].

The most common sources of sepsis are in descending order pneumonia, intra-abdominal-, urinary tract- and soft tissue infections[5]. Blood cultures are positive in only one third of cases, and up to a third of cases are culture negative from all body sites. The most commonly isolated Gram-positive bacterial pathogens are *Staphylococcus aureus* and *Streptococcus pneumoniae*, and the most common Gram-negative pathogens are *Escherichia coli*, *Klebsiella spp*, and *Pseudomonas aeruginosa*[7]. While Gram-positive infections had been reported as surpassing Gram-negative infections in recent years[8], a recent study encompassing 14000 ICU patients in 75 countries found that 62% of positive isolates were Gram-negative bacteria, *vs* 47% Gram-positive and 19% fungal[9].

Pathophysiology and host response

Sepsis occurs when the body's response to infection injures the host's tissues and organs. The deregulated host response during sepsis entails both excessive proinflammatory and immune suppressive anti-inflammatory components[7,10].

Immune cells can sense pathogens *via* so-called pattern-recognition receptors (PRRs), which recognize conserved motifs expressed by microorganisms called pathogen-associated molecular patterns or PAMPs[7,11]. Four classes of PRRs have been identified: Toll-like receptors (TLRs), C-type lectin receptors, RIG-I-like receptors and NOD-like receptors[11]. Activation of PRRs by PAMPs causes upregulation of inflammatory gene transcription and initiation of innate immunity, a response aimed at eliminating the invading pathogen. However, when bacteria overcome the ability of the innate immune system to clear the infection, resulting in progression to sepsis, the interactions between pathogens and PRRs advances into a deregulated response that no longer benefits the host. During such injurious host response inflammation can be perpetuated by stimulation of PRRs by so-called danger-associated molecular patterns (DAMPs or alarmins), which are endogenous molecules released by injured or dying cells[12]. Alarmins are also released during sterile injury such as after trauma or severe pancreatitis, which contributes to the concept that the pathogenesis of multiple organ failure in sepsis and non-infectious critical illness is not fundamentally different[5,13].

Cytokines are an important component of the "hyperinflammatory" response to severe infection. Experimental sepsis induced by systemic challenge with high bacterial doses is associated with enhanced release of multiple cytokines, and elimination or inhibition of several of these proinflammatory mediators [including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-12, IL-17, IL-18, interferon-γ, and macrophage migration inhibitory factor] improves survival in these models[14]. However and importantly, these systemic challenge models do not adequately mimic the clinical syndrome of sepsis. Many trials evaluating the efficacy of proinflammatory cytokine inhibition, especially targeting TNF-α and IL-1, or other anti-inflammatory strategies have failed[15]. Other proinflammatory mediators implicated in sepsis pathogenesis include high mobility group box 1 (HMGB1) and S100 proteins.

Activation of the complement systems forms a fundamental part of the innate immune response to infection[16]. Sepsis is associated with systemic activation of the complement system, which can be harmful in the setting of fulminant sepsis. Indeed, neutralization or genetic absence of complement factor C5a and its receptors results in increased survival during abdominal sepsis or endotoxemia in mice. Other hallmark features of the sepsis host response include activation of the coagulation system and vascular dysfunction. The most severe manifestation of coagulopathy is the syndrome of disseminated intravascular coagulation, with an

estimated incidence between 30%-50% in severe sepsis, caused by tissue factor-driven activation of coagulation with concurrent impairment of anticoagulant and fibrinolytic mechanisms[17]. Organ dysfunction in sepsis is at least in part caused by tissue hypoperfusion, secondary to hypotension, microvascular thrombosis and/or dysfunction of the vascular endothelium with loss of barrier function[5]. Mitochondrial dysfunction and altered cellular bioenergetics have been implicated in sepsis-induced organ dysfunction, although further research is warranted to establish a causal relationship[18].

While proinflammatory responses definitely contribute to sepsis pathogenesis, immune suppression is also a common feature in patients with sepsis[7,10]. Autopsy studies have revealed strong deficiencies of splenocytes harvested from patients who had died of sepsis to produce cytokines upon stimulation[19]. The mechanisms that underlie this phenomenon have not been fully elucidated, although likely anti-inflammatory cytokines, particularly IL-10 and transforming growth factor (TGF)-β, and inhibition of signalling by PRRs, partially due to epigenetic modifications of essential promoter regions, are involved. Moreover, apoptosis of immune cells has been implicated in immune dysfunction and mortality in sepsis[10]. Most cells that undergo enhanced apoptosis in sepsis are of lymphoid origin (B cells, CD4 T cells), but also dendritic cells are affected. Preclinical studies have suggested that enhanced apoptosis of lymphocytes contributes to sepsis lethality[10].

