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Stem Cell–based Therapies for Sepsis

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ABSTRACT

Sepsis is a life-threatening syndrome resulting in shock and organ dysfunction stemming from a microbial infection. Sepsis has a mortality of 40% and is implicated in half of all in-hospital deaths. The host immune response to microbial infection is critical, with early-phase sepsis characterized by a hyperinflammatory immune response, whereas the later phase of sepsis is often complicated by suppression. Sepsis has no treatment, and management remains supportive.

Stem cells constitute exciting potential therapeutic agents for sepsis. In this review, we examine the rationale for stem cells in sepsis, focusing on mesenchymal stem/stromal cells, which currently demonstrate the greatest therapeutic promise. We examine the preclinical evidence base and evaluate potential mechanisms of action of these cells that are important in the setting of sepsis. We discuss early-phase clinical trials and critically appraise translational barriers to the use of mesenchymal stem/stromal cells in patients with sepsis. **(Anesthesiology 2017; 127:1017-34)**

Sepsis: Extent of the Problem

Sepsis is a life-threatening syndrome resulting in shock and multiple organ dysfunction as a consequence of microbial infection.[1] Both pathogen and host factors influence the clinical presentation, severity, and ultimately patient outcome, including the nature and virulence of the microbial pathogen, which drives tissue invasion and toxin production, and the health status and comorbidities of the patient, which influence the host response. Patients frequently present with fever, shock, and dysfunction of one or more organs, including the lungs (acute respiratory distress syndrome), kidneys (acute kidney injury), brain (confusion, delirium, or coma), liver, and cardiovascular system (shock or myocardial dysfunction).

Infections of the lungs, abdominal cavity, urinary tract, and soft tissue constitute the most common sources of sepsis.[2] *Escherichia coli*, *Klebsiella spp*, and *Pseudomonas aeruginosa* constitute the dominant Gram-negative pathogens, whereas *Staphylococcus aureus* and *Streptococcus pneumoniae* are the most common Gram-positive pathogens isolated.[3] Recently, a global study of 14,000 critically ill patients found that 62% of isolates were Gram-negative, whereas 47% were Gram-positive and 19% were fungal.[4] Fungal infections are an increasing source of severe sepsis, although in one third of cases the causative organism is not determined.

Sepsis exerts a significant socioeconomic impact and is now the leading cause of critical illness globally.[5,6] In 2011, sepsis was responsible for more than $20 billion (5.2%) of hospital costs[7] and a quarter of a million estimated deaths in the United States annually.[8] The reported incidence of sepsis is increasing,[9,10] perhaps because of changing patient demographics, with advanced age, more comorbidities, impaired immunity, and increasing clinician diagnosis and recognition of sepsis all playing a role.[11] Sepsis has an overall mortality of 40%[8,12] and may cause half of all in-hospital deaths in the United States.[13] Furthermore, long-term follow-up studies demonstrate that sepsis survivors continue to have a higher mortality in the 5 yr after sepsis.[14] In addition, survivors of sepsis endure long-term psychologic, cognitive, and physical impairments.[15]

Sepsis: Role of the Immune Response

The host immune response, specifically the loss of immune homeostasis induced by the pathogen, is of critical importance to the initiation, evolution, and outcome from sepsis (fig. 1).[16] Patients in the early phases (hours to days) of sepsis present with fever, shock, and multiorgan failure, as well as evidence of a hyperinflammatory innate immune response. Pathogen-associated molecular patterns, which originate from microorganisms, are specific molecular signatures recognized as foreign to the host, and they bind to pattern recognition receptors expressed on innate immune cells and initiate and drive this initial hyperinflammatory phase.[17] Pattern recognition receptor activation

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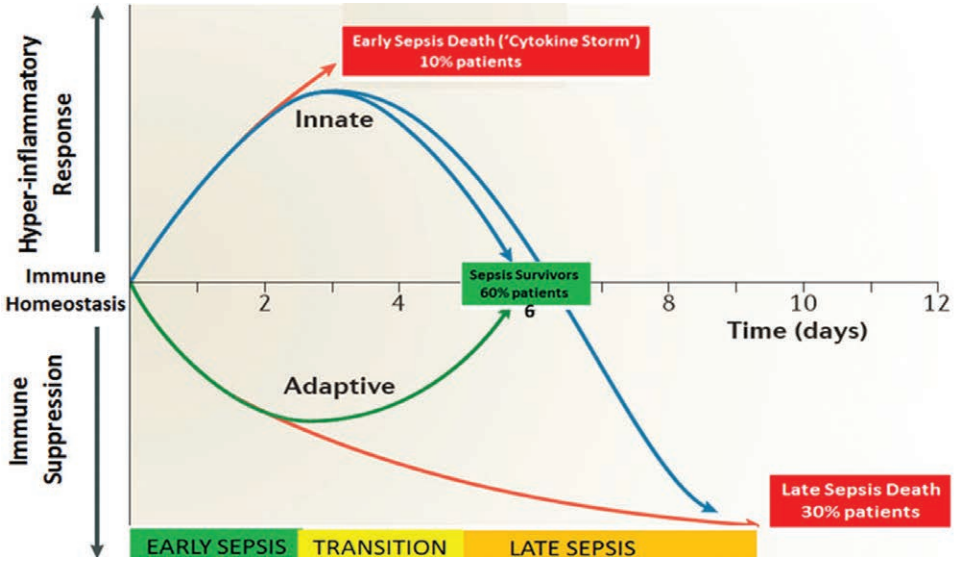

Fig. 1. The adaptive and innate host immune responses to sepsis. Patients in the early phases of sepsis present with fever, shock, and multiorgan failure, as well as evidence of a hyperinflammatory innate immune response. Patients surviving this phase may recover or progress to a later phase of sepsis resulting in a more immunosuppressed profile, characterized by functional deficits of the immune system and by superinfection and poor outcome. Adapted and reprinted by permission from Macmillan Publishers Ltd: Hotchkiss *et al*.,[16] Nature Review Immunology, copyright 2013.

generates diverse proinflammatory molecule expression including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-6, IL-8, and interferon (IFN)-γ, as well as antiinflammatory cytokines such as IL-10.[18] This process is further driven by the release of damage-associated molecular patterns from injured tissues and cells.[17] This exuberant production of proinflammatory cytokines and other soluble mediators, coupled with the demonstration that injection of these mediators into animals could recapitulate some of the effects seen in sepsis, led to the concept of the "cytokine storm" as being responsible for early sepsis-related multiple organ failure and death.[19] Key advances have occurred in the management of patients in the early phase of sepsis, with earlier recognition facilitating prompt broad-spectrum antimicrobial therapy,[20] aggressive source control,[8] and goal-directed resuscitation, all contributing to improved outcome and a reduction in mortality.

Patients surviving the early phases of sepsis regain immune homeostasis, clear their infection, and recover, or they transition into a protracted immunosuppressive phase where sepsis persists[16,21,22] (fig. 1). The proportion of patients who enter this later phase of sepsis is increasing, in part due to the advances in early supportive care reducing early deaths, as well as changes in the patient population, which is now older[8] and has more comorbidities that render them immunosuppressed. Seventy percent of sepsis deaths now occur in this phase,[16] which is characterized by opportunistic pathogen superinfections,[23] latent viral reactivation,[24] and evidence for profound immunosuppression.[21,25]

Sepsis Management: Current State of the Art

Although fewer patients die in the early hyperinflammatory phase of sepsis, the increasing numbers of people experiencing severe sepsis, coupled by the failure to improve outcomes from the later phases of sepsis, means that the mortality burden of sepsis continues to increase.[5,6,8,12,13] There are no therapies that directly modify the pathophysiology and injury mechanisms underlying sepsis. The focus of research over the last four decades has been on suppressing the early proinflammatory response to sepsis.[19] To date, there have been more than 40 unsuccessful clinical trials of agents that reduce pathogen recognition and/or block proinflammatory cytokines and/or inflammation-signaling pathways.[26,27]

A number of important insights have emerged from these efforts to find a therapy for sepsis. First, the traditional paradigm of sepsis as a hyperinflammatory disorder that led to the testing of interventions to suppress the immune response is likely an oversimplification, as discussed above. Second, given the complexity of the host response to sepsis, inhibition of a single mediator, however important to the injury process, is unlikely to be effective. Third, the timing of therapeutic interventions may be important. Although steroids can attenuate the early inflammatory response, these drugs have been demonstrated to worsen later immune suppression and increase mortality.[28] In contrast, encouraging results have been reported from early-phase studies of immune stimulation strategies to reverse specific immune defects in late sepsis, such as administration of granulocyte macrophage colony stimulating factor[29] and interferon-β1a,[30] suggesting that the optimal therapeutic approach may vary considerably depending on the stage of sepsis. Fourth, sepsis is a heterogeneous disease, and identification of sepsis subphenotypes or endotypes, as has been demonstrated recently for acute respiratory distress syndrome (ARDS),[31]

may allow for focusing of therapeutic interventions on specific sepsis subpopulations more likely to benefit.

