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# Concise Review: Mesenchymal Stromal/Stem Cells: A New Treatment for Sepsis and Septic Shock?

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**Key Words.** Mesenchymal stromal/stem cells • Sepsis and septic shock • Organs failures • Immunomodulation • Treatment

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## ABSTRACT

**Sepsis and septic shock are the leading cause of admission and mortality in non-coronary intensive care units. Currently, however, no specific treatments are available for this syndrome. Due to the failure of conventional treatments, in recent years, research is focussing on innovative therapeutic agents, including cell therapy. One particular type of cells, mesenchymal stromal/stem cells (MSCs), has raised hopes for the treatment of sepsis. Indeed, their immunomodulatory properties, antimicrobial activity and capacity of protection against organ failure confer MSCs with a major advantage to treat the immune and inflammatory dysfunctions associated with sepsis and septic shock. After a brief description of the pathophysiology of sepsis and septic shock, the latest advances in the use of MSCs to treat sepsis will be presented.** STEM CELLS *2017; 00:000–000*

## SIGNIFICANCE STATEMENT

Sepsis and septic shock is currently a major public health issue due to the number of deaths worldwide and the lack of effective treatment. Although its incidence has not stopped growing in recent years reaching 50 to 100 cases per 100,000 inhabitants, there is currently no specific treatment. Recently, several preclinical studies have shown that mesenchymal stromal/stem cells (MSCs) have a positive impact on the symptoms and mortality associated with sepsis. However, their action is still not clearly elucidated. This manuscript aims at reviewing recent studies concerning MSCs use in sepsis and highlighting their mechanisms in this pathology.

## SEPSIS AND SEPTIC SHOCK

When a pathogen breaches the body's natural barriers and enters into the body, it activates the innate immune system via specific conserved molecular patterns known as Pathogen-Associated Molecular Patterns (PAMPs). Binding of PAMPs to pattern recognition receptors (PRRs) expressed on the cell surface of the innate immune system triggers an inflammatory response. Furthermore, the cellular damage and apoptosis caused by pathogens induce the release of molecules called Damaged-Associated Molecular Patterns, which also bind to PRR and increase the triggering of inflammation [1]. Under certain conditions, such as predisposing genetic factors, associated comorbidity, or virulent pathogens, the inflammatory phase may become disproportionate and lead to sepsis and to septic shock the more severe form.

The pathophysiology of sepsis and septic shock has been questioned in recent years due to the failures of anti-inflammatory therapies. These failures have given rise to a new theory: septic shock is a dynamic model in a perpetually mixed state with concomitant inflammatory and anti-inflammatory states. Indeed, Osuchowski et al. and Remick showed simultaneous production of inflammatory and anti-inflammatory cytokines in mice in both the acute and late phases of sepsis [2, 3]. In humans, Novotny et al. made the same observation by showing a concomitant increase in the levels of interleukin (IL)-6 and IL10 in the first 2 days of sepsis [4].

Apart from the presence of pro- and anti-inflammatory cytokines in the acute phase, a form of immune paralysis has been demonstrated in the first days following shock. Indeed, many studies showed an increase in the apoptosis mechanism that affects cytotoxic T lymphocytes, whether CD4+ or CD8+, as well as B lymphocytes, natural killer (NK), and dendritic cells. Only T regulatory lymphocytes (Treg), which are definitely more resistant, seem to be relatively spared by this phenomenon [5].

This quantitative depletion of the immune reserves is associated with cell energy. Indeed, it has been shown that dendritic cells have a reduced ability to present antigens to T lymphocytes, that macrophages are less able to synthesize

pro-inflammatory cytokines, that NK and T lymphocytes present reduced cytotoxic functions, and that neutrophils produce fewer reactive oxygen species and nitric oxide [6–8].

On the other hand, immune response to pathogen attack is disproportionate. The massive arrival of neutrophils, monocytes, and macrophages to fight the pathogen leads to tissue damage and impairment of the vascular endothelium. This results in organ failure, coagulation disorders, and hypotension due to fluid leakage from the vascular compartment [7, 9–11].

Until recently, the definition of septic shock was based on the concept of excessive inflammatory response to an infection, but recent research has highlighted the narrowness of this definition. Sepsis is now characterized as a dysfunction of the host response to an infection, leading to organ failure and presenting a threat to life [12]. Clinically, the diagnosis is made in the event of suspected infection and an increase in the Sequential Organ Failure Assessment (SOFA) score of at least 2 points. A simplified score (quick SOFA, also known as qSOFA) has been established to facilitate diagnosis in settings other than intensive care units [13]. Septic shock is now defined as being a case of sepsis where particularly serious circulatory, cellular, and metabolic abnormalities considerably increase the risk of mortality. Clinically, it is characterized by hyperlactatemia ($>$2 mmol $l^{-1}$) and failure to maintain blood pressure above 65 mmHg by adequate resuscitation, leading to use of vasopressors [13].

