RESEARCH REVIEW

Advances in Mesenchymal Stem Cell Research in Sepsis

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Background. Sepsis remains a source of morbidity and mortality in the postoperative patient despite appropriate resuscitative and antimicrobial approaches. Recent research has focused upon additional interventions such as exogenous cell-based therapy. Mesenchymal stem cells (MSCs) exhibit multiple beneficial properties through their capacity for homing, attenuating the inflammatory response, modulating immune cells, and promoting tissue healing. Recent animal trials have provided evidence that MSCs may be useful therapeutic adjuncts.

Materials and Methods. A directed search of recent medical literature was performed utilizing PubMed to examine the pathophysiology of sepsis, mechanisms of mesenchymal stem cell interaction with host cells, sepsis animal models, and recent trials utilizing stem cells in sepsis.

Results. MSCs continue to show promise in the treatment of sepsis by their intrinsic ability to home to injured tissue, secrete paracrine signals to limit systemic and local inflammation, decrease apoptosis in threatened tissues, stimulate neoangiogenesis, activate resident stem cells, beneficially modulate immune cells, and exhibit direct antimicrobial activity. These effects are associated with reduced organ dysfunction and improved survival in animal models.

Conclusion. Research utilizing animal models of sepsis has provided a greater understanding of the beneficial properties of MSCs. Their capacity to home to sites of injury and use paracrine mechanisms to change the local environment to ultimately improve organ function and survival make MSCs attractive in the treatment of sepsis. Future studies are needed to further evaluate the complex interactions between MSCs and host tissues.

Key Words: mesenchymal stem cells; cell-based therapy; sepsis; animal sepsis models.

INTRODUCTION

The sepsis syndrome is defined by widespread inflammation, host immune dysfunction, dysregulation of the coagulation cascade, and endothelial dysfunction in response to invading pathogens. Sepsis represents a continuum of disease, which is first evidenced by the systemic inflammatory response syndrome (SIRS). This may then progress to septic shock, significant multiorgan dysfunction, and potentially results in death [1]. It is an especially common source of morbidity and mortality among critically ill patients and postoperative patients in the intensive care unit [2]. Even with appropriate antibiotic and resuscitative therapies, sepsis carries a 30% mortality rate and significant morbidity associated with organ failure [3, 4]. Sepsis incurs a staggering $16.7 billion cost in the US health economy with over 750,000 annual cases and greater than 200,000 deaths each year [3].

While sepsis remains one of the leading causes of death in the United States, years of research have begun elucidating its pathophysiologic mechanisms. Invading pathogen components such as lipopolysaccharide (LPS) interact with host immune cell Toll-like receptors (TLRs) to induce a proinflammatory cytokine phase characterized by systemically elevated interleukins (IL), chemokines, and immune and endothelial cell adhesion molecules responsible for cellular trafficking [5]. In sepsis, the exorbitant inflammatory response...
activates a number of pathways, which collectively produce the systemic hypotension, end-organ ischemia, and multi-organ dysfunction observed clinically [6–8]. Reactive oxygen species and proinflammatory cytokines secreted by neutrophils and macrophages mediate end-organ damage as much as the causative pathogens [9, 10]. As sepsis progresses, a subsequent anti-inflammatory phase ensues, which is characterized by further immune dysregulation, altered cytokine profiles, and prolonged organ dysfunction, eventually leading to organ damage by cellular apoptotic pathways [11].

Prompted by the morbidity and mortality resulting from sepsis and the shortfalls of our current therapeutic regimens, researchers in recent years have turned to cell-based therapy as a novel approach for managing sepsis. Stem cells are self-renewing undifferentiated precursors capable of differentiating into multiple cell types and providing an unending supply of healthy cellular units for damaged tissue. An ever growing variety of stem cell types and sources have been described to date and a number of phase I, II, and III clinical trials have been undertaken to evaluate their clinical utility in a variety of human diseases [12]. While it was initially believed that the greatest potential of stem cells resided in their ability to engraft and differentiate into the cell types of injured organs, it has now been well established that they also exhibit a range of beneficial abilities, including homing to sites of injury, down-regulating the inflammatory cascade, preventing apoptosis in threatened tissues, promoting neoangiogenesis, activating resident stem cells, and modulating the activity of multiple immune cell types [13–17]. These features make stem cells an extremely attractive therapy in addressing both the proinflammatory response of early sepsis as well as the widespread organ dysfunction which develops thereafter.

This manuscript will focus upon expanding the understanding of the functions of mesenchymal stem cells (MSCs) in the treatment of sepsis. MSCs (also known as mesenchymal stromal cells) are non-hematopoietic precursor cells derived from a variety of tissues, including bone marrow, adipose, placenta, and umbilical cord, and have the potential to differentiate along osteogenic, chondrogenic, and adipogenic lineages [18, 19]. MSCs offer several advantages over other types of stem cells in that they are easily harvested and rapidly expanded in culture, which has translated into their preferential usage in preclinical animal models of sepsis. Prominent features of MSCs include their ability to home to injured tissues, versatile paracrine signaling effects, immunomodulatory capacity, and potential for direct antimicrobial effects [13, 20–23]. Furthermore, recent evidence has demonstrated that these individual effects are involved in mitigating organ dysfunction and improving survival in animal models of sepsis, which suggests their potential utility in treating septic patients. This article will also highlight the current animal models employed by researchers to evaluate the functions of MSCs and examine how the new information provided by recent animal trials support MSCs as a novel cell-based therapy for sepsis.