An additional concern is the ongoing emergence of pathogens resistant to multiple antimicrobial therapies. Taken together with the failure to date of drug therapy trials, these concerns suggest a need to consider alternative therapeutic approaches, aimed at attenuating the proinflammatory response while enhancing host immune function and tissue reparative capacity. Stem cells constitute an emerging therapeutic candidate that might meet these requirements and consequently are emerging as potential therapeutic agents for sepsis.

Stem Cells: Classification and Therapeutic Potential

Stem cells (regardless of age of donor or source tissue) are undifferentiated cells with the capacity to self-renew and/or generate more than one differentiated functional daughter cell type. There is a hierarchy of "stemness," from pluripotent cells to multipotent cells and to progenitor cells, where the capacity to differentiate into different cell lineages is progressively reduced. Hematopoietic stem cells used for treatment of blood disorders, for example, are pluripotent cells that generate platelets, erythrocytes, and a wide variety of leukocyte types. Another important classification is in relation to their tissue source, that is, whether they are derived from embryonic or adult tissues and, in the latter case, which specific tissues they originate from. The cell type for which there is the most interest as a therapy for sepsis at present is mesenchymal stem/stromal cells (MSCs). These cells have multiple potential advantages, including their convenient isolation from multiple adult tissues and relatively easy culture expansion, which make them strong therapeutic candidates in patients with sepsis. There are exciting preclinical data supporting their use, and early-phase clinical trials are in progress. Consequently, we focus on MSCs in this review.

Therapeutic Potential of MSCs for Sepsis

The therapeutic potential of MSCs for sepsis is supported by several factors. First, MSCs are relatively immune privileged (low expression of cell-surface human leukocyte antigen class I and II molecules), they do not induce a classical cytotoxic T cell (rejection) response, and they can therefore be used as an allogeneic therapy without the need for immunosuppression. Second, they modulate diverse aspects of the host immune response. Although trials of agents that directly inhibit aspects of the immune response to sepsis have been unsuccessful, MSCs exert a more complex profile of immune effects. Importantly, MSCs may reprogram the immune system to reduce host tissue damage while preserving the immune response to microorganisms. Third, MSCs may enhance tissue repair and restoration after sepsis,[32–34] restoring endothelial barrier function, mediated partly by secretion of factors that enhance resolution of tissue injury. Fourth, sepsis and septic shock frequently progress to dysfunction and failure of multiple organs. MSCs may decrease injury and/or restore function in diverse organs, including the liver, kidneys, heart, and lungs. Fifth, MSCs may directly enhance host bactericidal capacity by increasing macrophage bacterial phagocytosis and killing[35] and increasing secretion

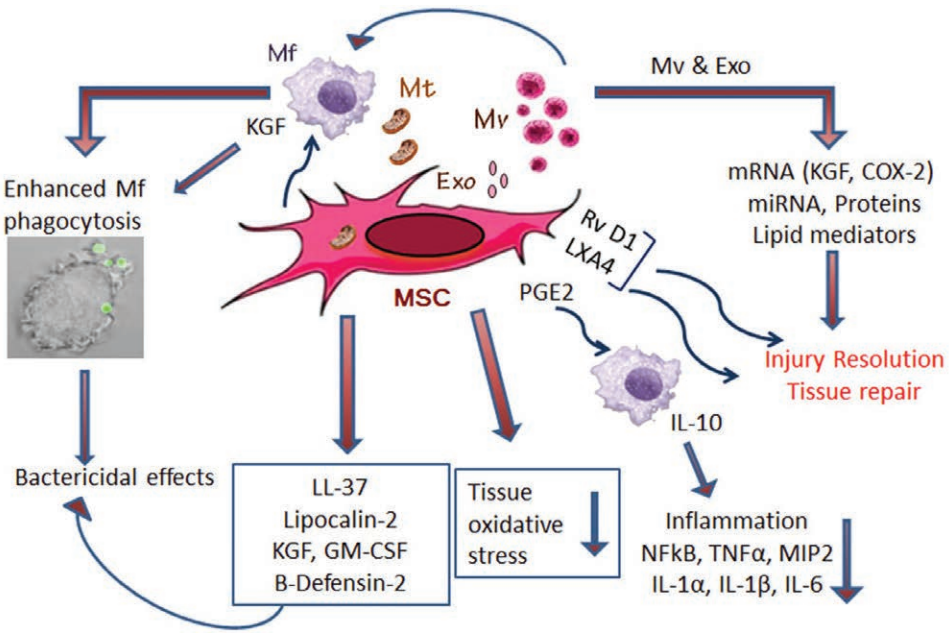

Fig. 2. Factors secreted from mesenchymal stem/stromal cells (MSCs) of importance for reducing severity of pneumonia and systemic sepsis. Exo = exosomes; GM-CSF = granulocyte-macrophage colony stimulating factor; KGF = keratinocyte growth factor; LXA4 = lipoxin A4; Mf = macrophages; MIP2 = macrophage inflammatory protein 2; Mt = mitochondria; Mv = microvesicles; PGE2 = prostaglandin E2; RvD1 = resolving D1; TNF = tumor necrosis factor.

of antimicrobial peptides.[36] Sixth, MSCs are well studied in clinical trials, with a growing safety record in patients. Concerns regarding the potential for long-term effects are mitigated by the fact that they disappear from the tissues within days of administration, although their effects often outlast their residence time in the tissues.

MSCs: Insights from Preclinical Sepsis Models

Pulmonary Sepsis

Initial studies demonstrated the potential for MSC therapy to decrease injury after pulmonary endotoxin instillation in murine models.[37] This, and subsequent studies in this model, provided important mechanistic insights, elucidating the role of MSC-secreted mediators, including keratinocyte growth factor,[38] TNF-α–induced protein-6, and lipoxin A4[39] in modulating the immune response to endotoxin (reducing TNF-α and macrophage inflammatory protein-2 and increasing IL-10 concentrations) and in promoting injury resolution and repair. The MSC secretome and MSC-derived microvesicles,[40] as well as embryonic stem cell–derived MSCs,[41] also effectively attenuated endotoxin-induced injury.[40] Mitochondrial transfer from MSCs to the pulmonary epithelium appears important in reducing the severity of injury caused by pulmonary endotoxin instillation[42] (fig. 2).

