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Gender differences in sepsis
Cardiovascular and immunological aspects

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During sepsis, a complex network of cytokine, immune, and endothelial cell interactions occur and disturbances in the microcirculation cause organ dysfunction or even failure leading to high mortality in those patients. In this respect, numerous experimental and clinical studies indicate sex-specific differences in infectious diseases and sepsis.

Female gender has been demonstrated to be protective under such conditions, whereas male gender may be deleterious due to a diminished cell-mediated immune response and cardiovascular functions. Male sex hormones, i.e., androgens, have been shown to be suppressive on cell-mediated immune responses. In contrast, female sex hormones exhibit protective effects which may contribute to the natural advantages of females under septic conditions. Thus, the hormonal status has to be considered when treating septic patients.

Therefore, potential therapies could be derived from this knowledge. In this respect, administration of female sex hormones (estrogens and their precursors) may exert beneficial effects. Alternatively, blockade of male sex hormone receptors could result in maintained immune responses under adverse circulatory conditions. Finally, administration of agents that influence enzymes synthesizing female sex hormones which attenuate the levels of pro-inflammatory agents might exert salutary effects in septic patients. Prospective patient studies are required for transferring those important experimental findings into the clinical arena.

Introduction

Recently there has been increasing interest in the immunoneuroendocrine system, including the relationship between gender, sex hormones, and their effects on pathophysiological parameters and the immune response following adverse circulatory conditions, i.e., hemorrhage[1] and sepsis.[2-4] These research efforts may disclose important links between steroid hormones and the immune system, thereby offering chances for novel therapeutic options. Several clinical and experimental studies demonstrated significant effects of sex hormones on cell mediated immune responses.[5,6] With respect to humoral immune responses, a remarkable preponderance of females are susceptible to autoimmune diseases, i.e., systemic lupus erythematosus (SLE), Hashimoto thyroiditis, rheumatoid arthritis, and primary biliary cirrhosis.[7-9] These have been documented in both human and experimental studies.[10,11] Male sex steroids appear to be immunodepressive whereas female sex steroids increase the activity of humoral immune responses.[12-14] This concept was also evident in a mouse model of autoimmune lupus. Female mice of this strain normally develop lupus erythematosus. Administration of male sex steroids prior to maturation prevented the disease development. Interestingly, castration of male mice resulted in the development of autoimmune diseases that are not evident in normal male mice of this strain.[15] Also, in humans, lower antigen expression and increased levels of active estrogen metabolites were evident in women suffering from SLE when compared with age-matched healthy female controls.[16] Similar effects of sex steroids have been demonstrated on cell-mediated immune responses.[17]

The above mentioned studies collectively suggest that male and female sex steroids have immune-modulating properties in humoral as well as cell-mediated immune responses under normal conditions and in various disease processes.

Effect of Gender on Sepsis and Septic Shock Associated Morbidity and Mortality

Epidemiological studies demonstrate gender differences with respect to the development of septic complications and multiple organ failure in trauma victims. In this respect, Offner et al. identified male gender as an independent risk factor for the development of severe infection in surgical patients.[18] McGowan et al. also reported a significantly higher incidence of bacteremic infections in males compared with females.[19]

A retrospective analysis incorporating 30 286 severely injured trauma victims (injury severity score, ISS, >15) displayed a higher incidence of pneumonia in males.[20] However, no differences in the mortality of pneumonia were evident in this study.[20] In addition to the incidence of sepsis, the clinical course also appears to be affected by gender. A retrospective analysis of 261 255 consecutive patients, reviewing the data available in the APACHE database, revealed an increased mortality in males younger than 50 y compared with women of the same age group.[21] This difference was not evident in patients older than 50 y.[21] A prospective observational cohort study of 2183 patients with community-acquired pneumonia, however, also revealed a lower survival in older men.[22] This was associated with an increased expression of TNF, IL-6, IL-10, D-dimer, antithrombin-III, and factor IX in men with pulmonary infection.[22] Conversely, anti-inflammatory

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mediators were increased in females which may contribute to the better prognosis.[22] Support for the advantage of female gender in sepsis comes from a prospective trial demonstrating an increased survival in females with sepsis compared with male patients (survival rate: 74% in women vs. 31% in men).[23] An analysis of 373 370 patients from the US National Trauma Data Bank similarly found male gender to be an independent prognostic variable for patients' outcome in sepsis using multivariate analysis.[24] In pediatric patients, gender-specific responses to sepsis have also been documented. Nonetheless, this was beyond the scope of the present manuscript.

Experimental studies in mice revealed a significantly increased survival rate of proestrus female mice following polymicrobial sepsis induced by cecal ligation and puncture (CLP) compared with male animals.[25] Moreover, the susceptibility to sepsis was also lower in proestrus female mice compared with males following trauma and severe blood loss.[26]

In summary, the above studies collectively suggest that male gender is a risk factor for the development of infectious complications after trauma. In patients with manifested sepsis, females are better positioned to survive the insult. This gender difference may only be evident in patients younger than 50 y, suggesting that postmenopausal women are no longer in an advantaged condition.