MSC

MSCs have emerged in recent years as therapeutic tools based on four important features: (1) differentiation potential; (2) capacity to modulate immune responses; (3) pro-angiogenic and repair promoting capacities; and (4) low immunogenicity; the latter feature may allow allogeneic treatments. MSCs have been found in a variety of adult tissues of mesodermal origin, such as bone marrow, adipose tissue, placenta, umbilical cord, dental pulp or synovium[20-25]. Although sharing the main characteristics, differences between MSCs from different sources can be found, for instance at the RNA and protein expression profiles levels[26-28]. MSCs are considered a promising tool for cell therapy, in particular for inflammatory diseases, based on their immunomodulatory properties and paracrine effects through trophic factors with anti-fibrotic, anti-apoptotic or pro-angiogenic properties[29,30]. MSCs regulate the function of a broad range of immune cells[30-37], and are activated by inflammatory mediators released from activated immune cells (*i.e.*, IFNγ, IL1β and TNFα)[38,39]. The mechanisms involved in the immunoregulatory activity of MSCs are still under investigation but rely on both cell contact-dependent mechanisms (*i.e.*, Jagged1-Notch1 interaction, Fas-Fas-L interaction)[40,41] and paracrine effects through the release of soluble factors including hepatocyte growth factor, prostaglandin-E2 (PGE2), TGF-β1, nitric oxide (NO), IL-10, IL-6, heme oxygenase-1 (HO-1), HLA-G5 or the enzymatic activity of indoleamine 2,3-dioxygenase[42]. In addition to the direct effect of these soluble factors, MSCs may also modulate immune responses through the generation of immune cells with regulatory phenotype, including regulatory T cells or anti-inflammatory macrophages[43-45].

MSCs have also been reported to show antimicrobial activities against different pathogens upon activation with inflammatory cytokines[46]. Noteworthy in the context of sepsis, the functionality of MSCs can also be modulated by activators of TLRs[47]. It has been described that MSCs can be polarized *in vitro* towards either anti-inflammatory or pro-inflammatory phenotypes, depending on the TLR ligand time/concentration used for activation[48]. Furthermore, it has been recently described that interaction of gastrointestinal bacteria (*Salmonella typhimurium* or *Lactobacillus acidophilus*) with MSCs increased their capacity to inhibit T lymphocyte proliferation *in vitro* through a PGE2-dependent mechanism, indicating that bacteria may also enhance the immunomodulating properties of MSCs[49].

MSCs can sense inflammatory signals through the expression of cytokine/chemokine receptors and integrins, and subsequently migrate to sites of inflammation[50]. Moreover, homing of systemically administered MSCs to lymphoid organs (draining lymph nodes and spleen) and the subsequent generation of functional Tregs have also been reported[51-53]. MSCs do not long-term engraft at the inflammation site and cells seem to be cleared shortly after administration. This suggests that transient effects through soluble factors and cell-to-cell contacts play a main role in MSC-mediated initial controlling and balancing of local inflammation.

Allogeneic MSCs are regarded as a preferred source for treatment as they would allow treatment with a ready to use, off-the-shelf product, available for a large number of patients, specially, in acute life threatening indications like sepsis in which isolation and expansion of autologous MSCs is not an option. In that context, MSCs are considered immune privileged as they express constitutively only low levels of cell-surface HLA class Ⅰ molecules and lack expression of HLA class Ⅱ, CD40, CD80 and CD86 which would lead to reduced activation of the innate and adaptive immune responses[54]. This immune privilege of MSCs therefore supports the feasibility of allogeneic treatments without the requirement of suppression of host immunity[55,56]. However the immunogenic features of MSCs are currently under review as there is some evidence of immunogenicity in experimental animal models that coincides with immunomodulary effects by MSCs[57].