MSCs also demonstrate efficacy in relevant preclinical models of bacterial pneumonia (table 1) induced by Gram-negative organisms such as *Escherichia coli,*[43–45] *Klebsiella pneumonia,*[46] and *Pseudomonas aeuroginosa*.[47,48] In the early phases of lung sepsis, several groups including ours have shown that human MSCs derived from bone marrow and umbilical cord tissues reduce *E. coli* lung injury, decreasing lung bacterial load, enhancing lung function, and reducing mortality in rodent, murine, and ovine models.[44,49–53] The potential for MSCs to attenuate pneumonia induced by Gram-positive organisms, including *Staphylococcus aureus* and *Streptococcus pneumoniae*, has also been demonstrated.[48] MSCs exert antimicrobial effects against methicillin-resistant *S. aureus* in a rodent model of pouch infection[54] and in an infected wound model[55] and can directly inhibit the growth of *S aureus*.[56] Microvesicles secreted by MSCs are also effective in attenuating bacterial pneumonia in mice *via* mechanisms including enhanced macrophage phagocytosis, mediated in part through the expression of keratinocyte growth factor and cyclooxygenase-2 messenger RNA (mRNA) in the injured alveoli.[53]

Systemic Sepsis

MSCs demonstrate efficacy in several preclinical systemic sepsis models (table 2). MSCs from the bone marrow,[41] adipose tissue,[57] and macrophages cocultured with adipose tissue MSCs[58] decreased systemic endotoxemia-induced lung injury, attenuated renal cell apoptosis, and decreased multiorgan injury in rodents. Bone marrow MSCs significantly reduced cytokine and chemokine (IL-1β, -6, -10, Chemokine [C-C motif] ligand-5 [CCL-5], and TNF-α) concentrations and improved survival after cecal ligation and puncture in mice, a key preclinical model of abdominal polymicrobial sepsis.[35,59] MSCs maintained their efficacy when administered 6 h after cecal ligation and puncture-induced polymicrobial sepsis in mice.[35] Bone marrow MSCs attenuated murine sepsis–induced kidney injury by decreasing the proinflammatory response and enhancing macrophage phagocytosis, with reductions in renal mRNA levels of IL-6, IL-17, TNF-α, IFN-γ, Chemokine (C-X-C motif) ligand (CXCL)-1, CXCL-2, CXCL-5, CCL-2 and CCL-3.[60,61] The effect was also seen in methicillin-resistant *S. aureus* systemic infection, with reduced bacterial load and expression of cytokines and chemokines.[54] In a genome-wide microarray analysis of septic animals, MSCs decreased transcription of proinflammatory genes while increasing transcription of genes relating to tissue repair and endothelial integrity and maintaining transcriptional pathways responsible for cellular bioenergetics[62] (fig. 3). MSCs can also reduce the cytokine response induced by Staphylococcal enterotoxin B in mice, although it did not increase survival in this model.[63]

Viral Infection

In vitro studies demonstrate that human MSCs exhibit antiviral effects, such as inhibition of virus-specific $CD8^+$ T-cell proliferation,[64] which is mediated through indoleamine 2,3-dioxygenase secretion.[64,65] MSCs did not attenuate moderate[66] or severe[67] H1N1 (PR8 strain) influenza-induced lung injury in mice. In the study by Gotts *et al.*,[66] MSCs modestly reduced viral load but failed to reduce disruption of the alveolar–capillary barrier in mouse lungs and severity of lung injury. In contrast, MSC therapy attenuated H9N2 avian influenza virus–induced acute lung inflammation and injury in mice[68] *via* reduction in TNF-α, IFN-γ, IL-1α, and IL-6, as well as an increase in IL-10. This suggests that the efficacy of MSCs for influenza may be strain dependent, although additional studies are needed to further understand these issues.

Immunomodulatory Effects of MSCs

MSCs exert multiple modulatory effects on diverse aspects of the immune response that are of direct relevance to their therapeutic potential for sepsis (fig. 3). In genome transcriptional studies in murine systemic sepsis models, MSC therapy has been demonstrated to modulate transcription of up to 13% of the murine genome, with immune response–related effects including the following: (1) down-regulation of toll-like receptor, nuclear factor-κB, and IL-6 signaling pathways; (2) up-regulation of nuclear factor of activated T cell–related genes; (3) up-regulation of genes involved in antigen presentation, phagocytosis, bacterial killing, complement, and coagulation regulation including platelet activation; and (4) enhancement of genes involved in cell-to-cell interactions and in the regulation of endothelial integrity.[35,62]

Table 1. Selected Preclinical Studies Examining Mechanisms of Action of MSCs in Pneumonia Sepsis

Study	Species	Sepsis Model	Cell Therapy	MSC Delivery Route and Timing of Administration	Effect and Mechanism of Action
Gupta *et al.*, 2007[37]	Mouse	LPS	mBM-MSC	IV, 4 h postinjury	Improved survival, reduced lung water and protein, increased IL-10, and reduced TNF-α/MIP-2 in BAL and serum
Lee *et al.*, 2009[38]	Human	LPS on *ex vivo* human lung	hBM-MSC	IV, 1 h postinjury	Restoration of AFC through KGF-dependent, sodium-dependent alveolar fluid transport
Krasnodembskaya *et al.*, 2010[36]	Mouse	*E. coli* pneumonia	hMSC	IV, 4 h postinjury	Secretion of the antimicrobial peptide LL-37 resulting in increased bacterial clearance
Kim *et al.*, 2011[51]	Mouse	*E. coli* pneumonia	hUC-MSC	IT, 3 h postinjury	MSCs attenuate *E. coli*–induced ALI by down-regulating the inflammatory process and enhancing bacterial clearance
Islam *et al.*, 2012[42]	Mouse	LPS	m/hBM-MSC	IT, at time of injury	BMSC's protect against ALI by reconstituting alveolar bioenergetics through Cx43-dependent alveolar attachment and mitochondrial transfer
Gupta *et al.*, 2012[43]	Mouse	*E. coli* pneumonia	mMSC	IV, 4 h postinjury	mMSCs reduced the severity of *E. coli* pneumonia, in part *via* an antimicrobial peptide lipocalin-2–dependent mechanism
Lee *et al.*, 2013[95]	Human	*E. coli* injury on lungs (*ex vivo*)	hMSCs	1 or 2 h postinjury	MSCs restored AFC, reduced inflammation, and exerted antimicrobial activity, partly *via* KGF secretion
Elman *et al.*, 2014[88]	Mouse	LPS	hBM/AdMSC	IP, at time of injury	BM-MSC provided greater survival benefit and multiorgan protection *versus* AdMSC
Asmussen *et al.*, 2014[52]	Sheep	*P. aeruginosa*	hMSC	IV, more than 1 h	hMSCs were well tolerated, improved oxygenation, and decreased pulmonary edema
Shalaby *et al.*, 2014[45]	Mouse	*E. coli* pneumonia	mBM-MSC	IV, 24 h preinjury or 12 h postinjury	MSCs increased survival and reduced pulmonary edema and lung injury; increased antioxidant activity with reduction in MPO activity and malondialdehyde levels
Hackstein *et al.*, 2015[46]	Mouse	*K. pneumonia*	mBM-MSC	IT, 4 h postinjury	MSCs increased survival; reduced alveolitis, protein leakage, and TNF-α and IL-12 p70 expression; reduced lung dendritic cell, IL-17$^+$- and IFN-γ$^+$-CD4$^+$ T cells
Zhu *et al.*, 2014[40]	Mouse	LPS	hBM-MSC MV	IT, 12 h postinjury	Reduced inflammatory cell, cytokine, and protein levels in lung, reduced EVLW, increased KGF levels, reduced proinflammatory cytokines, and increased IL-10 levels
Devaney *et al.*, 2015[44]	Rat	*E. coli* pneumonia	hBM-MSC	IV, 30 min postinjury	Reduced lung injury and lung bacterial burden *via* enhanced macrophage phagocytosis and increased alveolar LL-37 concentrations
Monsel *et al.*, 2015[53]	Mouse	*E. coli* pneumonia	hBM-MSC or MV	IV, 4 h postinjury	MVs derived from hMSCs were as effective as the parent stem cells; they improved survival through KGF secretion and enhanced monocyte phagocytosis of bacteria; MVs from poly (I:C) prestimulated MSCs and further enhanced the therapeutic effects
Hao *et al.*, 2015[41]	Mouse	LPS	hBM/ES-MSC	IT, at time of injury	Reduced inflammatory cell, cytokine, and protein levels in lung; reduced EVLW; and reduced MMP-9 secretion with BM *vs.* ES-MSC
Mao *et al.*, 2015[47]	Mouse	*P. aeruginosa*	mAd-MSC	IT, 1 h postinjury	Inhibition of overproduction of PGE2 leading to improved macrophage phagocytosis, reduced alveolar neutrophil accumulation, and reduced levels of MPO, MIP-2, and total protein in BALF
Sutton *et al.*, 2016[48]	Mouse	*P. aeruginosa*, *S. aureus*, *S. pneumoniae*	hBM/Ad- MSC	IT, 24 h postinjury	MSCs produced LL-37 with antimicrobial activity *in vitro* against all 3 bacteria and *in vivo* against *P. aeruginosa* and *S. aureus*; enhanced antimicrobial effectiveness in presence of MSCs
Sung *et al.*, 2016[114]	Mouse	*E. coli* pneumonia	hUC-MSC	IT, 3 h postinjury	β-Defensin-2 secreted by the MSCs *via* the TLR-4 signaling pathway mediates microbicidal effects against *E. coli*, both *in vitro* and *in vivo*
Jackson *et al.*, 2016[96]	Mouse	*E. coli* pneumonia	hBM-MSC	IV or IN, 4 h postinjury	Mitochondrial transfer from BM-MSCs to the AM *via* tunneling nanotubes leads to enhancement in their phagocytic activity and mediates the antimicrobial effect of MSCs in ARDS
Curley *et al.*, 2016[50]	Rat	*E. coli* pneumonia	hUC-MSC	IV, 30 min postinjury	UC-MSCs grown in xeno-free conditions are as effective as BM-MSCs; they improve survival, enhance bacterial clearance, and reduce *E. coli*–induced oxidant injury