Currently, there is no specific treatment for sepsis and septic shock. Management is only symptomatic and consists of infusion in antibiotics and catecholamine. However, mesenchymal stromal/stem cells (MSCs) seem to be of great interest for sepsis and septic shock treatment.

## Mesenchymal Stromal/Stem Cells

MSCs, named mesenchymal stem cell in vivo and mesenchymal stromal cell in vitro, are mesodermal stem cells, which can differentiate into osteocytes, adipocytes, and chondrocytes, and, under certain culture conditions, into cells of non-mesodermal origin [14–17]. They express surface markers such as CD73, CD90, and CD105, and are negative for CD34, CD45, CD14 or CD11B, CD79A or CD19, and HLA-DR markers. Presence of MSCs has been described in bone marrow, adipose tissue [18], lung [19], heart [20], synovial membrane, trabecular bone, periosteum, skeletal muscle, dental pulp [21, 22] menstrual blood [23], and also in different fetal tissues, including the amniotic fluid and membrane [24], placenta [25], umbilical cord blood [26], and Wharton's Jelly (WJ) [27].

MSCs exhibit many immunomodulatory properties demonstrated in vivo as well as in vitro. Considering their effect on both adaptive and innate immunities, they are able to modulate inflammation by modulating cytokine and chemokine synthesis by the cells of the immune system. Their action requires soluble factors, exosomes, as well as cell–cell interactions [28, 29].

MSCs are an attractive therapeutic candidate for several reasons. MSC use is not limited by ethical laws as are embryonic stem cells. They are present in several tissues and their isolation and expansion are both easy and fast. They are devoid of MHC class II antigens and express only low levels of MHC class I antigens, allowing their use in an allogeneic setting due to their low immunogenicity [30]. Several clinical trials have reported no adverse event after MSC infusion, describing those cells as safe for clinical administration [31]. Finally, different preclinical studies have shown a beneficial action in a high number of indications and especially in sepsis and septic shock.

## MSCs and Sepsis/Septic Shock

### MSCs Improve Survival During Sepsis and Septic Shock

Different studies recently reported a reduction in mortality in animal models of sepsis and septic shock after administration of MSCs (Fig. 1; Table 1). Although this varies with the animal model used or the time of injection, the mortality rate seems to improve by approximately 30% when MSC treatment is infused [32–34]. This clear advantage provided by MSCs is closely associated with their action on the host macrophages. Indeed, Németh et al., using a mouse cecum ligation and puncture (CLP) model, show that MSCs have no effect on survival when the mice are depleted of macrophages, whereas their beneficial action is maintained in those depleted of T, B, or NK lymphocytes [34].

MSCs appear to improve survival, but what are the conditions, the dose, and the frequency required? This question seems reasonable in light of the variability of their action depending on the dose used. Indeed, Gonzalez-Rey et al. [39] showed a significant reduction in mortality in a model of lethal endotoxemia, with 60% survivors at 96 hours, only when a high dose of MSCs ($1 \times 10^6$) was administered. Although a smaller dose ($3 \times 10^5$) improved the length of survival compared with controls, it did not prevent death in any of the animals. Similarly, repeated MSC administration may turn out to be ineffective or even harmful. Indeed, Chang et al. [36] compared the action of apoptotic MSCs to "healthy" MSCs in sprague-dawley (SD) rats in sepsis (a CLP model) and demonstrated that the "healthy" MSCs had no effect on survival. The authors attributed this surprising failure to the triple injection of MSCs. According to them, it may have induced a hypersensitization of the rats, leading to inefficacy of the healthy MSCs, whereas the apoptotic MSCs, being less immunogenic and hence less likely to cause hypersensitivity, were able to maintain a beneficial effect on survival. Conversely, Hall et al. did demonstrate significant improvement in survival of mice subjected to CLP following triple injection with MSCs [55].

Apart from the MSC dose, concomitant administration of antibiotic therapy seems important. Mei et al. [33] noted significant improvement in survival only when imipenem was injected at the same time. Similarly, Wu et al. [52] failed to show protective action of umbilical cord MSCs when no antibiotic was co-administered. Evidence of synergistic action of MSCs with antibiotics has also been reported by Alcayaga-Miranda et al. [35]. In that study, the authors randomly assigned mice subjected to CLP into four groups: one untreated group, one group given MSCs isolated from menstrual blood, one group given norfloxacin, and one group given norfloxacin and MSCs simultaneously. All of the treated groups, regardless of treatment received, showed significantly improved survival compared with controls. However, the MSC + norfloxacin group showed higher survival than groups treated with MSCs or norfloxacin alone, indicating a cumulative beneficial effect of MSCs associated to antibiotics.