MATERIALS AND METHODS

A literature search was performed using the PubMed database in the English language until June 2011. Keywords included in this search were the subject heading terms “sepsis,” “stem cells,” “mesenchymal stem cells,” and “animal sepsis models.” Other studies were then identified using references cited in relevant review articles and in publications found using the abovementioned search parameters.

MESENCHYMAL STEM CELL HOMING IN SEPSIS

Intravenous and intra-peritoneal delivery of MSCs is efficacious because of their intrinsic ability to migrate along chemotactic gradients to injured tissues, such as lung [24], myocardium [25], brain [26], liver [27], and kidney [28]. A recently published study demonstrated that intravenously administered MSCs migrated with greatest affinity to the lung and liver after LPS-induced endotoxemia [29], while another study showed the greatest concentration of MSCs in the lung, kidney, and spleen in response to polymicrobial sepsis [30]. This ability to home to diverse sites of injury makes MSCs particularly appealing in the treatment of the multi-organ injury and dysfunction observed in sepsis. The homing of MSCs shares many similarities with leukocyte tracking, adhesion through expression of specific selectins and integrins, and subsequent transmigration into injured tissues. Several studies have identified a variety of cell surface adhesion molecules expressed by MSCs [31–33]. One of the more important adhesion molecules is vascular cell adhesion molecule (VCAM)-1, which has been shown to be up-regulated in MSCs after exposure to tumor necrosis factor (TNF)-α [32]. Several in vitro studies have also demonstrated that the capacity of MSCs to transmigrate through the extracellular matrix is dependent upon an inflammatory cytokine-mediated MSC modulation of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinase (TIMPs) [34–36].

While the full complexity of endogenous MSC mobilization, homing, and transmigration processes has yet to be fully illuminated, it appears that several potent stimulators of mobilization are granulocyte colony stimulating factor (G-CSF) [37] and hypoxia [38]. Monocyte chemoattractant protein (MCP)-1 and MCP-3, stem cell factor, vascular endothelial growth factor (VEGF), IL6, and IL-8 have also been posited as likely chemoattractants in the homing of MSCs [13, 23, 39–41], and a variety of chemokine receptors have been
which is yielding an improved capacity for homing. Mokine receptors is currently an active area of research. Genetic modifications of MSCs to affect expression of chemokine receptors may represent the capacity of MSCs to home to the diverse tissues affected in sepsis. Pretreatment and genetic modifications of MSCs to affect expression of chemokine receptors is currently an active area of research which is yielding an improved capacity for homing. The homing of MSCs along an SDF-1 gradient was corroborated by several studies have implicated the expression of an array of other MSC chemokine receptors. Von Lutthau et al. demonstrated that CCR1, CCR4, CCR7, CXCR5, and CCR10 were also involved in MSC homing. Ringe and colleagues determined that MSCs express CCR2, CCR8, CXCR1, CXCR2, and CXCR3. A more recent study included CCR5 and CCR9 in the homing of MSCs to inflamed tissues after infusion. This variable range of chemokine receptor expression may represent the capacity of MSCs to home to the diverse tissues affected in sepsis. Pretreatment and genetic modifications of MSCs to affect expression of chemokine receptors is currently an active area of research which is yielding an improved capacity for homing and may provide added benefits to the efficacy of MSCs in the cell-based therapy of sepsis in the future.

MESENCHYMAL STEM CELL PARACRINE SIGNALING IN SEPSIS

MSCs have been shown to promote regeneration of injured tissue, prevent the loss of threatened tissues, and improve overall tissue function following insults such as ischemia or bacterial infection. An abundance of research suggests that these activities now appear to be as much related to MSC cytokine profile as to their long-term engraftment or differentiation. In rapidly evolving diseases such as sepsis, the interaction of MSCs with neighboring cells and tissues may assume an even greater importance in determining host survival. MSCs provide benefit through: (1) anti-inflammatory effects, (2) anti-apoptotic effects, (3) neoangiogenesis, (4) activation of resident stem cells, and (5) immunomodulatory effects on various immune cells.

Anti-Inflammatory Effects

MSCs protect the host by dampening the immune response to sepsis. The immune system responds to bacterial infection by activating an initial proinflammatory cascade that peaks within days of the inciting infection. While the institution of aggressive supportive strategies now allows the majority of septic patients to survive this initial proinflammatory phase, organ damage resulting from this phase remains a significant cause of morbidity. MSCs ameliorate this potential injury through an overall reduction in both local and systemic inflammation by a balanced decrease in proinflammatory cytokine production and an increase in anti-inflammatory cytokine production. Important players in the anti-inflammatory cytokine profile of MSCs include transforming growth factor (TGF)-β, IL-10, IL-13, and TNF-α stimulated gene/protein 6 (TSG-6). MSC-mediated down-regulation of proinflammatory cytokines TNF-α, IL-1, and IL-6 also contributes to the diminished inflammatory milieu. The reduced inflammatory state is an especially important consideration for the cytokine dysregulation observed in sepsis.