Ad = adipose-derived; AFC = alveolar fluid clearance; ALI = acute lung injury; AM = alveolar macrophages; ARDS = acute respiratory distress syndrome; BM = bone marrow; Cx43 = connexin 43; ES = embryonic stem; EVLW = extravascular lung water; h = human; IL = interleukin; IN = intranasal; IP = intraperitoneal; IT = intratracheal; IV = intravenous; LPS = lipopolysaccharide; m = murine; MMP-9 = matrix metallopeptidase 9; MSC = mesenchymal stem/stromal cell; MV = microvesicles; r = rat; TLR = toll-like receptor; TNF = tumor necrosis factor; UC = umbilical cord.

Table 2. Selected Preclinical Studies Examining Mechanisms of Action of MSCs in Systemic Sepsis

Study	Animal	Sepsis Model	Cell Therapy	MSC Delivery Route and Timing of Administration	Effect and Mechanism of Action
Nemeth *et al.*, 2009[59]	Mouse	CLP	BMSC	IV, 24 h pre/postinjury	PGE2-dependent reprogramming of macrophage to increase production of IL-10
Mei *et al.*, 2010[35]	Mouse	CLP	mMSC	IV, 6 h postinjury	Modification of inflammatory gene transcriptional activity, down-regulation of the acute inflammatory response, and up-regulation of pathways relevant to phagocytosis and bacterial clearance
DosSantos *et al.*, 2012[62]	Mouse	CLP	mMSC	IV, 6 h pre/postinjury	On transcriptional analysis, MSCs: (1) attenuated sepsis-induced mitochondrial-related functional derangement; (2) decreased endotoxin/toll-like receptor innate immune proinflammatory transcriptional responses; and (3) coordinated expression of transcriptional programs implicated in the preservation of endothelial/vascular integrity
Krasnodembskaya *et al.*, 2012[49]	Mouse	GNPS	hMSC	IV, 1 h postinjury	Increased animal survival and bacterial clearance secondary to enhanced monocyte phagocytosis
Shin *et al.*, 2013[57]	Rat	LPS	hAd-MSCs	IV, 30 min postinjury	hAd-MSCs decreased the level of inflammatory cytokines in serum and in the lung, reduced inflammatory changes in the lung, prevented apoptosis in the kidney, and improved multiorgan injury
Kim *et al.*, 2014[63]	Mouse	SEB	m/h BM-MSC	IV, 1 h preinjury or 3 h postinjury	Reduced levels of IL-2, IL-6, and TNF-α but no improvement in survival in therapeutic group
Luo *et al.*, 2014[60]	Mouse	CLP	mMSC	IV, 3 h postinjury	Improved survival and sepsis-related acute kidney injury, possibly by inhibition of IL-17 production and immunomodulation
Chao *et al.*, 2014[71]	Rat	CLP	hUC-MSCs and hBM-MSCs	IV, 4 h postinjury	MSCs increased circulating $CD3^+CD4^+CD25^+$ Treg cells and Treg cells/T cells ratio, enhanced Treg cell suppressive function, and decreased serum levels of IL-6 and TNF-α
Wang *et al.*, 2015[119]	Mouse	CLP	mMSCs or exosomes	IV, 1 h postinjury	MSC mediated cardioprotection mainly through exosomal transfer of miR-223 to macrophages and cardiomyocytes
Alcayaga-Miranda *et al.*, 2015[150]	Mouse	CLP	Men-MSCs	IP, 3 h postinjury	Men-MSCs in synergy with antibiotics improved the survival rate, enhanced bacterial clearance, and reduced organ injuries
Hu *et al.*, 2016[58]	Mouse	LPS	hAd-MSCs	IV, at time of injury	Ad-MSCs, as well as macrophages educated by Ad-MSCs, decreased lung inflammation, pulmonary edema, and inflammatory cytokine response in LPS-induced systemic response

Ad = adipose tissue; AM = alveolar macrophages; CD = cluster of differentiation; CLP = cecal ligation and puncture; GNPS = Gram-negative polymicrobial sepsis; h = human; IL = interleukin; IN = intranasal; IP = intraperitoneal; IT = intratracheal; IV = intravenous; LPS = lipopolysaccharide; m = murine; Men = menstrual-derived; miRNA = microRNA; PGE2 = prostaglandin E2; r = rat; SEB = Staphylococcal enterotoxin B (SEB); TNF = tumor necrosis factor; Treg = regulatory T cells.