MSC IN EXPERIMENTAL MODELS OF SEPSIS

Sepsis being a disease that results as a consequence of deregulated inflammatory and immune responses against an infection can lead to tissue damage, multiorgan failure and death. Interest in investigating the therapeutic effect of MSCs on experimental models of sepsis emerged recently, and is based on their immunomodulatory properties[58]. A description of the therapeutic effects of MSCs in experimental small animal models of sepsis and the mechanisms involved are described in the following sections. A summary of the data is provided in Table 1.

Experimental models of sepsis

In order to study sepsis pathophysiology, animal models of sepsis have been established. These models are normally used to preliminarily test potential therapeutic treatments prior to human clinical trials. On the basis of the initiating agent, sepsis models can be divided into three categories: toxaemia models (exogenous administration of a bacterial toxin, such as lipopolysaccharide), bacterial infection models (exogenous administration of a bacteria) and host barrier disruption models (alteration of the animal's endogenous colonic protective barrier allowing bacterial leakage)[59]. These experimental models have in common high inflammatory responses against endotoxins or bacteria, subsequent organ injury and failure and, as a consequence, high mortality rates within few hours or days. All models have contributed significantly to our understanding of sepsis pathophysiology, although no single one fully mimics the course of human disease. Two limitations of sepsis models compared to human disease are the timing of disease progression (the progression to multiorgan failure and death occurs in hours to days in most animal models, whereas in human sepsis this occurs in days to weeks) and lack of supportive therapeutic intervention (*i.e.*, intubation and mechanical ventilation, fluid therapy), in particular in small animal models. Therefore, extrapolation of efficacy results obtained in small sepsis animal models to the human disease has to be made with caution[58,59].

The toxemia models involve the administration by intraperitoneal or intravenous injection of a bacterial toxin. Thus, a single injection of high dose LPS (normally 10-20 mg/kg) is the most commonly used toxaemia model (LPS model). LPS administration induces a very rapid and transient increase in systemic cytokine levels, hypodynamic cardiovascular activity and a shock-like state. The injection of LPS may result within hours in high mortality rates that may vary with the dose, type of LPS, age and strain of animal. The bacterial infection models consist on an exogenous bacterial infection and the severity of the model may vary depending on the bacterial strain (*i.e.*, *Escherichia coli*, *Pseudomonas aeruginosa*) and route of infection (intravenous, intraperitoneal, intratracheal) used. The clinical progression of the disease is rapid with hypodynamic cardiovascular state, high cytokine levels and progression towards death within hours. The host-barrier disruption models require the surgical disruption of the shielding barrier that protects sterile compartments from pathogens, allowing bacteria to spread. These models have become the most relevant sepsis models because they create a focus of infection that can disseminate throughout the body, mimicking the human situation. The caecal ligation and puncture model (CLP) is considered to be one of the most clinically relevant models for sepsis research. The model involves surgical ligation of the distal cecum with suture followed by one or two small punctures distal to the ligation. This allows the leakage of intestinal content into the peritoneal cavity, which results in polymicrobial sepsis (several bacterial species can be found in the blood and other organs of CLP animals). Technical variations (needle size and number of punctures) can influence the severity of the CLP model (mortality within hours or several days)[59,60].

Effect of MSC treatment on mortality and organ injury induced by sepsis

The therapeutic effect of MSC treatment has been tested using different sepsis animal models, MSC types, dosing, timing and routes of administration. These studies have consistently reported improvement on survival rates of animals treated with MSCs (Table 1). In mice, one single dose of between 3×10^5 and 10^6 MSCs administered by intraperitoneal, intravenous or intratracheal route was able to significantly reduce sepsis-related mortality in LPS, CLP, *P. aeruginosa* peritonitis and *E.coli* pneumonia mouse models[52,61-68]. Similar therapeutic effects have been observed using autologous, allogeneic or xenogeneic MSCs[52,61]. Noteworthy, treatment with fibroblasts has not been reported to increase survival of septic mice, despite the shared immunomodulating properties of fibroblasts with MSCs[61,63,64].