In sepsis, the lungs are particularly sensitive to injury and intra-alveolar neutrophil-mediated inflammation represents a significant underlying mechanism. A number of acute lung injury studies have shown that MSCs reduce lung inflammation by inhibiting the transmigration of neutrophils into alveoli and have even improved survival. Several studies have implicated prostaglandin E2 (PGE2) as an essential soluble factor secreted by MSCs to achieve their anti-inflammatory effects as well. In the gut, Gonzalez et al. demonstrated that injection of human adipose-derived MSCs into septic mice reduced the septic inflammatory response and mortality by decreasing proinflammatory cytokine expression while increasing anti-inflammatory IL-10. Furthermore, these cytokine effects could be achieved from MSC-conditioned-medium alone thereby emphasizing their definitive paracrine nature. Németh et al. further emphasized the importance of MSC-secreted PGE2 in stimulating IL-10 production by resident macrophages in order to assuage the excessive inflammatory state observed in sepsis. This up-regulation of macrophage IL-10 synthesis was responsible for increased survival rates and an improvement in renal and liver function.

MSCs may also decrease proinflammatory mediator expression from innate immune cells in part through a negative feedback loop. A recent study demonstrated that the interaction of TSG-6 secreted by MSCs with the CD44 receptor of resident macrophages effectively down-regulated nuclear factor (NF)-κB signaling to decrease expression of TNF-α. This inhibition of macrophage proinflammatory cytokine synthesis essentially short circuits the early intraperitoneal inflammatory response in sepsis and TSG-6 represents yet another anti-inflammatory factor secreted by MSCs. These recent studies have offered greater insight into...
the complexity of MSC anti-inflammatory mechanisms in multiple injured organs and may provide novel approaches in the management of sepsis.

**Anti-Apoptotic Effects**

While an anti-inflammatory response may be beneficial in early sepsis, sepsis induces an inappropriate immunosuppression, which serves no clear purpose as the syndrome progresses. Similarly, while many apoptotic events are beneficial to an organism in response to damage, apoptosis seen in sepsis also lacks clear benefit. A septic environment results in widespread apoptosis of cells with high turnover rates such as lymphocytes and gastrointestinal cells. This cell death occurs independently of direct infectious agent contact and is thought to be due to cytokines such as TNF which result in the activation of caspase systems. This system-wide apoptosis results in the profound lymphopenia associated with poor sepsis outcomes, and helps establish sepsis’ second, immunosuppressive phase, which is often fatal. Thus, much research is underway to prevent apoptosis in progressing sepsis [63].

MSCs have the capacity to oppose this apoptosis. In several myocardial infarction/reperfusion studies, it has been demonstrated that MSCs are capable of promoting the survival of threatened cells along the border of the myocardial infarct zone [13, 64–66]. Stem cells possess anti-apoptotic mechanisms such as up-regulating DNA-repair, down-regulating mitochondrial death pathways, increasing antioxidant activity, and altering anti- and pro-apoptotic protein expression [67–69]. These mechanisms would be especially important in sepsis, where mitochondrial damage, oxidative stress, and apoptosis have clearly been implicated in pathology [7, 11, 70]. Manukyan et al. showed that MSCs elicited an improvement in the ratio of anti-apoptotic bcl-xL to pro-apoptotic bax in endotoxemic cardiac tissue, which correlated with reduced cardiac dysfunction [71]. While prior MSC studies have demonstrated sexual dimorphism between male and female donor MSCs [72–74], this experiment is the first to demonstrate the greater efficacy of female MSCs in an endotoxemia model. Recently, Yagi et al. demonstrated that compared with controls, MSCs significantly reduced the number of apoptotic cells found in the lungs and kidneys of endotoxemic rats [75]. Similarly, Mei et al. revealed the capacity of MSCs to prevent apoptotic cell death in the lung and kidneys of mice after cecal ligation and puncture (CLP) [76]. As renal failure and respiratory failure represent key limiting factors in predicting post-sepsis survival, employing MSCs as cell-based therapy to reduce injury to these organs is an attractive possibility. While MSC-mediated alterations in the bcl-xL/bax ratio have been demonstrated in infarction/reperfusion and endotoxemia models, ongoing research will be needed to evaluate whether MSCs exert anti-apoptotic effects through regulation of caspases and mitochondrial death pathways in sepsis as has been shown in the ischemia/reperfusion model [77, 78].

**Neoangiogenic Effects**

The neoangiogenic properties of MSCs are still being elucidated, but several studies have demonstrated MSC expression of angiogenic cytokines [53, 79, 80]. MSC-secreted paracrine factors like VEGF, basic fibroblast growth factor (FGF2), hepatocyte growth factor (HGF), and angiopoietin (Ang)-1 promote neovascularization of injured tissues [17, 72, 81]. This may be especially important in sepsis, where microvascular congestion and coagulation dysfunction produce multi-organ ischemia. It has been established that TNF-α, LPS, or hypoxia exposure increase the quantity of VEGF, FGF2, and HGF produced by MSCs and, thus, enhance their neoangiogenic potential upon injured tissues [82, 83]. Fan et al. have revealed that the neoangiogenic activity of MSCs is induced by hypoxia and is contingent upon a balanced ratio of MSC-secreted VEGF to pigmented epithelial-derived factor (PEDF) [84]. Tang and colleagues demonstrated that MSCs assuaged the effects of myocardial infarction in a rat model by increasing vascular regeneration through secretion of FGF-1, VEGF, and SDF-1 [85]. Several other studies have revealed MSCs capacity to improve tissue vascularity by promoting endothelial cell sprout formation through soluble factor secretion [86, 87]. MSCs have been shown to increase their secretion of angiogenic growth factors in infarction/reperfusion injury and when exposed to LPS or inflammatory mediators in in vitro studies. Consequently, it is likely that a similar paracrine mechanism underlies their neoangiogenic potential in sepsis.