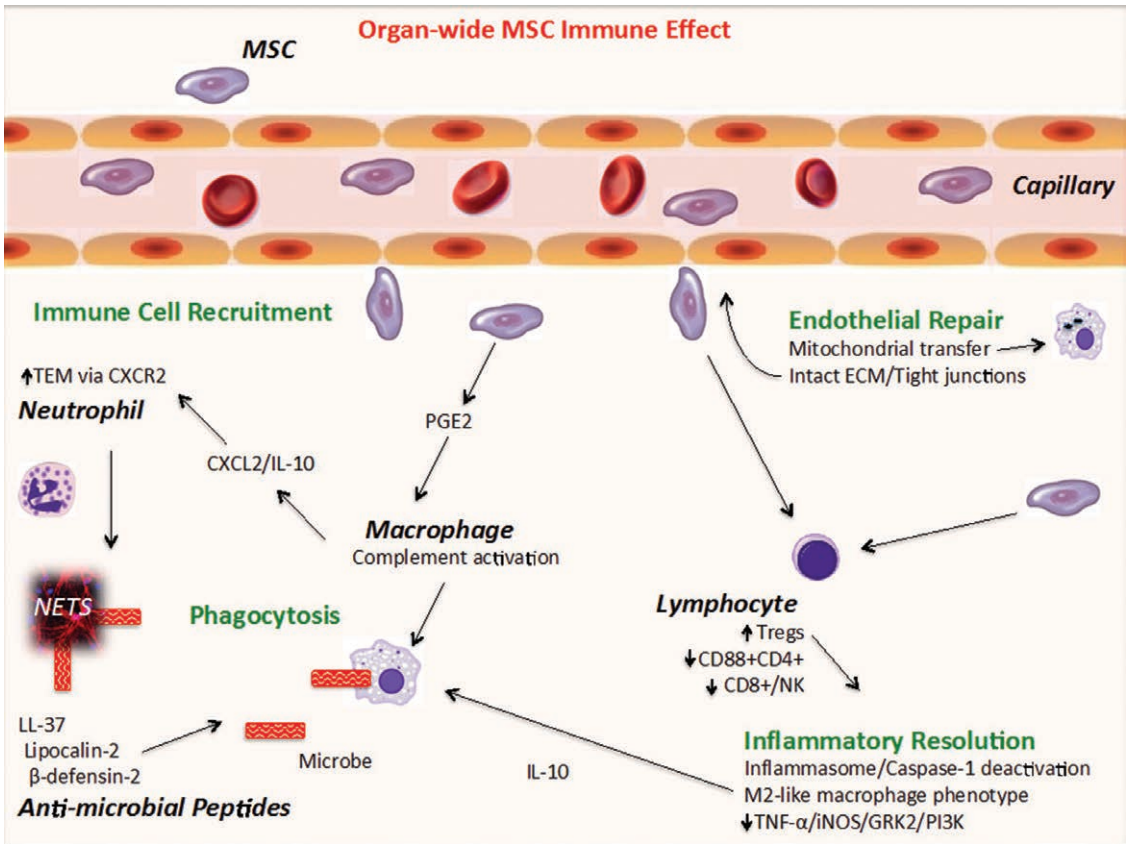

Fig. 3. Organ-wide mesenchymal stem/stromal cell (MSC) effect on immune cells. MSCs exert beneficial effects on organ systems through their interaction with cells of the innate and adaptive immune systems, subsequent controlled tissue inflammation, and vascular integrity. Enhanced endothelial repair, through mitochondrial transfer, allows for tissue inflammatory milieu gatekeeping. M2-like macrophages enhance complement activation, increase microbe phagocytosis and clearance, and increase targeted neutrophil recruitment to injured tissue *via* CXCL2. Neutrophil extracellular traps release enhanced phagocytosis along with MSC-secreted antimicrobial peptides. The targeted control of microbial invasion is further influenced by inflammasome/caspase-1 deactivation, downregulation of GRK2 and PI3K, reduced secretion of TNF-α and iNOS, and an increased regulatory T-lymphocyte presence. ECM = extracellular matrix; GRK2 = G protein–coupled receptor kinase 2; IL-10 = interleukin-10; iNOS = inducible nitric oxide synthase; KGF = keratinocyte growth factor; MSC = mesenchymal stem cell; NETS = neutrophil extracellular traps; NK = natural killer cell; PGE2 = prostaglandin-E2; PI3K = phosphatidylinositol-4,5-bisphosphate 3-kinase; TEM = transendothelial migration; Treg = regulatory T cell.

Effects on Humoral Immune Response

Cytokines released from immune cells play key roles in the regulation of the host immune response. These intercellular messengers are the source of soluble regulatory signals that govern inflammatory responses to pathogens and tissue injury. Multiple studies demonstrate that MSCs decrease the proinflammatory cytokine response (TNF-α, IFN-γ, and IL-1α, -1β, -6, -12, and -17)[35,37,57,60,69–79] while increasing concentrations of the antiinflammatory agents, including IL-1 receptor antagonist IL-10, cyclooxygenase-2, and prostaglandin E2.[59,70,72,74,80,81]

Effects on the Inflammasome

The inflammasome was first described by Martinon *et al.*[82] in 2002 and is a key component of the innate immune system. It is a multiprotein oligomer of pattern recognition receptors and sensors that regulate the activation of caspase-1 and a subsequent inflammatory response to infectious microbes and molecules derived from the host innate immune system. Several variants exist, and much interest centers on their role in health and in a host of inflammatory disorders. These structures may be on the membrane surface of myeloid cells, for example, toll-like receptors and C-type lectin receptors, or inside the cytoplasm, for example, nucleotide-binding oligomerization domain-like receptors MSCs appear to regulate inflammatory function. Miao *et al.*[81] showed that MSCs can regulate the NLRP3 inflammasome in Kupffer cells *via* secretion of prostaglandin E2, leading to increased Kupffer cell production of IL-10. This reduced inflammasome activation and reduces the inflammatory response and ensuing organ dysfunction.[81]

Effects on Neutrophil Response

Pathogen-associated molecular patterns, released from pathogens in infected tissue, bind to pattern recognition

receptors, initiating a cascade of events and generating chemotactic (*e.g.*, CXCL-2) and haplotactic gradients, which recruit activated neutrophils to the affected area. Neutrophils then attempt eradication of the offending microorganism *via* phagocytosis, the release of neutrophil extracellular traps, and the release of antimicrobial peptides.[83] Neutrophil extracellular traps are structures released from neutrophils comprising a core of chromatin DNA and histones, surrounded by specific antimicrobial proteins (lactoferrin, cathepsin G, defensins, LL-37, and bacterial permeability increasing protein), proteases (neutrophil elastase, proteinase-3, and gelatinase), and reactive oxygen species–generating enzymes (myeloperoxidase).[83] Neutrophil extracellular traps are extremely efficient in pathogen trapping, killing, and prevention of pathogen dissemination. This neutrophil response is generally advantageous and central to effective source control and pathogen eradication. However, when uncontrolled, such as in severe sepsis, activated neutrophils can migrate from inflamed tissues to other, noninfected tissue and organ systems (termed *reverse migration*), causing widespread host injury and organ dysfunction, potentially culminating in multiorgan dysfunction syndrome.[84,85] In severe ongoing sepsis, infected tissues may have inadequate or dysfunctional neutrophils that are insufficient for source control due to neutrophil C-X-C motif chemokine receptor-2 downregulation, whereas an abundance of activated neutrophils contribute to injury in distant, healthy tissue due to neutrophil C-C motif chemokine receptor upregulation.[84,86,87]

Multiple preclinical sepsis animal models demonstrate the potential for MSC therapy to alter neutrophil function to reduce host injury while maintaining bactericidal function.[59,61] MSCs reduce neutrophil infiltration into the lung, liver, gut, and kidney, reducing injury and improving organ function in preclinical sepsis models.[35,59–61,88,89] MSCs also enhance neutrophil-mediated phagocytosis, making them more effective in the clearance of bacteria.[61] Neutrophil depletion, using anti-Ly6G antibody, totally abolished the protective effect of MSCs in systemic sepsis,[61] highlighting the pivotal MSC–neutrophil interaction to the resolution of sepsis.

Effects on Monocyte/Macrophage Response

Macrophages are present in almost all tissues, where they coordinate developmental, metabolic, and immunologic functions and thus contribute to the maintenance of homeostasis.[90] Macrophage dysfunction plays a key role in the pathogenesis of multiple diseases,[91] including sepsis, and therefore these cells represent attractive therapeutic targets.