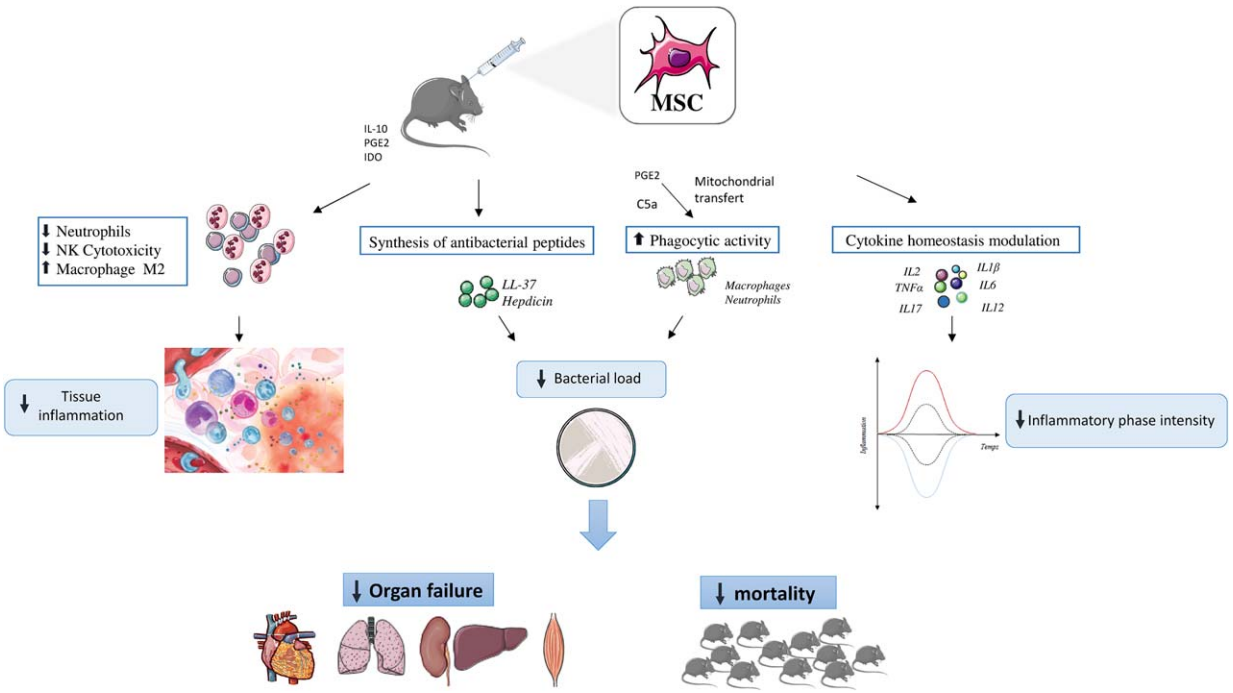


**Figure 1.** Mesenchymal stromal/stem cell (MSC) action during sepsis and septic shock. The action of MSCs during sepsis and septic shock is extensive. Their immunomodulatory capacity decreases tissue inflammation by regulating cytokine homeostasis and decreasing the traffic of immune cells into organs. Their antibacterial capacities are mediated by direct action on the bacterial load through secreting antibacterial peptides and by indirect action through increasing the phagocytic activity of macrophages and neutrophils. These properties allow MSCs to reduce organ failure and mortality associated with sepsis and septic shock. Abbreviations: IL, interleukin; LL-37, Cathelicidin LL-37; IDO, Indoleamine 2, 3-dioxygenase; NK, natural killer; PGE, prostaglandin; TNF, tumor necrosis factor.

## MSCs Are Capable of Modulating Inflammation

Many studies using mouse models of endotoxemia or peritonitis have demonstrated the ability of MSCs to reduce plasma levels of IL6, IL1β, IL12, IL2, and IL17 [37, 39, 44, 46, 56]. Gonzalez-Rey et al. [39] also showed that MSCs were able to decrease the tissue concentration of tumor necrosis factor (TNF)-α, IL6, IL1β, and IL12 in the lung, liver, and intestine, whereas Mei et al. [33] demonstrated a similar action in bronchoalveolar fluid.

However, not all cytokines seem to be as sensitive to the action of MSCs. Indeed, apart from the study by Luo et al. [44], where a non-significant reduction was noted, and the study of Liu et al., where a significant reduction was noted only 6 hours after induction of peritonitis [43], they seem to have little effect on interferon (IFN)-γ when administered intravenously [34, 37, 56]. Only two studies showed a significant reduction in IFNγ during sepsis following administration of MSCs, but these were administered locally, by the intraperitoneal route [39, 52].

Several authors agree that MSCs can reduce TNFα levels [34, 35, 37, 39, 44, 46, 56]. However, time after sepsis induction when TNFα level is reduced varies from one study to another. Luo et al. have shown a decrease in TNFα level 24 hours after sepsis induction, whereas Alcayaga-Miranda et al. have shown a significant decrease in TFNα level 40 hours after sepsis induction [35, 44]. Nevertheless, MSC sources and injection times are not the same in these studies.