Despite these cited studies regarding gender-dimorphic responses following sepsis, conflicting studies with varied conclusions have also been published. In this regard, a huge cohort of trauma victims involving 22 332 patients did not detect gender differences in patient outcome.[27]

In the text that follows, we will offer potential explanations for this conflicting data. In particular, sex hormones and not gender itself have been shown to be responsible for gender-specific findings following sepsis. In this respect, differences in plasma sex hormone levels due to the stage of the menstrual cycle or age might explain why some clinical studies have failed to demonstrate gender differences. Stratification for hormone levels due to menstrual cycle or menopause has not been included in those clinical analyses investigating gender-specific outcomes in these patients.

Gender-Specific Immune Response after Sepsis

As outlined above, several clinical studies indicate a differential outcome in patients after septic conditions with respect to patients' gender. As a potential mechanism for this observation, a sex-specific expression of pro- and anti-inflammatory cytokines has been found in surgical patients at the molecular level.

Significantly elevated levels of pro-inflammatory cytokines (i.e., IL-6, procalcitonin) have been described in sepsis in male compared with female patients.[28-30] The importance of IL-6 has recently been emphasized in a clinical study by Wang et al. which demonstrated the predictive role of this cytokine for the severity of septic episodes.[31]

Similarly, experimental studies described an increased early cytokine response in males after the induction of endotoxemia. Four hours after LPS injection, significantly increased IL-1 levels were found in male as compared with female mice.[32]

Conversely, Ongaro et al. showed an elevated LPS-induced TNF-α secretion in plasma of adult male rats after androgen receptor antagonism or testosterone depletion with neonatal flutamide treatment or prepubertal orchidectomy, respectively.[33] In this regard, our studies have shown that flutamide administration following trauma increases aromatase activity which promotes the synthesis of 17β-estradiol from testosterone.[34-36] Thus, the salutary effects of flutamide in males may not be mediated by androgen receptor antagonism but due to increased estrogen levels. Similarly, stimulatory effects of estradiol on the responsiveness of human peripheral blood mononuclear cells to LPS in vitro have been demonstrated.[37] Thus, this early immune response may contribute to the enhanced immune resistance of females in subsequent phases of sepsis.

Despite these findings, gender-specific differences in the activation of lymphocytes remain controversial.[38-40] While Wichmann et al. reported a gender dimorphism in those cells following abdominal trauma,[38] Majetschak et al. failed to demonstrate differences in the cytokine response after activation of the immune system.[39] In a model of experimental sepsis in mice, protective effects of a precursor of testosterone, androstenedione, as indicated by decreased levels of pro-inflammatory cytokines in animals treated with this drug, have been found.[41] It should be noted, however, that testosterone is an intermediate and is converted to 17β-estradiol by aromatase and to 5α-dihydrotestosterone (DHT; active compound) by 5α-reductase. Thus, depending upon the prevailing enzymatic milieu, testosterone may be converted to 17β-estradiol or DHT.

Sepsis is often the consequence of operative interventions. In their clinical study, Wichmann et al. reported a significant depression of immune competent cells following surgery in men.[38] As a potential explanation, this study describes a sex-specific expression of different types of lymphocytes as well as natural killer cells in the postoperative course.[38]

In addition to a gender-specific immune response following sepsis, differences in survival have been demonstrated in various sepsis models. In this respect, in an experimental "two-hit" model of trauma-hemorrhage and subsequent sepsis, male gender and age have recently been shown to increase mortality rates.[42] Moreover, maintained immune responses in proestrus females were associated with significantly improved survival following cecal ligation and puncture.[25]

One explanation might be the fact that the number of resting resident leukocytes occupying the peritoneal and pleural cavities is higher in female than male rats, comprising more T and B lymphocytes, as recently shown by Scotland et al.[43] This altered immune cell composition of the female peritoneum is controlled by elevated tissue chemokine expression. Female resident macrophages also exhibit greater Toll-like receptor (TLR)-4 expression, enhanced phagocytosis, and NADPH oxidase-mediated bacterial killing.[40] On the other hand, addition of testosterone to isolated mouse macrophages decreased TLR-4 expression and sensitivity to a TLR-4-specific ligand as demonstrated by Rettew et al.[44] This enhanced immunological competence of local immune cells in females led to improved antimicrobial host defense after polymicrobial sepsis as indicated by Newsome et al.[45] They

demonstrated that intraperitoneal application of the biological modifier poly-(1,6)-b-d-glucopyranosyl-(1,3)-b-d-glucopyranose glucan (PGG glucan) at the onset of sepsis enhanced survival in female mice over a 10-d period, but survival in males was improved for only 24 h. This effect was associated with decreased IL-6 and IL-10 levels and reduced bacterial burden in the liver in female compared with male mice.