Much work has focused on the potential for MSCs to modulate macrophage function and phenotype.[49,92] Macrophages, on stimulation, become activated into one of two phenotypes, namely classically activated M1 macrophages that were considered proinflammatory and play a key role in phagocytosis and killing of pathogens, and alternately activated M2 macrophages, with a more prorepair/resolution phenotype, that contribute to clearance of dead/injured host cells and tissue repair. A key effect of MSCs on macrophages may be their ability to favor development into an M2-like phenotype,[44,49] with improved phagocytic activity and capacity for resolution of inflammation and injury repair.[93] In a murine systemic sepsis model, MSCs secreted prostaglandin E2, which reprogrammed macrophages to the M2-like phenotype. Prostaglandin E2 increased macrophage production of IL-10, which reduced neutrophil transendothelial migration and neutrophil-induced organ damage and increased intravascular neutrophil and monocyte numbers, improving organ function and reducing pathogen load.[35,49,59–61,70,72,73,88,89] MSCs can increase intravascular monocyte phagocytic potential *via* complement activation, increasing C5a levels, with subsequent CD11B up-regulation, both crucial for effective pathogen clearance.[49,94] They also have an ability to enhance macrophage phagocytosis *via* several mechanisms, including secreted factors such as keratinocyte growth factor[95] and mitochondrial transfer (from MSC to macrophage), either *via* direct cell–cell contact (*via* tunneling nanotubules) or indirectly (*via* exosomes).[96] MSCs attenuate lipopolysaccharide-induced macrophage apoptosis *via* inhibition of the Wnt/β-catenin pathway.[97]

Alteration of M1 macrophages to the M2 phenotype has been demonstrated to be important to injury resolution.[98] More recently, emerging data have observed a wider spectrum of macrophage phenotypes.[91] It appears that macrophages are activated to a spectrum of phenotypes depending on macrophage origin, current tissue of residence, and whether exposed previously to the same insult,[90] and activation patterns display an element of temporal and spatial plasticity. Consequently, the effects of MSCs on macrophage phenotype may vary considerably based on these factors.

Effects on Adaptive Immune Response

The impact of MSCs on the T-cell response during sepsis has received limited attention. If anything, the well-described suppressive actions of MSCs on T-cell effector pathways in, for example, transplant studies, have been considered a potential concern in sepsis.[99] Specifically, MSCs inhibit effector T-cell activation and can increase regulatory T-cell numbers,[71,100] while suppressing proliferation of $CD4^+$ T-helper cells, $CD8^+$ cytotoxic T lymphocytes, and natural killer cells.[99,101–103] These effects may be direct or may occur indirectly *via* effects on dendritic cells and/or other antigen-presenting cells.[99]

The potential for MSCs to modulate regulatory T-cell function is of particular interest in the setting of sepsis and deserves additional attention. Regulatory T cells are a subpopulation of T cells that modulate the immune system, maintaining self-antigen tolerance and preventing autoimmune disease. They are classically considered to constitute a double-edged sword in infection, limiting inflammation and host tissue injury potentially at the price of reduced bacterial clearance.[104] Regulatory T cells appear to have

a role in suppressing the hyperinflammatory response to sepsis[71] *via* suppression of the activation of autoreactive T effector cells.[105] In contrast, in mice subjected to cecal ligation and puncture-induced sepsis, adoptive transfer of activated regulatory T cells enhanced bacterial clearance and increased animal survival,[106] suggesting that the presence of regulatory T cells is essential to bacterial clearance and sepsis resolution.[107] MSCs have been demonstrated to induce regulatory T-cell populations in multiple inflammatory models[108–110] and during sepsis,[71] potentially providing a mechanism by which MSCs may enhance sepsis resolution. MSCs may also modulate the activity of natural killer cells, alter dendritic cell differentiation,[111] and regulate B-cell function *via* mechanisms that are not well understood.

Mechanisms by which MSCs Exert Their Effects

Although much of the early enthusiasm for stem cells as a therapy derived from the concept that these cells could (trans-)differentiate to replace injured cells, this has been clearly demonstrated not to be a core mechanism by which MSCs exert their effects. Instead, MSCs work by multiple mechanisms, some of which require contact between the MSC and the target cells, while others are mediated *via* secreted products, both mediators and cell products, such as microvesicles and exosomes (figs. 2 and 3).

Cell Contact-dependent Effects

Although MSC engraftment is not required for efficacy in preclinical sepsis models, it seems that migration of MSCs to the site of injury and their retention there, at least for a short period, is required for efficacy after intrapulmonary endotoxin instillation.[112] In murine endotoxemic sepsis, Xu *et al.*[113] found that MSCs reduced lung inflammation and injury *via* a direct cell-to-cell contact-dependent mechanism. MSCs can bind to alveolar epithelial cells at connexin-43–positive gap junctions and transfer cellular products, including mitochondria, to increase cellular ATP levels, reducing epithelial cell dysfunction and mortality.[42]

MSC Secretome

MSCs secrete multiple antimicrobial peptides, such as lipocalin-2,[43] β-defensin-2,[114] and LL-37.[36,44] Other immunomodulatory mediators in the MSC secretome include prostaglandin E2,[59] transforming growth factor-β,[115] indoleamine 2,3-dioxygenase,[116] IL-1 receptor antagonist,[117] TNF-α–induced protein-6,[79] and IL-10[37]. MSC attenuation of cecal ligation and puncture sepsis is mediated in part *via* prostaglandin E2 secretion, which altered the host macrophage phenotype to an M2-like state.[59] Endotoxin-induced stimulation of the toll-like receptor 4 expressed by the MSCs increases MCS production of prostaglandin E2 and cyclooxygenase 2.

MSC-derived Extracellular Vesicles

MSCs also release subcellular particles, termed *extracellular vesicles*, which incorporate cellular components, including mitochondria[42] and gene products (*i.e.*, mRNA and microRNAs).[53] Two types of extracellular vesicles exist, namely microvesicles, which are in the 50- to 1000-nm range, and exosomes, which are in the 40- to 100-nm range. Microvesicles from MSCs decrease lung[53] and kidney injury.[118] These microvesicles decreased pulmonary edema, reduced the alveolar influx of neutrophils, and decreased alveolar macrophage inflammatory protein-2 concentrations after endotoxin-induced acute lung injury in mice,[40] mainly through keratinocyte growth factor mRNA transferred to the injured alveolar epithelium. MSC-derived microvesicles decreased murine *E. coli*–induced severe pneumonia.[53] MSC-derived exosomes exerted cardioprotective effects in polymicrobial sepsis through miR-223 transfer to cardiomyocytes and to macrophages, reducing the inflammatory response and enhancing survival of recipient cells.[119] More recently, human-induced pluripotent stem cell–derived MSC exosomes had significant hepatoprotective effects in a hepatocellular injury model secondary to a combination of inflammatory response suppression, oxidative stress amelioration, and reduced apoptosis.[120]

Strategies to Enhance MSC Efficacy

MSCs are activated by inflammatory mediators (including IFN-γ, IL-1β, and TNF-α) released from stimulated immune cells, potentially enhancing MSC function in sepsis.[101] MSCs can also be modulated by toll-like receptor activators,[121] which can polarize MSCs *in vitro* toward either a proinflammatory (MSC1) or antiinflammatory (MSC2) phenotype, depending on the specific receptor activator ligand.[122] Activation of umbilical cord–derived MSCs with poly (I:C), a toll-like receptor-3 ligand, increased their efficacy in murine cecal ligation and puncture-induced systemic sepsis *via* inhibition of microRNA-143, which increased MSC expression of cyclooxygenase-2, leading to increased prostaglandin E2 production and enhanced MSC effects on macrophage function.[89]

Overexpression of potentially therapeutic proteins is another strategy used to enhance MSC efficacy. MSCs overexpressing angiopoietin 1 were more effective than naive MSCs in reducing endotoxin-induced alveolar inflammation and lung permeability.[123] Several gene overexpression strategies, using genes such as angiotensin-converting enzyme 2,[124] fibroblast growth factor 2,[125] and keratinocyte growth factor,[126] have been demonstrated to enhance MSC efficacy in attenuating endotoxin-induced lung injury. MSCs transduced with E-prostanoid 2 receptor demonstrate enhanced homing to the injured lung, decreasing lung inflammation and reducing permeability.[127] MSCs that overexpress the orphan receptor tyrosine kinase ROR2 further improved MSC-mediated protection against epithelial impairment in

ARDS.[128] Although these studies demonstrate useful proof-of-concept, additional studies in live bacterial models of pulmonary and systemic sepsis, with greater characterization of the effects on the immune response, would greatly enhance the translational potential of these approaches.