Finally, IL10 seems to be a subject of debate. Indeed, several studies attest to the ability of MSCs to increase the level of IL10 during sepsis [34, 39, 44, 46, 53]. Although the ability of MSCs themselves to produce IL10 has not been clearly established [57], it would seem that they can increase the production of this cytokine by monocytes [34]. Moreover, Németh et al. have proposed a mechanism of action to explain this effect [34]. According to them, the interaction of bacterial compounds with the toll-like receptors (TLRs) of MSCs may result in an intracellular signaling cascade and translocation of NFκB, which may lead to the synthesis of COX2 by MSCs. Overexpression of this enzyme would then induce the release of prostanglandine E2 (PGE2), which would bind to the EP2 and EP4 membrane receptors of macrophages, and trigger the synthesis of anti-inflammatory IL10. This theory has, however, been called into question by Mei et al.'s team, which showed not an increase, but a decrease in plasma levels of IL10 following administration of MSCs [33]. The discrepancy of their results might be explained, according to them, by a later treatment (6 hours after the beginning of the sepsis in Mei's study versus at the time of induction or as a prophylaxis by Németh et al.'s team), and more precisely, at a time when IL10 levels, already high, could not be further increased.

However, this hypothesis is not confirmed by the results of another study [44], which reported an increase in plasma IL10 following injection of MSCs 3 hours after the onset of sepsis. Controversial results are reported by the team of Alcayaga-Miranda et al. [35] showing a definite reduction in IL10 in a model of peritonitis treated 3 hours after the onset of sepsis.

This modulation of cytokine homeostasis by MSCs seems closely linked to stimulation of their TLRs. In their study published in 2013, Zhao et al. demonstrated a stronger immunomodulatory ability in umbilical cord MSCs if these were stimulated with 10 μg $ml^{-1}$ poly (I:C), a TLR3 ligand [54]. In vivo, using the CLP murine model, they observed a significant reduction in plasma levels of IL6, TNFα, and murine homolog of human

 

RANTES (CCL5) in animals treated with MSCs previously stimulated by poly (I:C). Conversely, animals treated with unstimulated MSCs showed lowered IL6 and TNFα levels, but no significant difference compared with controls, and unchanged chemokine levels compared with the control group. The authors also observed improved survival, bacterial clearance, biochemical parameters, and the number of necrotic cells contained in the peritoneum of mice treated with poly (I:C)-MSCs compared with animals that received a dose of conventional MSCs. Similarly, Song et al. showed recently that pretreatment of MSC with IL1β induces an increase in the level of anti-inflammatory miR146 contained in MSC exosomes. Transferred into the macrophages, miR146 induces their polarization into anti-inflammatory M2 macrophages, by blocking the pro-inflammatory signaling pathways IL-1R-associated kinase (IRAK1), TNF receptor-associated factor 6 (TRAF6), and Interferon regulatory factor 5 (IRF5) [48].

## MSCs and Antibacterial Action

Gonzalez-Rey et al. were the first to demonstrate a reduction in the number of bacterial colony forming units (CFU) in the blood, liver, spleen, and peritoneal fluid of septic mice treated with MSCs from adipose tissue [39]. Although MSCs lack the ability of phagocytosis [33], they seem to be able to increase bacterial clearance through monocyte reprogramming [33, 41]. This reprogramming is, however, dependent on the complex environment associated with sepsis. Mei et al.'s team showed a clear increase in phagocyte activity only when the cells were from septic mice treated with MSCs, whereas a coculture of MSCs and "non-septic" monocytes induced no change in phagocytosis [33]. Krasnodembskaya et al. goes further, showing the presence of a higher concentration of the anaphylatoxin C5a in mice given MSCs [42]. They suppose that the increased amount of C5a is partly responsible for the increase in phagocytic activity by causing increased expression of the CD11b receptor on monocytes. Another mechanism is involved in the antimicrobial effect of MSCs: mitochondrial transfer. Jackson et al. recently showed that the ability of MSCs to transfer their mitochondria to macrophages, inducing an increase in both their mitochondrial metabolism and their phagocytic index. Associated mainly with direct contact, transfer of mitochondria from MSCs to macrophages is enabled by the formation of cytoplasmic bridges as well as by the release of exosomes. Inhibiting transfer causes a reduction in the antibacterial effect of MSCs [40].

Moreover, the same team recently showed that mitochondrial transfer can occur via MSC extracellular vesicles (EVs). In a clinical relevant model of lung injury, they demonstrated that the transfer of functional mitochondria in EVs is responsible for MSC anti-inflammatory- and phagocytic-enhancing effects on macrophages [58]. Similarly, Monsel et al. demonstrated in a murine model of pneumonia an increase in survival after administration of EV by increasing monocyte phagocytosis while decreasing inflammatory cytokine secretion [59].

In addition to their action on macrophages, MSCs influence phagocytosis by neutrophils. In vitro, an increase in the phagocytic index of neutrophils for *Escherichia coli* was shown when they were cocultured with MSCs [55]. This finding was also demonstrated in vivo: MSCs failed to enhance bacterial clearance in mice depleted of neutrophils and in septic shock. However, the role of neutrophils in the antibacterial capacity of MSCs has been studied by Jackson et al. [40]. After intranasal administration of $3.5 \times 10^6$ CFU of *E. coli* to mice depleted of neutrophils, the authors observed an increase in the number of bacterial CFU in the bronchoalveolar fluid compared with control mice, but saw that this increase was partially abolished when MSCs were injected, suggesting an antibacterial action unconnected with neutrophils. Conversely, depletion of alveolar macrophages abolished the protective action of MSCs.