**Activation of Resident Stem Cells**

The panoply of growth factors secreted by MSCs are critical for neoangiogenesis potential, but may also be involved in the mobilization of resident stem cell populations now known to exist in the adult heart, lung, liver, and kidney [19]. As these organs are affected deleteriously in the septic patient, the possibility of MSC-secreted VEGF, HGF, and insulin-like growth factor (IGF)-1 to stimulate resident stem cell proliferation provides another potential mechanism by which MSCs may ameliorate the morbidity of organ dysfunction [13, 17]. VEGF was found to be a key mobilizer of cardiac stem cells, which resulted in improved cardiac function following acute regional infarction [88].
Mazhari and Hare demonstrated that MSCs could mobilize an endogenous population of cardiac stem cells through complex paracrine and cell-to-cell interactions to improve ejection fraction after myocardial infarction [54]. More recently, Zisa et al. revealed that VEGF is a critical paracrine factor secreted by MSCs to mediate cardiac regeneration [89], and it is possible that this is achieved in part through a VEGF-dependent mobilization of cardiac stem cells as has been observed in a non-MSC study [90]. As end-organ hypoxia represents one of the key derangements underlying the pathophysiology of sepsis, these infarction studies offer insight into how MSCs may mobilize resident stem cells in a variety of organs. Further research will be needed to fully evaluate to what extent MSCs affect the mobilization and proliferation of resident stem cell populations in other organ systems affected by sepsis.

Immunomodulatory Effects

A further group of studies suggests that MSCs interact with cells of the innate immune system to protect the septic host as shown in Fig. 1. MSCs exhibit an ability to promote bacterial killing and clearance through paracrine interactions with local immune cells. A study by Kim and Hematti suggested that macrophages cocultured with MSCs are induced to express an alternative cytokine profile to increase their phagocytic activity and improve tissue repair and bacterial clearance [91]. This effect was corroborated more recently by the research of Mei and colleagues who analyzed the effects of MSCs in a CLP model of sepsis in mice [76]. They found that MSCs were capable of up-regulating genes in macrophages responsible for phagocytosis and this resulted in significantly improved bacterial clearance and survival. Gonzalez-Rey et al. demonstrated in a similar model that treatment with MSCs significantly improved bacterial clearance from injured organs, further manifesting the capacity of MSCs to up-regulate the phagocytic activity of resident macrophages [46]. Improving phagocytosis by resident immune cells to reduce bacterial burden represents a promising characteristic of MSCs, which could provide benefit in addressing both the early and later phases of sepsis.

In addition to this enhancement of phagocytosis, MSCs display some immunosuppressive characteristics that may be quite important in sepsis. Soluble paracrine signals from MSCs have been shown to down-regulate
inflammatory cytokine production by macrophages [30, 52, 61], and inhibit neutrophil chemotaxis [16, 58]. During inflammatory states such as sepsis, neutrophils are activated to release proinflammatory cytokines, migrate into tissues en masse, and subsequently release caustic enzymes and reactive oxygen species, which contribute to end-organ dysfunction [9, 92–94]. Several studies utilizing animal models of sepsis and endotoxin-mediated acute lung injury have demonstrated that MSC administration decreased transmigration of neutrophils and subsequent injury to lung [16, 46], liver [30, 46], and kidneys [30], and that decreasing local infiltration and activation of neutrophils correlated with improved morbidity and mortality. This reduction in morbidity and mortality may also be related to the ability of MSCs to inhibit neutrophil respiratory burst and apoptotic functions likely by an IL-6-dependent mechanism [95]. In addition, recent in vivo peritonitis and endotoxemia studies have demonstrated a decrease in proinflammatory and an increase anti-inflammatory mediator production by macrophages following MSC stimulation [10, 30, 62]. This overall picture of depressing the initial proinflammatory cascade response by reprogramming macrophages and reducing neutrophil migration and subsequent activation in tissues demonstrates the unique utility of MSCs as a therapeutic possibility in sepsis.

However, MSCs have also displayed a variety of sometimes contradictory paracrine effects upon adaptive immunity. While MSCs have been shown to arrest B-cell maturation, impair isotype-switching, and inhibit chemotaxis in some studies [96], another study demonstrated that MSCs can up-regulate antibody secretion [97]. Additional research has had conflicting results regarding the effects of MSC-derived soluble factors such as TGF-β, HGF, PGE2, and nitric oxide (NO) in impairing T-cell proliferation and cytokine production [98–101]. In addition, Aggarwal and Pittenger found that MSCs diminished proinflammatory cytokine secretion by T-helper (Th1) cells and increased secretion of IL-4 by Th2 cells [52]. Further research suggests that MSC activation may favor the formation of T-regulatory cells [60, 102, 103] and decrease the cytotoxic effects of cytolytic T-cells (CTLs) [104], which in turn may diminish the inflammatory milieu of sepsis. Of further interest, MSCs appear to interact with the innate immune system in conflicting ways. MSCs have been shown to beneficially suppress TNF-α secretion by dendritic cells as well as suppress dendritic cell maturation and antigen presentation, which could be deleterious in mounting an appropriate immune response to invading pathogens [52, 61, 105, 106]. Similarly, MSCs have also been shown to inhibit the activation and cytotoxic effects of natural killer (NK) cells through paracrine mechanisms, which may be detrimental to responding to infection [59, 107]. The full complexity of the interactions of MSCs with innate and adaptive immune cells in sepsis has yet to be fully defined, and further research will be needed to resolve conflicting studies.