Insights from Clinical Studies of MSC Therapy

Despite multiple clinical trials of MSCs in diverse disease conditions, the evidence for therapeutic efficacy remains scant. An open-label phase I dose escalation trial for early septic shock, led by Drs McIntyre and Stewart at the University of Ottawa (Ottawa, Ontario, Canada), is due to publish preliminary results at the time of writing (table 3). The Cellular Immunotherapy for Septic Shock trial is a safety and dose escalation study of freshly cultured (*i.e.*, not cryopreserved) allogeneic bone marrow MSCs for patients fulfilling clinical criteria for septic shock within 24 h of intensive care unit admission. MSC doses of 0.3- to 3.0-million cells per kilogram were used in the study. The investigators also enrolled a historical cohort that met study eligibility criteria to examine the adverse advents risk (NCT02421484). This key safety study will inform the design of larger-scale phase II septic shock trials that will determine the efficacy of MSCs for sepsis.

A pilot study for a randomized, interventional trial, assessing the effect of MSCs on organ failure during septic shock, is due to commence enrollment in France (NCT02883803). A clinical trial of bone marrow MSCs recently concluded in Russia, which assessed neutropenic patients with septic shock (NCT01849237), demonstrated potentially promising results, although mortality was high in both groups.[129] Of relevance to sepsis, a phase I trial of MSCs in ARDS has been published,[39] a phase II trial has recently completed in the United States (NCT02097641), and a second is in progress in the Republic of Korea (NCT02112500).

However, despite multiple clinical studies of MSCs for diverse disease processes, there are no large-scale clinical trials demonstrating efficacy of MSC therapy. The study of MSC therapy for graft-*versus*-host disease, an immunologic condition with parallels to sepsis, is instructive. MSC therapy has been investigated for the prevention[130] and treatment[131] of graft-*versus*-host disease for more than 15 yr, and it is licensed for clinical use in certain countries, yet the clinical efficacy and mechanisms of action remain unclear. In acute graft-*versus*-host disease, ambiguity arose after failure of a phase III trial in the United States in 2009 (NCT00366145) to reach its clinical endpoint.[132] This unexpected result contradicted European literature, with several smaller positive phase II trials emerging contemporaneously.[133–136] Much discussion has focused on potential dissimilarities between large-scale, industrial-produced MSCs (used in a U.S. phase III trial) and smaller-scale MSC production in academic centers used in phase II trials, as a potential explanation for the contrasting trial results.[137] Issues such as MSC donor variation, cell expansion techniques, immunogenicity of transfused products, and

Table 3. Selected Clinical Studies Examining Effects of MSCs of Relevance to Sepsis

Study	Phase	Status	Cell Therapy	MSC Delivery Route and Timing of Administration	Findings/Comments
Russian Clinical Trial of Mesenchymal Cells in Patients with Septic Shock and Severe Neutropenia (RUMCESS) (NCT01849237)	1	Completed	MSC (undefined)	IV, within 10 h of onset of septic shock in severely neutropenic patients	Improved 28-day survival, benefit lost at day 90
Cellular Immunotherapy for Septic Shock (CISS) Trial (NCT02421484)	1	In progress	BM-MSC	IV, within 48 h of developing severe sepsis	Recruitment in progress
Effects of Administration of Mesenchymal Stem Cells on Organ Failure during the Septic Shock (CSM choc) (NCT02883803)	2	Not yet open	MSC (undefined)	IV, within 12 h of presentation with septic shock and organ failure (two or more organs)	Recruitment in progress
Human Mesenchymal Stem Cells for Acute Respiratory Distress Syndrome (START) Trial (NCT01775774)	1	Completed	BM-MSC	IV, within 96 h of ARDS diagnosis	Intervention well tolerated
Human Mesenchymal Stem Cells for Acute Respiratory Distress Syndrome (START-2) Trial (NCT02097641)	2	In progress	BM-MSC	IV, within 96 h of ARDS diagnosis	Recruitment in progress
Mesenchymal Stem Cell in Patients with Acute Severe Respiratory Failure (STEL-LAR) Trial (NCT02112500)	2	In progress	BM-MSC	IV, patients requiring mechanical ventilation for 7 or more days	Recruitment in progress

ARDS = acute respiratory distress syndrome; BM = bone marrow; MSC = mesenchymal stem cell.

cryopreservation techniques are all challenges to large-scale MSC production. Currently, a number of graft-*versus*-host disease trials using MSCs have either recently completed (NCT01222039) or are currently enrolling (NCT01765634 and NCT01765660) to address these issues.

Other clinical trials that have not proven clinical efficacy include a trial of autologous MSCs in patients with myocardial ischemia[138] and a trial of allogeneic MSCs in patients with chronic obstructive airways disease.[139] These trials highlight potential translational challenges that lie ahead with regard to MSC therapy for sepsis.

Challenges to Clinical Translation of MSCs for Sepsis

Considerable barriers and knowledge gaps exist that significantly impede the clinical translation of MSCs for patients with sepsis. These issues will need to be better understood to enhance the likelihood of successful clinical efficacy studies. These challenges can be divided into those that relate MSCs as a therapy and that relate to sepsis as a disease target.

Challenges Relating to MSCs as a Therapy

Heterogeneity of MSC Populations

There is no single marker or characteristic that identifies a cell as an MSC. The International Society for Cell and Gene Therapy (Vancouver, British Columbia, Canada) first defined MSCs for cellular therapy in 2006 based on the presence of three specific criteria, namely: (1) adherence to plastic; (2) the expression of, or lack thereof, certain surface molecules; and (3) their capacity for differentiation.[140] Even if MSCs are sorted by consensus positive and negative surface markers, the resulting population is functionally heterogeneous.[141] Consequently, MSC preparations produced by different cell-manufacturing facilities may differ in subtle but important ways, meaning that effects seen with one particular MSC product may not be seen with another, increasing the risk of therapeutic heterogeneity and failure of clinical translation. A more specific and robust approach to defining an MSC is imperative to reduce this heterogeneity.[142,143]

Clonal derivation of MSCs has been described and may yield a more homogenous MSC population for subsequent expansion.[144] Another approach to decrease MSC heterogeneity may be to generate them from homogenous populations of induced pluripotent stem cells.[145] Proof of concept for this approach has been demonstrated, with induced pluripotent stem cell–derived MSCs demonstrating comparable efficacy to bone marrow–derived cells in preclinical models of corneal injury.[146]

MSC Quality Assurance Issues Challenges

An advantage of MSCs as a therapy is their ease of passage and culture *in vitro*. However, this brings challenges, including the risk that repeated passaging can alter the MSC phenotype, ultimately resulting in reduced therapeutic efficacy.[147] Repeated *in vitro* cellular passage can result in chromosomal damage, telomere shortening, and even malignant transformation.[148] MSCs that have undergone cryopreservation and storage (generally used in clinical studies) may be less effective than fresh MSCs (used generally in preclinical studies), and this may explain inconsistencies in results between clinical and preclinical studies.[149] Optimizing cryopreservation strategies for MSCs that maintain cell viability, potency, and efficacy is an important translational challenge. The criteria for the selection of donors for the establishment of master cell banks, which are then used to manufacture the MSC batches for clinical use and release criteria for the release of cell batches for clinical use are specified by regulatory agencies such as the U.S. Food and Drug Administration. Compliance with these criteria is a key issue in ensuring that cells of the highest quality are used in the clinical setting.