Phagocyte reprogramming is not the only mechanism that explains increased bacterial clearance during treatment with MSCs. It has been shown that culture supernatant from MSCs previously activated by a suspension of *E. coli* reduces bacterial growth. Indeed, MSCs are able to secrete two antibacterial peptides: LL-37 [38, 42, 44] and hepcidin [35]. However, this synthetic ability seems to depend on MSC culture conditions. Oxygen level, in particular, seems to impact hepcidin expression [35].

Finally, Alcayaga-Miranda et al. reported a lower level of bacteremia in mice with sepsis given MSCs compared with those given standard antibiotic therapy [35]. This finding makes it possible to discern a genuine antibiotic effect of MSCs in their own right.

## MSCs and Organ Failure

It is now accepted that MSCs can moderate many types of organ failure, particularly respiratory failure (Fig. 2). Having proved their efficacy in mouse models of respiratory failure, MSCs administered during sepsis or septic shock have shown to be beneficial in improving and stabilizing arterial and pulmonary pressure [60], reducing cell infiltration [41, 45, 53, 60], pulmonary edema [41], and moderating ambient inflammation by lowering the levels of albumin, IgM, cytokines (IL6, IL1β, murine homolog of CCL-2 (JE), murine homolog of human IL-8 (KC), and CCL5), and total protein detectable in the bronchoalveolar fluid [33, 45]. Thus, MSCs make it possible to reduce inflammatory [33, 45, 53] and respiratory failure scores [34, 49].

Apart from the lungs, the kidneys also benefit from the protective action of MSCs during sepis/septic shock. By lowering urea creatinine levels [33, 34, 44] and enabling a smaller influx of neutrophils and a reduction in the number of apoptotic cells [33, 53], MSCs promote improvement in inflammatory scores and in the tubular necrosis [33, 34, 44, 53, 55] leading to renal failure. However, this protective action of MSCs on the kidney was not reported by Alcayaga-Miranda et al., who found no improvement in creatinine and urea levels nor in the histology of mice treated with MSCs following CLP [35].

Other markers of organ failure also seem to improve in the presence of MSCs, such as transaminases, and levels of bilirubin [34, 35, 46] and amylase [46]. Improvement in liver injury by MSCs seems to be related to a decrease in cytotoxicity and the capacity of liver NK cells to produce inflammatory cytokines in an iNOS- and Indoleamine 2,3- dioxygenase (IDO)-dependent manner [61, 62]. MSC infusion also improves coagulopathy with lower levels of Von Willebrand factor and tissue factor in the plasma of treated animals [49].

Furthermore, MSCs induce cardioprotection during sepsis. Weil et al., using a mouse model of sepsis induced by intravenous infusion of lipopolysaccharide (LPS) from *Salmonella typhimurium*, showed a smaller reduction in ventricular ejection

 