The effects on survival might depend on the dose (low/high, one/multiple) and the timing of administration (early/late after insult). Gonzalez-Rey *et al*[52] reported that one dose of 10^6 human ASCs administered intraperitoneally 30 min after LPS injection in mice had a higher protective effect on mortality than one dose of 3×10^5 cells. Mei *et al*[62] found that intravenous administration of one dose of 2.5×10^5 mouse BM-MSCs after 6 h of CLP did not significantly protect mice, unless MSCs were administered concomitantly with antibiotics. These results might be related to different experimental settings because, compared to other studies carried out in CLP mouse models, Mei *et al*[62] administered a lower dose of MSCs (2.5×10^5 *vs* 10^6 cells) and at a later time point (6 h after CLP *vs* a range between 24 h before and 4 h after CLP)[52,61,66,68]. On the other hand, Hall *et al* reported that three intravenous

Table 1 Efficacy preclinical studies on experimental models of sepsis using mesenchymal stem cells, mesenchymal stem cell-conditioned medium or mesenchymal stem cell-derived macrophages

Animal model	MSC type	Route/time	Dose	Number of doses	Therapeutic effects					MoA	Ref.
					Survival	Cytokines	Inflammatory infiltration	Organ injury	Bacterial load		
LPS, mouse	hASCs (xeno)	I.P/after 0.5 h or I.P/after 0.5 h	3×10^5 10^6	1 1	Improved Improved	Reduced pro-inflammatory cytokines in serum, liver, lung and intestine Increased anti-inflammatory cytokine (IL10) in liver, lung and intestine	Reduced lymphocyte, neutrophil and macrophage infiltration in peritoneum, liver, lung and intestine	ND	ND	ND	Gonzalez-Rey *et al*[52]
LPS, mouse	hBM-MSCs (xeno) normal/ senescent	I.P/after 0.5 h	10^6	1	Improved only by normal cells	Reduced pro-inflammatory cytokines in serum and lungs (normal cells) Reduced anti-inflammatory cytokine (IL10) in serum (normal cells)	ND	ND	ND	ND	Sepúlveda *et al*[67]
LPS, mouse	hASC/BM-MSC-CM	I.P/at 0 h	1 mL CM (from 2×10^6 cells per milliliter)	1	Improved only by hBM-MSC CM	ND	Reduced neutrophil infiltration in kidney (hBM-MSC CM)	Improved kidney, liver and lung damage (hBM-MSC CM)	ND	ND	Elman *et al*[65]
LPS, rat	hASCs (xeno)	I.V/after 0.5 h	2×10^6	1	ND	Reduced pro-inflammatory cytokines in lung No effect on anti-inflammatory cytokine (IL10)	ND	Improved kidney, liver and lung damage	ND	ND	Shin *et al*[75]
LPS, rat	hBM-MSCs (xeno)	I.M/at 0 h	2×10^6	1	ND	ND	ND	Improved kidney, liver and lung damage	ND	ND	Yagi *et al*[73]
LPS, rat	hBM-MSCs (xeno)	I.M/at 0 h	2×10^6	1	ND	Reduced pro-inflammatory cytokines in serum	Reduced neutrophil and macrophage infiltration in kidney, liver and lung	ND	ND	MSC release of sTNFR1	Yagi *et al*[76]
LPS, rat	mBM-MSCs (xeno)	I.P/after 1 h	2×10^6	1	ND	Reduced pro-inflammatory cytokines in serum and myocardium Increased anti-inflammatory cytokine (IL10) in serum but not in myocardium	ND	Improved myocardial damage Cells from female donors showed higher effect	ND	Higher expression of anti-apoptotic proteins in myocardium	Manukyan *et al*[71]
LPS, rat	mBM-MSCs (xeno)	I.P/after 1 h	2×10^6	1	ND	Reduced pro-inflammatory cytokines in serum and myocardium Increased anti-inflammatory cytokine (IL10) in serum but not in myocardium	ND	Improved myocardial damage	ND	ND	Weil *et al*[72]
LPS, rat	rBM-MSCs (auto)	I.V/after 1 h	2.5×10^6	1	ND	Reduced pro-inflammatory cytokines in serum and myocardium Increased anti-inflammatory cytokine (IL10) in serum but not in myocardium	ND	Improved myocardial damage	ND	ND	Weil *et al*[74]