Optimal MSC Tissue Source

MSCs can be isolated from many tissues and organs. The bone marrow remains the standard tissue source of MSCs, and most preclinical and early-phase clinical sepsis studies use bone marrow MSCs. Other, potentially more plentiful sources of MSCs, including the umbilical cord and adipose tissues, are receiving increasing attention as potentially more feasible sources of cells for clinical use. Umbilical cords have the additional advantage of being a plentiful source; they are a waste biologic product and donor heterogeneity is reduced. Menstrual-derived MSCs, when combined with antibiotic therapy, synergistically improved the survival rate in mouse cecal ligation and puncture-induced sepsis, enhancing bacterial clearance and reducing organ injury.[150]

Interestingly, with regard to sepsis, there appears to be differential immunomodulatory effects of MSCs derived from differing tissues. MSCs derived from the Wharton's jelly of the umbilical cord attenuated increases in proinflammatory cytokines IL-1α, IL-6, and IFN-γ but did not modulate the response of antiinflammatory cytokines IL-4 and IL-10 in rats with cecal ligation and puncture-induced polymicrobial sepsis.[73] Mouse adipose tissue MSCs protected mice from *P. aeruginosa* pulmonary infection by reducing lung bacterial load, neutrophil, and macrophage inflammatory protein-2 levels.[47] Adipose tissue MSCs also enhanced the phagocytic and bactericidal abilities of mouse bone marrow–derived macrophages *in vitro* by inhibiting prostaglandin E2 signaling.[47] Interestingly, it was observed that when prostaglandin E2 was administered to adipose tissue MSCs, their protective effects were negated.[47] This contrasts with those effects observed with mouse bone marrow MSCs. Previous studies have suggested that bone marrow MSCs release prostaglandin E2, which enhances phagocytic ability and bacterial clearance by macrophages and stimulates them to release antiinflammatory IL-10.[59,151] These differential immunomodulatory effects of MSCs, depending on their tissue source, may be important to consider when determining the optimal MSC for clinical testing.

Mechanisms of Action Relevant to Sepsis

The roles and relevance of the different mechanisms of action of MSCs in sepsis remain incompletely understood. Specifically, we need to better understand which MSC mechanisms of action are most relevant to sepsis and to develop strategies to enhance these effects. The most relevant MSC effects will likely differ based on the etiology, source, and phase of sepsis, highlighting the need to better characterize the biology of sepsis. Multiple secreted products, including prostaglandin E2,[59] keratinocyte growth factor,[53] and LL-37,[36] exert therapeutic effects in preclinical sepsis models. Other potentially important effects are cell contact dependent, such as alteration of macrophage phenotype and phagocytic capacity. MSC-derived microvesicles play an important role *via* mechanisms involving transfer of mitochondria and nucleic acids.[53] The injury microenvironment may further modulate MSC behavior.[152] Evaluating the relative importance in sepsis of these diverse mechanisms of action will be important for maximizing the therapeutic efficacy of MSCs for sepsis.

MSC Dose and Timing

The majority of preclinical studies have used intravenous cell delivery. Local or regional cell delivery, for example, intraperitoneal for abdominal sepsis or intrapulmonary for pneumonia, is a feasible alternative option that may minimize systemic effects. However, the best dose and dosing regimen for MSCs in patients with sepsis are not known. Extrapolation from preclinical studies or from human studies for other conditions may be of limited relevance to patients with sepsis. The Cellular Immunotherapy for Septic Shock phase I study in sepsis is testing the safety of doses up to 3 million cells per kilogram. The optimal dose of MSCs may differ substantially in different disease states. The effect of factors such as the stage of illness, type of MSCs, route of cell delivery, viability and purity of MSCs, and condition of the patient, are all poorly understood. The timing of MSC therapy is also relevant, with preclinical studies to date generally focused on early MSC delivery. Characterization of MSC efficacy in later-phase sepsis, which is characterized by immune suppression, is a priority. The safety of repeated doses remains to be determined, with evidence to suggest that repeated administration does elicit an immune response.[153]

MSC Safety Concerns

Although there is considerable experience in administering MSCs to patients, sepsis presents a number of safety concerns. Infusional toxicity is a concern during intravenous administration due to the risk of MSC clumping into microemboli that could obstruct the pulmonary circulation. Encouragingly, no infusional toxicities were seen in patients with ARDS in a recent phase I dose escalation clinical study.[39] In the longer term, MSCs could potentially enhance tumorigenesis either by direct malignant transformation of MSCs or indirectly by facilitating growth of other tumor cells. Reassuringly, increased tumorigenesis has not been reported in the more than 6,000 patients who have received MSCs in clinical trials to date.[154]

Challenges Relating to Sepsis

Population Heterogeneity

Sepsis is not a disease but rather a syndrome defined by a set of consensus clinical criteria that lump together patients who vary considerably in terms of their underlying biology, the source and nature of the inciting agent(s) and the host response, and a varying severity of illness. Some patients fulfilling clinical criteria for sepsis will not have a pathogen as the underlying inciting agent. The sepsis diagnostic criteria are useful in enabling rapid identification and early resuscitation and organ support of severely ill patients with sepsis. In addition, patients in different phases of sepsis may respond very differently to a therapeutic intervention. Consequently, this heterogeneity constitutes an impediment in identifying effective therapeutic strategies, especially where these strategies may have potentially harmful as well as beneficial effects.[155] This heterogeneity of treatment effect may explain some negative trials in sepsis to date, whereby a treatment may have benefit in a particular patient subset, for example, severe sepsis with organ failure, but be ineffective or even harmful to patients with less severe sepsis.

It is very unlikely that MSC therapy will be useful in all patients with sepsis. Identification of patient subgroups within the population with sepsis that are more likely to respond to MSC therapy, and testing MSCs in these patient groups, will be necessary. In this regard, the identification of subphenotypes or endotypes within the sepsis population, as has been done in patients with ARDS by Calfee *et al.*,[31] would be a key advance. A related approach, termed *theranostics*, involves identifying biomarkers of therapeutic responsiveness. Man *et al.*[156] used this approach to identify potential subgroups of patients in the trial of Drotrecogin Alfa (Activated) in Adults with Septic Shock (PROWESS-SHOCK) that may have benefited from activated protein-C therapy.[157] Similarly, Wong *et al.*[158] identified a pediatric septic shock subgroup that had a higher mortality from corticosteroid administration. Based on our current elucidation of the biologic effects of MSCs, therapy might be more likely to be effective in patients with a hyperinflammatory phenotype.

Conclusions

Preclinical studies have demonstrated the therapeutic potential of MSCs for sepsis. The mechanisms of action of MSCs are increasingly well characterized and include modulation of the immune response, reduction of host injury from the proinflammatory response while augmenting bacterial clearance by indirect and direct mechanisms of action, and enhanced resolution of inflammation and enhanced tissue repair after injury. Although we await evidence of MSC benefit in patients with sepsis, phase I to II studies are underway, and

initial reports are encouraging. However, significant hurdles still exist, both in terms of MSCs as a therapy and sepsis as a therapeutic target, which need to be overcome if the therapeutic potential of MSCs is to be realized. Addressing these ongoing knowledge gaps will help us to fully harness the therapeutic promise of MSCs for our patients with sepsis.

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Competing Interests

The authors declare no competing interests.

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ANESTHESIOLOGY REFLECTIONS FROM THE WOOD LIBRARY-MUSEUM

A "Dopeless" Diamond Belied Koca Nola's Cocaine-laced Content

Because it had been widely used in soft drinks in the 1890s, cocaine gained rapid acceptance as a local anesthetic. In 1904 Thomas H. Austin founded the Koca Nola Company, a soft-drink firm that produced copycat cocaine-laced beverages in Atlanta, the hometown of the largest cola company in the world at that time. By 1907 the Koca Nola diamond logo (*above*) had been trademarked. On the diamond, Koca Nola is touted as "The Great Tonic" that is not only "Delicious" but "Dopeless." Unfortunately for the beverage company, federal chemists isolated cocaine in a jug of their Koca Nola. Consequently, in March of 1910, the United States Department of Agriculture published its "Notice of Judgment" that Koca Nola had violated the 1906 Food and Drugs Act by "Adulteration and Misbranding" its beverage, which, yes, still contained cocaine. Although its logo was "dopeless," apparently Koca Nola was not. Bankrupt by 1910, the Koca Nola Company did not completely disappear until 8 yr later. (Copyright © the American Society of Anesthesiologists' Wood Library-Museum of Anesthesiology.)

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