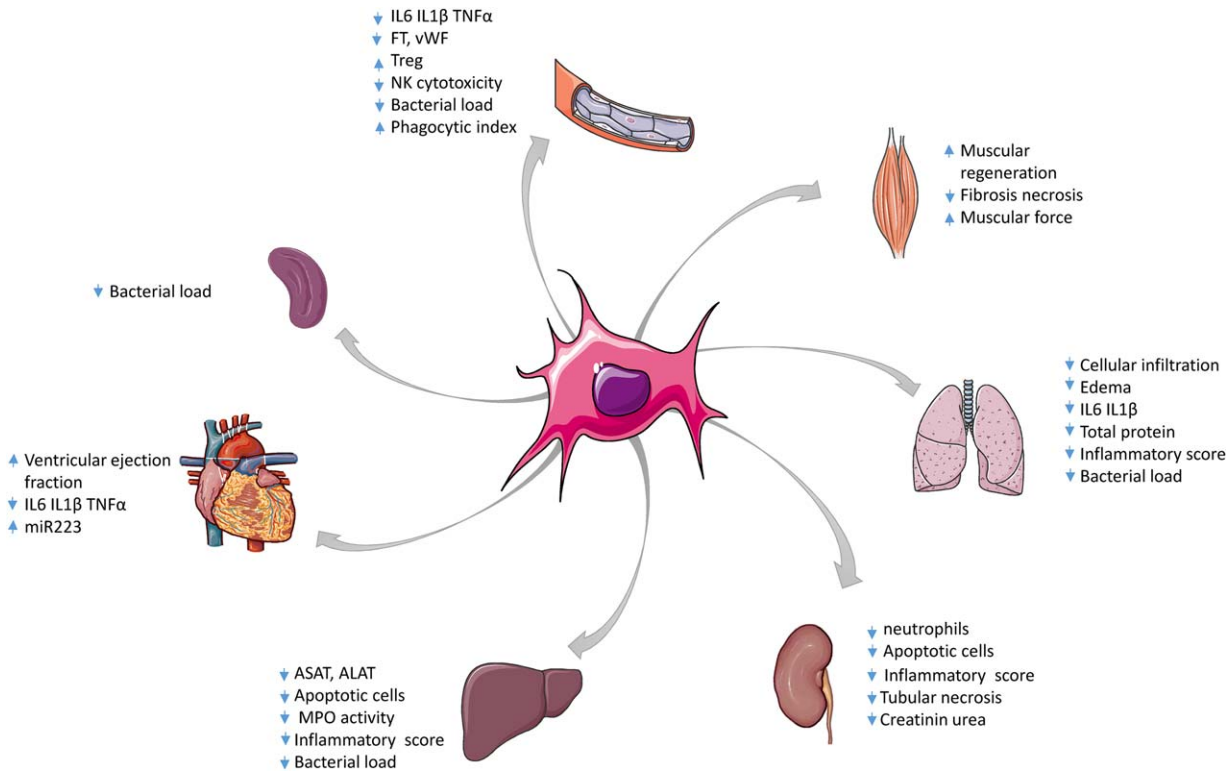


**Figure 2.** Organ failure and mesenchymal stromal/stem cells (MSCs). Several studies have shown that MSCs protect organs from the deleterious effects of sepsis and septic shock. Their ability to decrease bacterial load and pro-inflammatory cytokines and to enhance coagulation enables MSCs to reduce pulmonary, hepatic, microvascular, renal, cardiac, and muscle failure. Abbreviations: ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; IL, interleukin; MPO, myeloperoxidase; NK, natural killer; TNF, tumor necrosis factor.

fraction and shortening fraction 6 hours after sepsis when they administered murine MSCs [51]. This protective action may be partly due to the ability of MSCs to reduce levels of IL6, IL1β, and TNFα in both plasma and cardiac tissue. Wang et al. go further, finding a direct relationship between MSC miR-223 and the cardioprotective role of MSCs. Indeed, they observed an inhibition of the beneficial effect of MSCs removed from the femur of miR-223 knockout mice. Conversely, they demonstrated the ability of MSCs from mi-R223$^{+/+}$ mice to transfer their mi-R223 to the host via exosome secretion [50]. Expression of this micro-RNA is correlated with the severity of sepsis: the weaker its expression, the stronger the manifestation of sepsis-associated inflammation and mortality [63, 64].

Finally, myopathy is a common occurrence during sepsis and septic shock [65]. Four percent of patients are affected. The physiopathology of this phenomenon is complex and poorly known, although possible causes include loss of membrane excitability, mitochondrial dysfunction, proteolysis, and disordered intracellular calcium homeostasis, leading to an alteration in or even a loss of function of contractile proteins. Recently, Rocheteau et al. demonstrated clear dysfunction of the satellite stem cells needed to regenerate skeletal muscle during septic shock [47]. On simultaneously mimicking severe sepsis by the CLP technique and myopathy by injecting notexin, they observed that sepsis led to a clear aggravation of the muscular disorder attributable to early and sustained dysfunction of satellite cells, and particularly an impairment of their mitochondria. However, after intramuscular injection of a dose of $0.3 \times 10^6$ MSCs, they observed the restoration of the mitochondrial parameters of the satellite cells (membrane potential, ATP level, etc.), leading to improved muscular regeneration and reduction in the fibrosis and necrosis associated with notexin injection and enabling superior recovery of muscular strength of treated versus control mice.

## Limitations In Vivo Studies

The actions of MSCs during sepsis and septic shock are unclear. Indeed, the absence of any standardized experimental protocols leads to a variation in the severity and etiology of sepsis. Thus CLP, considered as the "gold standard," mimics human peritonitis by causing polymicrobial sepsis, whereas administration of LPS or bacterial suspensions results in a more reproducible but less clinically relevant sepsis with a single bacterial etiology [66].

Furthermore, for a given sepsis induction technique, the procedures vary between the studies, and this is particularly true for the CLP technique. Thus, the position of the ligature, depending on its distance from the distal pole, will induce 50% mortality in mice at 10 days or 100% mortality at 3 days [67]. The gauge of the needle used in this same procedure, as well as the number of perforations made in the cecum, will produce a more or less severe sepsis. Similarly, the species used, as well as the strain, will have a direct impact on mortality [68].