CLP, mouse	hASCs (xeno) mASCs (auto/allo)	I.P/after 4 h	10^6	1	Improved	Reduced pro-inflammatory cytokines in serum, liver, lung and intestine Increased anti-inflammatory cytokine (IL10) in liver, lung and intestine	Reduced lymphocyte, neutrophil and macrophage infiltration in peritoneum, liver, lung and intestine	ND	ND	ND	Gonzalez-Rey *et al*[52]
CLP, mouse	mBM-MSCs (auto/allo)	I.V/24 h prior or I.V/after 1 h	10^6 10^6	1 1	Improved Improved	Reduced pro-inflammatory cytokines in serum Increased anti. inflammatory cytokine (IL10) in serum	Reduced neutrophil infiltration in peritoneum, liver and kidney	Improved kidney, liver, pancreatic and spleen damage and vascular permeability	Reduced bacterial counts in blood	Anti-inflammatory Mph (IL10) induced by MSCs through PGE2	Németh *et al*[61]
CLP, mouse	mBM-MSCs (auto)	I.V/after 6 h	2.5×10^5	1	Improved	Reduced pro-inflammatory cytokines in serum and BAL No effect on anti. inflammatory cytokine (IL10) in serum and BAL	Reduced neutrophil infiltration in peritoneum, liver and kidney	Improved kidney and lung damage. No effect on liver and pancreatic damage	Reduced bacterial counts in spleen	Increased phagocytic activity of macrophages and neutrophils	Mei *et al*[62]
CLP, mouse	mBM-MSCs (auto)	I.V/after 2 h and I.V/after 24 h and I.V/after 48 h	5×10^5 2.5×10^5 2.5×10^5 total: 10^6	3	Improved	ND	Reduced neutrophil infiltration in bowel	Improved bowel, kidney, liver and spleen damage	Reduced bacterial counts in peritoneum and blood	Increased phagocytic activity of neutrophils	Hall *et al*[64]
CLP, mouse	mBM-MSCs (auto)	I.V/after 3 h	10^6	1	Improved	Reduced pro-inflammatory cytokines in serum Increased anti-inflammatory cytokine (IL10) in serum	Reduced neutrophil infiltration in kidney	Improved kidney damage	Reduced bacterial counts in blood	ND	Luo *et al*[66]
CLP, mouse	ASC-derived mouse Mph	I.P/after 4 h or I.P/after 6 h or I.P/after 12 h or I.P/after 24 h	10^6 10^6 10^6 10^6	1 1 1 1	Improved Improved Improved No effect	Reduced pro-inflammatory cytokines in serum (only treatment at 4 h tested)	Reduced lymphocyte, neutrophil and macrophage infiltration in peritoneum, lung, liver and intestine (only treatment at 4 h tested)	ND	ND	IL10 secreted by Mph	Anderson *et al*[77]
CLP, mouse	hUC-MSCs (xeno) wt/Poly I:C preactivated	I.V/after 1 h	10^6	1	Improved Better preactivated	Reduced pro-inflammatory cytokines in plasma Better preactivated	Reduced inflammatory infiltration in kidney, liver and lung	Improved kidney, liver and pancreatic damage Better preactivated	Reduced bacterial counts in peritoneum and blood Better preactivated	Poly I:C inhibition of MiR-143 expression by MSCs	Zhao *et al*[68]
CLP, rat	rASCs (auto) living/ apoptotic	I.P/after 0.5 h and I.P/after 6 h and I.P/after 18 h	1.2×10^6 1.2×10^6 1.2×10^6 total: 3.6×10^6	3	Higher mortality by living cells Improved by apoptotic cells	Reduced TNFα (apoptotic rASC treated rats)	ND	Improved kidney, liver, lung and myocardial damage (apoptotic rASCs)	ND	ND	Chang *et al*[69]

E.coli pneumonia, mouse	hBM-MSCs (xeno)	I.T/after 4 h	10^6	1	ND	Reduced pro-inflammatory cytokines in bronchoalveolar liquid (BAL)	Reduced neutrophil infiltration in BAL	Improved lung epithelial and endothelial permeability	Reduced bacterial counts in BAL	MSC release of the antimicrobial peptide LL-37	Krasnodemskaya *et al*[70]
P. aeruginosa peritonitis, mouse	hBM-MSCs (xeno)	I.V/after 1 h	10^6	1	Improved	No changes in serum or peritoneal fluid levels of pro or anti-inflammatory mediators	ND	ND	Reduced bacterial counts in peripheral blood, peritoneal fluid, lung, and spleen	Increased phagocytic activity of macrophages Generation of anti-inflammatory macrophages in spleen	Krasnodemskaya *et al*[63]