A partial answer to the above seemingly contradictory findings may lie with both the characteristics of the local environment as well as the timing of the septic injury. MSCs are known to express TLRs, which are major contributors to the initial proinflammatory phase but assume lesser importance as an infectious course progresses. Recent evidence suggests that certain TLRs, most notably TLR4, may play an important role in determining the immunosuppressive properties of MSCs although the findings remain controversial. While some studies suggest that ligation of TLR4 inhibits MSC-mediated immunosuppression, other studies report the opposite effects [108, 109]. In addition, a more recent study suggests that the specific TLR agonist may determine whether MSCs exhibit a more proinflammatory versus immunosuppressive phenotype [110]. Moreover, the local milieu of co-factors, immune cells, and ratio of stimulatory versus inhibitory molecules, among other characteristics of the local environment, may all work in a multifactorial fashion to determine the activity of MSCs in sepsis.

The immunosuppressive paracrine effects of MSCs remain to be fully elucidated and possibly represent a two-edged sword. It remains unclear to what extent the suppression of innate and/or adaptive immunity may be involved in their ability to provide benefit in sepsis. While suppression of cytokine production early in sepsis could be beneficial in reducing inflammation and organ damage from bacteria and innate immune cells, oversuppression of the protective elements of B-cell and T-cell activity could be detrimental in later stages of sepsis. It is likely that the timing of MSC administration as well as variability in local mediators play key roles and further studies will be required to more clearly establish the mechanism of immunomodulation.

**MESENCHYMAL STEM CELL ANTIMICROBIAL EFFECTS IN SEPSIS**

The protective effects of MSCs in sepsis may also include direct antimicrobial activity against invading pathogens. In an *in vivo* mouse pneumonia study, Krasnodembskaya *et al.* determined that MSCs secrete the anti-microbial peptide LL-37 in response to stimulation by *Escherichia coli*, thus equipping MSCs with an intrinsic bacterial killing mechanism [22]. Anti-LL37 antibodies only partially reduced the killing activity of MSCs evidenced by the fact that mice receiving MSCs and anti-LL37 still had reductions in bacterial growth.
in lung homogenates and bronchoalveolar lavage fluid. This partial reduction may indicate the presence of other unknown antimicrobial products. Through their killing of invading pathogens, the authors suggest that MSCs may play an intrinsic role in innate immunity. LL-37 achieves its antimicrobial effect by disrupting bacterial membranes [111]. It is also known to be involved in a variety of other functions including chemotaxis, angiogenesis, inflammatory cytokine regulation, wound healing, and chemokine stimulation [111–113]. While direct antimicrobial activity may prove important in the ability of MSCs to ameliorate the overwhelming infection that precipitates and propels sepsis, another study showed that isolated MSCs lacked the ability to directly kill *Escherichia coli* in vitro [46]. The disparity between the two studies may reflect the variation in local environment between *in vivo* and *in vitro* models. It may also reflect the necessity of signals from other host cells to stimulate MSC LL-37 production. Further studies utilizing animal models of sepsis will be required in order to elucidate whether MSC-derived LL-37 or other as-yet unidentified antimicrobial factors are capable of significantly contributing to bacterial clearance in sepsis.

**ANIMAL MODELS OF SEPSIS AND ENDOTOXEMIA**

Because sepsis as a syndrome represents a highly complex disease involving progressive coagulation abnormalities, inflammatory cytokine aberrations, and microcirculatory dysfunction, establishing adequate animal models is a quite challenging endeavor. Several experimental animal models of sepsis have been utilized by researchers in order to evaluate the efficacy of MSCs as cell-based therapy. Foremost among these are the cecal ligation puncture (CLP) model, which is considered the gold standard, the endotoxemia model, and the colon ascendens stent peritonitis (CASP) model [114]. These models share in common the exposure of the abdominal peritoneum to microbial factors associated with the inciting events leading to postoperative sepsis.

Each animal model has its own utility in the lab and varies in its capacity to imitate the multifaceted clinical picture of human sepsis. As denoted in Fig. 2, the CLP model involves surgical ligation of the distal cecum with suture followed by one or two small punctures being introduced distal to the ligation. This allows for leakage of gastrointestinal contents into the peritoneal cavity in a controlled and limited fashion, which results in a polymicrobial sepsis. Comparatively, the endotoxemia model involves no intra-abdominal surgery and instead consists of the injection of specific concentrations of LPS 

via

an intraperitoneal or tail vein approach. In the CASP model, a stent is inserted through the bowel wall into the lumen of the ascending colon and fixed in place to allow seepage of gastrointestinal contents into the peritoneal cavity to produce polymicrobial sepsis. The CASP procedure is more technically challenging than the CLP or the endotoxemia models and is, therefore, the least utilized in sepsis studies. Of note, in CASP the animal mortality correlates with stent diameter [114]. Determining which model to utilize is contingent upon the nature of the outcomes being evaluated by the researcher.