Apart from the technique of sepsis induction, the procedures for using MSCs vary widely from one study to another. They can be equally well administered by the intravenous, intraperitoneal, or pulmonary route, while it seems obvious that, as in any treatment, the route of administration is of importance in the associated

 

**Table 1.** Preclinical studies using MSCs during sepsis/septic shock

| Authors | Sepsis induction | MSCs used | Dose | Time of MSC injection | Injection route |
|---|---|---|---|---|---|
| Alcayaga-Miranda et al. [35] | CLP | Mens-MSC | $7.5 \times 10^5$ | H + 3 | IP or IV |
| Chang et al. [36] | CLP | Autologous mAD-AMSC or autologous mAD-HMSC | $1.2 \times 10^6$ | H+0.5 and H+6 and H+18 | IP |
| Chao et al. [37] | CLP | hBM-MSC or hWJ-MSC | $5 \times 10^6$ | H+4 | IV |
| Condor et al. [32] | CLP | hWJ-MSC | $1 \times 10^6$ | H+6 | IP |
| Devaney et al. [38] | *Escherichia Coli* | hBM-MSC | $1 \times 10^7$ kg$^{-1}$ or $2 \times 10^7$ kg$^{-1}$ or $5 \times 10^6$ kg$^{-1}$ or $2 \times 10^6$ kg$^{-1}$ | H+0.5 | IV or intra-tracheal |
| Gonzalez-Rey et al. [39] | CLP | hAD-MSC or allogeneic mAD-MSC | $1 \times 10^6$ | H+4 | IP |
| | LPS | hAD-MSC | $3 \times 10^5$or $1 \times 10^6$ | H+0.5 | IP |
| Hall 2013 [55] | CLP | Autologous mBM-MSC | $2.5 \times 10^5$ | H+2, H+24, H+48 | IV |
| Jackson et al. [40] | *E. Coli* | hBM-MSC | $1 \times 10^6$ | H+4 | IV |
| Kim 2014 [56] | enterotoxin | m-MSC or h-MSC | $2.5 \times 10^5$ | H-3 or H-1 | IV |
| Krasnodembskaya et al. [41] | *E. Coli* | hBM-MSC | $1 \times 10^6$ | H+4 | Intra-tracheal |
| Krasnodembskaya et al. [42] | *P. Aeruginosa* | hBM-MSC | $1 \times 10^6$ | H+1 | IV |
| Liu et al. [43] | CLP | m-MSC | $1 \times 10^6$ | H0 | IV |
| Luo et al. [44] | CLP | Autologous mBM-MSC | $1 \times 10^6$ | H+3 | IV |
| Mei et al. [45] | LPS | Autologous mBM-MSC | $2.5 \times 10^5$ | H+ 0.5 | IV |
| Mei et al. [33] | CLP | Autologous mBM-MSC | $2.5 \times 10^5$ | H+6 | IV |
| Németh et al. [34] | CLP | Autologous and allogeneics mBM-MSC | $1 \times 10^6$ | H-24 or H0 or H+1 | IV |
| Pedrazza et al. [46] | *E. Coli* | mAD-MSC | $1 \times 10^6$ | H0 | IV |
| Rocheteau et al. [47] | CLP | Autologous mBM-MSC | 0.310 | H+6 | IM |
| Rojas et al. [60] | LPS | hBM-MSC | $4 \times 10^6$ or $10 \times 10^6$ or $40 \times 10^6$ | H+0.5 | Intra-bronchial |
| Song et al. [48] | CLP | hWJ-MSC | $1 \times 10^6$ | H+4 | IV |
| Tan et al. [49] | CLP | mBM-MSC | $1 \times 10^6$ | H+6 | IV |
| Wang et al. [50] | CLP | mBM-MSC | $1 \times 10^6$ | H+1 | IV |
| Weil et al. [51] | LPS | Autologous mBM-MSC | $2 \times 10^6$ | H+1 | IV |
| Wu et al. [52] | CLP | hWJ-MSC | $1 \times 10^6$ | H0 | IP |
| Yagi et al. [53] | LPS | hBM-MSC | $2 \times 10^6$ | H0 | IM |
| Zhao et al. [54] | CLP | hWJ-MSC | $1 \times 10^6$ | H+1 | IV |

MSC were considered autologous when they derived from the same species of mouse/rat.
Abbreviations: AMSC: apoptotic MSC; CLP, cecum ligation and puncture; hAD, human adipose; hBM, human bone marrow; HMSC: healthy MSC; hWJ, human Wharton's jelly; IV, intravenous; IM, intramuscular; IP, intraperitoneal; LPS, lipopolysaccharide; mAD, murine adipose; mBM, murine bone marrow; Mens-MSC: menstrual MSC; MSC, mesenchymal stromal/stem cell.

therapeutic effect. Similarly, the dose and concentration of cells administered can vary between the studies. The time of injection is also different; some use MSCs as a prophylactic, others as a curative treatment at different times after sepsis induction.

Finally, the source of MSCs used is not always the same, although it has been widely described that the immunomodulatory potency attributed to them varies with their tissue of origin [69].

## MSCs and Clinical Trials

Currently, of the 720 clinical trials involving MSCs being conducted throughout the world, 269 are dedicated to immune and inflammatory diseases. Among them, only a few relate to sepsis and septic shock (http://www.clinical trial.gov, accessed July 2017). The first is a Russian study that started in 2013 (NCT01849237). Its objective was to evaluate at 28 days the efficacy of MSCs administered at a dose of 1–2 $\times 10^6$ kg$^{-1}$ in 30 patients with combined septic shock and severe neutropenia. It concluded that MSC administration improved short-term survival but did not prevent organ failure-related death [70].