LPS: Lipopolysaccharide; CLP: Caecal ligation and puncture; h: Human; r: Rat; m: Mouse; ASCs: Adipose mesenchymal stem cells; BM-MSCs: Bone marrow mesenchymal stem cells; UC-MSCs: Umbilical cord mesenchymal stem cells; Mph: Macrophages; I.P: Intraperitoneal; I.V: Intravenous; I.T: Intratracheal; I.M: Intramuscular; ND: Not determined; CM: Conditioned medium.

administrations of 5×10^5, 2.5×10^5 and 2.5×10^5 mouse BM-MSCs at 2, 24 and 48 h after CLP, respectively, also reduced mortality rates on mice, although they did not compare with the effects of one single dose[64]. Unfortunately, so far, different timing of administration have only been compared in the same study by Németh *et al*[61] who reported that a prophylactic intravenous treatment with mouse BM-MSCs 24 h prior to CLP had similar therapeutic effects than a therapeutic treatment after 1 h of CLP. However, due to the urgent and acute condition of sepsis, a prophylactic treatment is clinically not feasible and comparing in the same study single *vs* multiple dosing and different time regimens (early or late after the induction of sepsis) might be very important in order to understand if there is a time window in which MSC treatment is most efficacious and, therefore, maximize the therapeutic benefit of MSC therapy.

In addition to the dose and time, the "fitness" of the cells at the time of administration might also affect the therapeutic effect of MSCs. Thus, Sepúlveda *et al*[67] found that while intraperitoneal administration of normal human BM-MSCs 30 min after LPS injection reduced mortality in an LPS sepsis model in mice, senescent human BM-MSCs failed to protect them, despite the fact they conserved the capacity to modulate the function of lymphocytes and macrophages *in vitro*. However, in contrast to these positive results, Chang *et al*[69] reported that three intraperitoneal administrations of 1.2×10^6 living rat ASCs (at 0.5, 6 and 18 h) did not reduce, but moderately increased, mortality in a rat model of CLP, whereas the administration of apoptotic rat ASCs protected rats from death. Further studies will be needed to better understand these results.

The effects of MSC treatment on survival are a consequence of the reduction of inflammation-associated organ injury and the improvement in organ function. MSC treatment has been reported to reduce damage in kidney (*i.e.*, reduced levels of apoptotic cells, serum creatinine and tubular injury score), liver (*i.e.*, reduced levels of apoptotic cells, serum liver enzymes and blood urea nitrogen), pancreatic (*i.e.*, reduced levels of serum amylase), spleen (*i.e.*, reduced levels of apoptotic cells), lung (*i.e.*, reduced levels of apoptotic cells and vascular leakage) and heart function (*i.e.*, improved cardiac depression) in a variety of sepsis models and experimental settings[61,62,64-66,68-75]. Improvement in organ damage correlates with reduction on neutrophil infiltration and myeloperoxidase (MPO) activity in target organs[52,61]. Notably, these effects can also be obtained by intraperitoneal administration of conditioned medium from BM-MSCs or ASCs in a LPS mouse model, suggesting that the therapeutic effects of MSCs might be mediated, at least in part, by soluble factors[65]. Of note, Manukyan *et al*[71] observed that female mouse BM-MSCs injected intraperitoneally had a better improvement of cardiac function of LPS septic rats than male mouse BM-MSCs. This effect correlated with a higher expression of the anti-apopotic protein Bcl-XL in the myocardium of female MSC treated rats.

No specific side effects of MSC treatment have been reported in sepsis models (only Chang *et al*[69] reported increased mortality when using living rat ASCs compared to the untreated group in a rat model of CLP). The fate of MSCs in sepsis models have been also investigated in some studies. When MSCs were administered intravenously, cells were always detected in the lungs and eventually, to a lesser extent, in spleen, liver, kidney or lymph nodes[61,66,74]. When MSCs were administered

intramuscularly, cells were only detected in the muscle up to 24 h after administration[76].