As LPS represents only one of a plethora of bacterial factors involved in initiating the progression to sepsis, the endotoxemia model has obvious limitations [114]. In an experiment comparing the two models, Remick et al. determined that both the CLP and endotoxemia models resulted in similar mortality rates, but that the time course of the CLP model more clearly reflected the cytokine profile and physiological course observed in sepsis. The endotoxemia model, on the other hand, elicited a more brisk and higher peaking elevation of serum cytokines than observed in the CLP model or in actual human sepsis; this LPS-stimulated cytokine stimulation reached its zenith after only several hours before declining [114, 115]. Therefore, while the CLP model is more clinically relevant and more accurately reflects both the physiology and time course of postoperative sepsis, the endotoxemia model allows for a more efficient and cost-effective way to determine quantifiable end effects like alterations in cytokine levels. If a study seeks to determine whether a potential therapy or a modification to a current therapy will provide a desired outcome, the endotoxemia model allows for a more rapid exaggeration of the immune response in order to detect even subtle alterations of specific factors. Once a therapy has been found to produce beneficial effects by an initial evaluation using the endotoxemia model, the CLP model allows for a more accurate representation of how the effects from the proposed therapy will impact clinical outcomes. Due to their individual value, both models continue to have a place in scientific experimentation.

A similar comparison between the relative utility of the CLP and CASP models has also been performed [116]. Maier and colleagues suggest that the punctures of the CLP method are more likely to produce intra-abdominal abscesses and adhesions. Conversely, they found that the CASP method resulted in more diffuse peritonitis which more closely mimicked the inflammatory and multi-organ dysfunction progression of postoperative sepsis. However, both of these methods suffer from a higher degree of user-dependent variation in results over those seen with the endotoxemia model. A study by Singleton and Wischmeyer demonstrated that outcomes in the CLP model are highly dependent upon the length of cecum ligated, thus necessitating...
precise repeatability [117]. Ligation was performed between 3 to 15 mm from the distal cecum with higher mortality rates and inflammatory cytokine elevations noted when 10 mm or greater was ligated. Puncture size also represents a user-dependent variable in the CLP model and is correlated with survival and inflammatory cytokine elevations [118]. Similarly, results from the CASP model are correlated with stent diameter. Two separate studies found mortality rates at 48 h post-CASP ranged from 50% using an 18 gauge (G) stent to 100% using a 14G stent [116, 119]. Based on the relative ease of reproducing the CLP model in comparison to the CASP model and its use in sepsis research for several decades [120], the CLP model remains the gold standard and most commonly employed model of human sepsis. Due to its greater technical difficulty, no MSC studies have utilized the CASP model to date, which represents an unexplored area of MSC research. Further studies will be required to evaluate the adequacy of these various animal models of sepsis and any interpretation of the efficacy of MSCs in cell-based therapy will need to be weighed against the limitations of each respective model.

STEM CELLS IN EXPERIMENTAL SEPSIS

Currently, MSCs are being employed in human clinical trials for a variety of diseases, including autoimmune disorders, gastrointestinal diseases, cancer, heart disease, diabetes, and neurodegenerative disorders [12]. MSCs can be easily harvested from a patient’s own bone marrow, clonally expanded in vitro within a few weeks, and then injected directly into the site of pathology or systemically into the bloodstream or peritoneal cavity. Autologous transplantation is preferable for planned interventions, as there is some evidence that suggests allogeneic rejection after transplantation [121]. However, because of MSC-specific major histocompatibility complex (MHC) profiles and immunomodulatory properties, allogeneic transplantation of stem cells remains a therapeutic possibility [122], and MHC-typed stem cell banks with ready-to-transplant reserves for acute therapies are also plausible. While there are currently no human trials underway to evaluate the efficacy or safety of MSCs in the management of sepsis, an expanding body of animal research data exists, which demonstrates the potential benefits of cell-based therapy for the septic patient (see Table 1) [15, 16, 30, 46, 71, 75, 76]. One of the first studies utilizing MSCs in a mouse endotoxemia model was performed by Xu and colleagues in 2007 [16]. They demonstrated that MSCs significantly reduced serum levels of proinflammatory cytokines early in sepsis and pathologic analysis revealed that this correlated with decreased endotoxin-induced lung injury. This study was limited in that the endotoxemia model, as discussed above, does not necessarily correlate with the progressive time course and multi-organ dysfunction observed clinically in human sepsis and in that it focused primarily on lung injury. Several more recent studies have further examined the degree to which MSCs provide a therapeutic benefit in sepsis.

The ability of MSCs to blunt the inflammatory phase in sepsis has been amply and consistently demonstrated in recent animal models of sepsis. Utilizing a murine CLP model, Németh et al. evaluated the effects of intravenously injected MSCs [30]. Their cytokine analysis corroborated the earlier findings of Xu et al. with significantly reduced serum TNF-α and IL-6, and significantly increased IL-10 levels. Other recent studies utilizing CLP and endotoxemia models have demonstrated similar reductions in proinflammatory...
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<tr>
<th>Author/Year</th>
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<th>MSC Route</th>
<th>MSC Delivery Timing</th>
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<tr>
<td>Németh et al. [30] 2009</td>
<td>CLP</td>
<td>Intravenous</td>
<td>1 h post-CLP</td>
<td>4 d for survival</td>
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<td>CLP</td>
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<td>4 h post-CLP</td>
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<td>Endotoxemia</td>
<td>Intraperitoneal</td>
<td>30 min post-LPS-injection</td>
<td>4 d for survival</td>
<td>Improved survival</td>
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<td>Mei et al. [76] 2010</td>
<td>CLP</td>
<td>Intravenous</td>
<td>6 h post-CLP</td>
<td>28 h</td>
<td>Improved survival*</td>
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<td>Weil et al. [15] 2010</td>
<td>Endotoxemia</td>
<td>Intraperitoneal</td>
<td>1 h post LPS-injection</td>
<td>6 h</td>
<td>Decreased proinflammatory cytokines</td>
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<td>Yagi et al. [75] 2010</td>
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<td>0 min post-LPS-injection</td>
<td>24 h</td>
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<td>Endotoxemia</td>
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<td>1 h post-LPS-injection</td>
<td>6 h</td>
<td>Decreased proinflammatory cytokines</td>
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*Improved survival was observed when MSCs were co-administered with daily imipenem compared to treatment with daily imipenem and saline alone. MSCs did not significantly improve survival in this study when administered alone compared to saline alone.
cytokines [15, 29, 46, 71, 76], and increased IL-10 levels [15, 29, 46]. These alterations to the cytokine profile occurred across studies despite varying routes and timing of MSC administration. Beyond improving the cytokine profiles of resident immune cells, it has also been demonstrated that MSCs can triple their own IL-10 synthesis after exposure to inflammatory serum [75]. This production of IL-10 by MSCs may contribute to their overall anti-inflammatory capacity.