The Canadian trial "Cellular Immunotherapy for Septic Shock: A Phase I Trial (CISS)," (NCT02421484) opened in 2015 and currently recruiting patients, is aimed at evaluating the toxicity of an infusion of allogeneic bone-marrow MSCs in nine patients with septic shock, by assessing the development of adverse effects. The protocol begins by infusing three patients with the lowest dose of MSCs: $0.3 \times 10^6$ cells per kilogram. In the absence of major adverse effects, the trial will continue with the infusion of MSCs at a higher dose: three patients will be given $1 \times 10^6$ cells per kilogram, whereas the final three will be given a dose of $3 \times 10^6$ cells per kilogram.

Finally, the Belgian company TiGénix has closed a phase I trial (NCT02328612). The aim of this study was to evaluate the impact of the prophylactic administration of MSCs from adipose tissue of allogeneic donors on inflammation associated with an infusion of bacterial endotoxin (LPS) administered to 32 healthy volunteers. Three doses of MSCs were studied: $0.25 \times 10^6$ cells per kilogram, $1 \times 10^6$ cells per kilogram, and $4 \times 10^6$ cells per kilogram. This study demonstrated safety of infusion of MSCs from adipose tissue. Following these results, TiGénix has started an international phase Ib/IIa

clinical trial (SEPCELL NCT03158727). Currently recruiting patients, this study evaluates the impact of MSCs versus placebo on acute bacterial community pneumonia in 180 patients.

MSCs are also studied in the indication of acute respiratory distress syndrome (ARDS), a frequent complication of sepsis and septic shock. A phase I clinical trial published in 2014 has demonstrated that administration of allogeneic adipose-derived MSCs as a treatment of ARDS appears to be safe and well tolerated (NTC01902082). However, the clinical effect at the dose of $1 \times 10^6$ cells per kilogram was weak [71]. Similarly, in a dose escalation pilot study (NCT01775774), Wilson et al. demonstrated that there were no adverse events after administration of allogeneic bone marrow MSCs and no significant difference in ARDS markers between groups [72]. However, the patient number in this study was low (overall, nine patients). A phase II efficacy trial enrolling 60 patients is ongoing.

Moreover, three clinical trials (phase I/II and II) (NCT02804945; NCT02112500; NCT02444455) are still recruiting and one will start soon in Ireland (NCT03042143). Results of these four studies will highlight the efficacy or not of MSCs on ARDS symptoms.

## Limitation of Clinical MSC Use in Sepsis and Septic Shock

MSCs seem to be good candidates for sepsis and septic shock treatment, but their use in this indication meets some limitations. As septic shock is a vital emergency, a banking of frozen allogeneic MSCs must be available. However, the effect of freezing/thawing on MSC properties is unclear. Some studies have demonstrated that immunomodulatory properties of frozen MSCs are decreased compared with fresh MSCs while others demonstrate no effect of freezing on MSC efficacy [73–76]. Moreover, MSCs present the same drawbacks as blood-derived medications. Their production depends on the donor; they are expensive and not exactly reproducible. Production time and quality depend on the tissue used and the donor's characteristics. MSCs derived from fetal tissues have increased the proliferative capacities compared with MSCs derived from adult tissues [77]. Moreover, it was recently demonstrated that obstetrical factors modulate the proliferation and differentiation capacities of MSCs [78]. Consequently, to standardize MSC preparation, donor selection criteria should be defined for each tissue source. Finally, the stability of MSC suspensions has to be considered and defined especially when thawed MSCs are used.

## Conclusion

There is currently no specific treatment for sepsis and septic shock, a major challenge to public health. Despite many clinical trials, even Phase III, conducted worldwide with promising innovating drugs, the management of sepsis and septic shock remains exclusively symptomatic. The latest advances in medical research have highlighted a particularly complex pathophysiology with a concurrent pro-inflammatory and anti-inflammatory syndrome. The difficulty of finding a therapeutic target in this perpetual mixed state has directed research toward innovative therapeutic agents, particularly MSCs. With their additional ability to modulate cytokine homeostasis, limit organ failure, and increase survival and bacterial clearance, they seem to be particularly well suited to treating sepsis and septic shock. However, the heterogeneity of experimental procedures has generated questions that are currently unanswered: what is the effective dose in the indication of sepsis and septic shock? What is the most appropriate route of administration? What is the best source of MSCs? The first few ongoing clinical trials will help to answer those questions and establish whether or not MSCs are on their way to becoming established as a genuine therapeutic innovation in the treatment of sepsis and septic shock.

## Author Contributions

C.L.: collection and/or assembly of data, data analysis and interpretation, manuscript writing; S.G.: conception and design, financial support, data analysis and interpretation, final approval of manuscript; L.R. and D.B.: conception and design, data analysis and interpretation, final approval of manuscript.

## Disclosure of Potential Conflicts of Interest

The authors indicate no potential conflicts of interest.

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