Effects of MSCs on inflammation induced by sepsis

The pathogenesis of sepsis is characterized by massive infiltration of immune cells in target organs and high pro-inflammatory cytokine levels systemically and locally, that can lead to tissue damage, multiple organ failure and death. Treatment with MSCs reduces the infiltration of neutrophils and monocyte/macrophages to target organs, including liver, lung, intestine and kidney[52,61,62,64-66,68,70,76]. Furthermore, MSC treatment has also been reported to reduce the levels of proinflammatory cytokines (*i.e.*, IFNγ, TNFα, IL1β or IL6) in several organs including serum, liver, lung, intestine and myocardium[52,61,66-68,70-72,74,76]. These anti-inflammatory effects can be enhanced by preactivation of UC-MSCs with Poly I:C which results in the inhibition of miR-143 expression by MSCs[68]. The reduction on the levels of anti-inflammatory cytokines was accompanied by the increase on the levels of the anti-inflammatory cytokine IL10[52,61,66-68,70-72,74,76], although other authors have reported either no effect on IL10 levels or even a reduction[62,63,67,75]. These differences might be related to differences in the experimental settings, such as the use of different animal models, MSCs, dosing and time of sample collection. Nevertheless, there is evidence that IL10 plays an important role in the therapeutic effects of MSCs in sepsis. Thus, injection of a neutralizing antibody against IL10 or IL10 receptor prior to CLP abrogated the therapeutic effects of mouse BM-MSCs[61]. *In vitro* studies showed that IL10 was not directly produced by MSC, but by macrophages through a mechanism that required MSC-secretion of PGE2[61,77]. Moreover, a role of IL10 in inhibiting the migration of neutrophils into the infected tissues has also been suggested[61]. In addition to IL10, other mediators of the therapeutic effect of MSCs have been identified. Thus, Yagi *et al*[73] observed that blockade of sTNFR1, which is released by MSCs in response to inflammation, partially impaired the anti-inflammatory effects of MSC treatment.

The MSC-mediated reprogramming of macrophages towards a regulatory and anti-inflammatory M2 phenotype has also been reported in sepsis models by other authors. Krasnodemskaya *et al*[63] observed a larger population of monocytes expressing CD206 (a marker of alternative activated M2 macrophages) in the spleen of MSC-treated mice and a higher phagocytic capacity of blood monocytes. Furthermore, Anderson *et al*[77] provided strong evidence of the important role that MSC-induced regulatory macrophages play in the therapeutic effects of ASCs in sepsis. The authors generated "ASC-mediated regulatory macrophages" (ASC-Mph) by *in vitro* culture of mouse bone marrow macrophages and ASCs (either mouse or human) and injected 10^6 ASC-Mph intraperitoneally in septic mice at different time points after CLP. These treatments resulted in reduced mortality rates when ASC-Mph were administered between 4 h and 12 h (but not at 24 h) after CLP by a mechanism that required the production of IL10 by ASC-Mph[77]. Moreover, these regulatory ASC-Mph also reduced levels of pro-inflammatory cytokines in serum and infiltration of inflammatory cells in the peritoneum, lung, liver and intestine. Finally, the relevance of monocytes/macrophages, but also neutrophils, in mediating the therapeutic effects of MSCs is highlighted by the fact that depletion of monocyte/macrophages (by using clodronate-filled liposomes) or neutrophils (by using anti-Ly6G antibody) completely abrogated the protective effects of MSCs *in vivo*[61,64].

The effects of MSC treatment on transcriptional inflammatory pathways in target organs of CLP septic mice treated with MSCs have been investigated by microarray analysis of total RNA expression. The results show that MSC treatment affects an ample range of transcriptional networks (it was estimated that up to a 13% of total murine genome was transcriptionally reprogrammed after MSC treatment compared to control septic mice including: (1) downregulation of TLR, NF-κB or IL6 signaling pathways; (2) upregulation of NF-AT-related genes; (3) upregulation of genes involved in phagocytosis, antigen presentation, bacterial killing, coagulation, complement regulation and platelet activation; and (4) upregulation of genes involved in cell-to-cell interaction and endothelial/vascular integrity[62,78].