Of additional importance, recent research has revealed that monocytes and macrophages may be among the more significant host cells responsible for these changes in the inflammatory mediator profile [10, 30, 91]. Moreover, the ability of MSCs to trigger this response in macrophages may require direct cell-to-cell contact as well as production of PGE2 [30]. These findings may be in part responsible for the elevated serum neutrophil counts and significantly reduced neutrophil myeloperoxidase (MPO) activity measured in MSC-treated septic mouse liver and kidneys [30]. Thus, by re-programming the earliest immune responder, i.e., the macrophage, MSCs appear to diminish the initial proinflammatory cascade and organ dysfunction observed clinically in the later phase of sepsis.

Several recent studies have also noted the ability of MSCs to improve bacterial clearance in animal models of sepsis [46, 76]. It has been previously noted that MSCs are capable of increasing the phagocytic activity of macrophages in vitro [91]. In a 2009 CLP model, Gonzalez-Rey et al. showed that the bacterial burden found in the peritoneum, spleen, liver, and blood of MSC-treated mice was determined to be significantly reduced at 24 h, thereby emphasizing the ability of MSCs to improve bacterial clearance [46]. In a similar model, Mei and colleagues showed that MSCs upregulated phagocytosis-promoting genes in macrophages, which correlated with significantly improved bacterial clearance from the spleen at 28 h [76]. By reducing the bacterial burden early in the course of sepsis, MSCs may attenuate not only the inflammatory response, but also reduce the magnitude of the inflammatory stimulus from invading pathogens.

Recent studies have also examined the effects of MSCs in attenuating septic organ dysfunction in animal models. One means of limiting organ injury has been demonstrated in the ability of MSCs to reduce neutrophil transmigration and MPO activity in the lungs, liver, and kidneys [16, 30]. One study revealed that the MPO activity 6 h post-LPS injection in mice was found to be significantly reduced in the lung, liver, and intestine of those also treated with MSCs [46]. Similar results were noted 18 h post-procedure in their corresponding CLP study. This reduction in neutrophil migration was associated with significantly improved survival in both models.

Improvement of organ function has also been demonstrated by analyses of serum markers and inflammatory histology of organs injured in animal models of sepsis and endotoxemia. Utilizing the endotoxemia model, Yagi et al. demonstrated that MSCs improve renal and liver function as well as reduce lung and liver inflammation [75]. Other recent studies have corroborated that MSC administration improves renal function [30, 76], liver function [30], and lung injury [16, 57, 76]. Reductions in renal apoptosis [76], pancreatic inflammation, splenic necrosis, and vascular injury [30] have also been observed following MSC administration in CLP models. Of note, several studies have demonstrated the efficacy of MSCs in improving cardiac function in the endotoxemia model independent of intraperitoneal or intravenous routes of administration [15, 29, 71]. This finding emphasizes that the beneficial effects of MSCs may be more related to their homing and paracrine signaling than to any direct effects dependent upon their route of administration. In addition, Mei and colleagues evaluated the efficacy of MSCs in reducing organ dysfunction when administered 6 h after a CLP procedure [76]. Of interest, while this study corroborated findings found in earlier ones, the improvement in functional markers did not reach significance for liver and pancreatic function and may reflect the fact that MSCs were administered at a later time period following the procedure than in prior studies. This suggests that the protective effect to be found from MSCs may in part be dependent on when they are administered during a septic course.

Administration of MSCs in animal models of sepsis has revealed that they not only improve organ function, but also confer a survival benefit. Németh et al. found that overall survival rate was significantly improved from 10% to 50% 4 days after CLP was performed on mice that received intravenous MSCs [30]. They also noted that this significant improvement in survival could be achieved even when MSCs were administered 1 h after the septic insult. Significantly improved survival from 20% to 80% at 10 d post-CLP was also observed by Gonzalez-Rey and colleagues when MSCs were administered intraperitoneally 4 h after CLP was performed [46]. Similarly, these authors found that survival at 4 d was improved from 0% to 60% with MSC administration in their endotoxemia model. Contrasting these positive results, Mei et al. found that when they administered MSCs intravenously 6 h after performing CLP, no significant survival benefit was achieved despite evidence of reduced inflammatory cytokines, improved organ function, and increased bacterial clearance [76]. These authors demonstrated significantly improved survival only when MSCs were co-treated with broad-spectrum antibiotics. This disparity between the results of Mei et al. and other
authors further implies a relationship between the timing of septic insult and MSC administration, and further studies will need to evaluate what timing provides maximal benefit.