Effect of MSCs on bacterial burden in sepsis

The mechanism by which MSCs protect from sepsis is not only limited to reducing the production of inflammatory cytokines and migration of inflammatory cells to infected organs, but also includes direct anti-microbial properties, as well as the improvement of the phagocytic properties of monocyte/macrophages and neutrophils. Gonzalez-Rey *et al*[52] and Németh *et al*[61] first reported a reduction on bacterial load in target organs (*i.e.*, peritoneal cavity, blood, spleen or liver) in MSC-treated septic mice, despite the MSC-mediated reduction of the inflammatory response. Krasnodembskaya *et al*[70] determined that MSC have intrinsic anti-microbial activity because they secrete the anti-microbial peptide LL-37 in response to the stimulation with *Escherichia coli* or *Pseudomonas aeruginosa*. Intratracheal administration of human BM-MSCs in a mouse pneumonia model highly reduced bacterial counts in bronchoalveolar lavage (BAL). However, when a LL-37 neutralizing antibody was also administered to mice, the anti-microbial effects of MSCs were only partially lost, suggesting that additional anti-microbial mechanisms might be involved. This potential direct killing of bacteria by MSCs needs to be further confirmed as Gonzalez-Rey *et al*[52] did not observe direct killing of *Escherichia coli* by MSCs *in vitro* in the absence of other cells.

In addition, the enhancement of the phagocytic properties of monocyte/macrophages and neutrophils have also been reported to improve bacterial clearance by MSCs. Noteworthy, MSCs seem not to have the capacity to phagocyte bacteria *in vitro*[62,64]. Mei *et al*[62] found that MSC treatment in a mouse CLP model increased the phagocytic capacity of peritoneal and spleen CD11b positive cells (mainly monocyte/macrophages and neutrophils) in MSC treated mice. Krasnodembskaya *et al*[63] observed a reduction on bacterial counts in several organs, but more significantly in peripheral blood of MSC treated mice infected with *Pseudomonas aeruginosa*, which was also associated to an increased capacity of peripheral blood monocytes to phagocyte bacteria. Hall *et al*[64] determined that MSCs, but not fibroblasts, also enhanced the phagocytic properties of neutrophils *in vitro* and in a CLP mouse model. In fact, depletion of neutrophils *in vivo* abrogated the ability of MSCs to promote bacterial clearance[64]. Notably, Németh *et al*[61] noticed that while infiltration of neutrophils to target organs was inhibited in MSC treated mice, their presence in circulation was concomitantly increased and suggested that this mechanism might help to clear bacteria from circulation and minimize organ injury due to leukocyte infiltration. Interestingly, preactivation with Poly I:C increased the *in vivo* anti-microbial effects of UC-MSCs in a CLP mouse model through a mechanism that requires the inhibition of the expression of miR-143[68].

CONCLUSION

Sepsis is a leading cause of death and the most frequent cause of death in non-coronary ICUs in the developed world and, despite improvement in treatments, the mortality of severe sepsis and septic shock remains very high, showing that current treatments are not sufficient to combat this syndrome. The use of MSCs in experimental animal models of sepsis has reported strong evidence of the therapeutic potential of MSC therapy in this indication. These studies have been mainly focused on the effects of MSCs on the pro-inflammatory phase of sepsis, while the effects of MSCs on the subsequent anti-inflammatory/immune exhaustion phase of the disease has not been elucidated so far and will need further investigation. The mechanisms by which MSCs improve survival in sepsis models rely on the collective effects of their immunomodulatory and anti-microbial properties: MSC treatment modulates inflammation in septic mice by a mechanism that requires the reprogramming of macrophages towards a more anti-inflammatory phenotype (release of anti-inflammatory IL10), resulting in reduced levels of pro-inflammatory cytokines in blood and organs and attenuated infiltration of immune cells in infected tissues (monocytes and neutrophils). Moreover, MSCs show direct (release of LL-37 peptide) and indirect (increase of phagocytic properties of monocyte/macrophages and neutrophils) anti-microbial effects. The combined effect of reducing both the inflammatory response and the bacterial burden results in an improvement of organ function and higher survival rates. The promising results obtained in these, small animal, preclinical efficacy studies are encouraging and suggest that MSCs might be a therapeutic option to treat sepsis in patients. Importantly, efficacy of MSCs in large animal models that better replicate the inflammatory response, organ failure and disease in humans (*e.g.*, sheep models) will be additionally relevant to support further testing of the therapeutic potential of allogeneic MSC treatment in humans. Such clinical trials should be prospective, controlled, and randomized so to guarantee a clear outcome of the MSC treatment effect. Moreover, taking into consideration the complexity and heterogeneity of sepsis and the poor results up to now in sepsis clinical trials, we believe that such trials should first be done in well defined and homogeneous sepsis patient populations.

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