While these studies amply demonstrate that MSCs exhibit the capacity to reduce the inflammatory response and assuage organ damage in various animal models of sepsis, further research will assuredly be needed to evaluate the effects of MSCs in the treatment of sepsis before commencing human trials. Because CLP and CASP models more clearly reflect the variability in the progression of polymicrobial human sepsis, more animal studies will need to be designed which implement these models. Future studies will also need to consider and compare the timeframe of administration of MSCs as well as the co-administration of adjunctive therapies such as fluid resuscitation, broad-spectrum antibiotics, low-dose glucocorticoids, or intense insulin therapy employed in human sepsis. While numerous studies have examined ex vivo modifications of MSCs through preconditioning and genetic modifications in the treatment of myocardial infarction [47, 123–130], these approaches have not yet been exploited in animal models of sepsis and represent an exciting possibility for future research.

**CONCLUSION**

Sepsis remains a threat to postoperative patients and carries a high degree of morbidity and mortality with associated fiscal burden to the US healthcare economy. Current therapeutic regimens are not sufficient in combating the more advanced aspects of the sepsis syndrome, which include shock and multi-organ failure. While there are no human trials evaluating the role of MSCs in sepsis to date, an expanding field of experimental research using animal models suggests that MSCs may provide a unique ability to modulate various aspects of the complex pathophysiology in human sepsis. MSCs possess the ability to home to sites of injury, exhibit the capacity to reduce the inflammatory response and assuage organ damage, stimulate neangiogenesis, activate resident stem cells populations, enhance bacterial clearance, reduce the deleterious activities of neutrophils in injured tissue, and favor the formation of regulatory lymphocytes, among others. Further benefits achieved by ex vivo preconditioning and genetic modifications of MSCs have yet to be explored in sepsis studies, but have shown great potential in other disease models. The potential to condition or activate specific MSC immunomodulatory profiles to tailor therapies in sepsis and across many disease fields remains promising; investigation into the timing of MSC administration and further establishing the mechanisms of MSC immunomodulation in sepsis are key to future successes. As recent animal models of sepsis have demonstrated the ability of MSCs to significantly suppress the inciting inflammatory phase of sepsis as well as the multi-organ dysfunction observed in the later phase of sepsis, MSCs represent a promising cell-based therapy, which continues to merit further investigation. Indeed, the ability of MSCs to interact with the immune system at multiple levels may represent a dynamic new treatment for the future septic patient.

**REFERENCES**

17. Wang M, Crisostomo PR, Herring C, et al. Human progenitor cells from bone marrow or adipose tissue produce VEGF,


38. Rochefort GY, Delorme B, Lopez A, et al. Multipotent mesenchymal stem cells are mobilized into peripheral blood by hypoxia. Stem Cells 2006;24:2202.


53. Kinnaird T, Stabile E, Burnett MS, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote proliferation of host macrophages to increase their interleukin-2 production. J Mol Cell Cardiol 2007;41(Suppl 1):S21.


55. Tanaka F, Tominaga K, Ochi M, et al. Exogenous administration of mesenchymal stem cells ameliorates dextran sulfate


94. Matthey MA, Zemans RL. The acute respiratory distress syn-

ymal stem cells inhibit neutrophil apoptosis: A model for neu-
trophil preservation in the bone marrow niche. Stem Cells 2008;26:151.

96. Corcione A, Benvenuto F, Ferretti E, et al. Human mesenchy-


98. Bowdish DM, Davidson DJ, Hancock RE. Immunomodulatory
properties of defensins and cathelicidins. Curr Top Microbiol
Immunol 2006;306:27.

99. Wittchen AK, Baue AE, Chaudry IH. Sepsis and septic
shock—A review of laboratory models and a proposal. J Surg

100. Nauta AJ, Westerhuis G, Kruisellbrink AB, et al. Donor-de-
rivled mesenchymal stem cells are immunogenic in an allogene-


mal stem cells inhibit neutrophil apoptosis: A model for neu-
trophil preservation in the bone marrow niche. Stem Cells 2008;26:151.

103. Bowdish DM, Davidson DJ, Hancock RE. Immunomodulatory
properties of defensins and cathelicidins. Curr Top Microbiol
Immunol 2006;306:27.

104. Wittchen AK, Baue AE, Chaudry IH. Sepsis and septic
shock—A review of laboratory models and a proposal. J Surg

105. Herrmann JL, Abarbanell AM, Weil BR, et al. Ablation of TNF-
a receptor influences mesenchymal stem cell-mediated cardiac pro-
tection against ischemia. Shock 2010;34:236.

106. Shu T, Zeng B, Ren X, et al. Over-expression of HO-1 on mesen-
chymal stem cells promotes angiogenesis and improves myo-
17:80.

stem cell-natural killer cell interactions: Evidence that ac-
tivated NK cells are capable of killing MSCs, whereas MSCs
can inhibit IL-2-induced NK-cell proliferation. Blood 2006;
107:1484.

chymal stem cell (MSC) paradigm: Polarization into a pro-inflamma-
tory MSC1 or an immunosuppressive MSC2 phenotype. PLoS One 2010;5:e10088.

mesenchymal stem cells improve myocardial recovery after is-