These gender-specific effects on different immune cell functions and compartments are potentially influenced by an X-chromosome mosaicism that exists naturally in females. Therefore, heterozygous cellular mosaicism presents a unique biological circumstance in females due to the fact that either the maternal or the paternal X-chromosomes are inactivated in each individual cell whereas males carry exclusively the maternal X-chromosome. Experimental studies revealed that this female X-chromosome mosaicism diversifies leukocyte responses during endotoxemia and may contribute to the dimorphic character of the inflammatory response.[46] Moreover, male and female sex hormone receptors have been identified on immune cells suggesting direct effects of androgen and estrogen. In particular, the estrogen receptor β expressed on immune cells plays a pivotal role in mediating immunoprotective effects of female sex hormones. In this respect, experimental studies indicate that the estrogen receptor β agonist WAY-202196 preserved gastrointestinal barrier function and improved outcome in models of systemic infection and inflammation.[47]

Sex Steroids Differently Affect the Cardiovascular System

Recent studies indicate that gender also affects cardiovascular responses.[1] Sex hormones have been demonstrated to affect outcomes after trauma-hemorrhage, ischemia/reperfusion (I/R) injury, and sepsis.[48,49] Castration of male rats two weeks prior to the onset of trauma-hemorrhage prevented the depression of myocardial function as evidenced by significantly higher values of heart performance in vivo.[50] Similarly, treatment of male rats with the androgen receptor antagonist flutamide has also been found to improve and restore cardiovascular responses following trauma and severe blood loss.[51] Recently, differences in the restitution of plasma and tissue volumes between males and proestrus females during and after trauma-hemorrhage have been found.[52] In this respect, proestrus females showed faster restitution of blood volume during and after trauma-hemorrhage, which might contribute to the improved immune and organ functions in females under adverse circulatory conditions.[40]

There is increasing evidence that administration of female sex steroids, i.e., estrogen, result in maintained cardiovascular functions through paracrine actions on immune cells, host tissue, and endothelial cell function.[53-55] In this respect, several studies involving males and ovariectomized females given exogenous estrogen indicated that females might be better positioned in mitigating against pathology from microcirculatory disorder and sepsis.[48,49] In particular, Sharawy et al. showed that independent of whether septic males or ovariectomized females were treated with agonists for the estrogen receptor (ER)-α or -β, a significantly reduced sepsis-induced leukocyte–endothelial interaction (rolling, adherent leukocytes and neutrophil extravasations) and improved intestinal muscular functional capillary density was observed.[56] Those results suggest that the observed effects of estradiol receptors on different phases of leukocyte recruitment with the improvement of the functional capillary density could partially explain salutary effects of estradiol on the intestinal microcirculation during sepsis.

Moreover, direct effects of sex hormones on myocardial function appear to be relevant for gender-specific cardiovascular functions in sepsis. In this respect, hearts from proestrus females displayed a significantly better post-ischemic functional recovery than males. Administration of estradiol in males and ovariectomized females improved post-ischemic myocardial functional recovery, reduced the production of TNF-α, IL-1β, and IL-6, and decreased the activation of p38 MAPK and caspase-3 when compared with their untreated counterparts.[49] These beneficial effects of estradiol were also evident in endotoxemia as shown by Zhu et al.[57] They demonstrated that male mice had greater myocardial dysfunction compared with female animals in a model of endotoxemia. Administration of estrogen reversed those alterations in the studies of Zhu et al.[57] Particular signaling pathways have been identified to be involved in this estrogen-mediated effect on myocardial function during endotoxemia. Generally, endotoxin induces Rac1 activation, which contributes to NADPH oxidase activity and phosphorylation of ERK1/2/p38 MAPK, leading to TNF-α expression in the heart. Estrogen attenuated this endotoxin-induced RAC 1 activation thereby reducing potentially detrimental myocardial TNF-α expression.[57]

Potential Therapeutic Strategies

Androgen receptor antagonists

Several animal studies have indicated that testosterone depletion exerts beneficial effects on immune responses after infectious diseases and sepsis.[58,59] In order to transfer those effects into the clinical arena, concepts mimicking castration by the use of the androgen receptor antagonist flutamide have been conducted, revealing modulation of a variety of pathways.[34-36] Administration of flutamide at a dosage of 25 mg/kg BW following trauma-hemorrhage and resuscitation normalized the depressed splenic and peritoneal macrophage cytokine release.[60] Additionally, LPS-induced TNF-α secretion in plasma in adult male rats after neonatal flutamide treatment was increased, indicates the depressive effects of testosterone.[33] In addition, flutamide administration on three consecutive days following trauma-hemorrhage not only restored the depressed splenocyte and splenic macrophage cytokine release even after the induction of subsequent sepsis, but also significantly decreased the mortality of post-hemorrhaged animals subjected to a subsequent septic challenge.[59] This maintenance of the immune system after flutamide treatment in male mice was also seen after induction of heatstroke in another animal model associated with massive inflammation. In this model flutamide administration resulted in improved survival.[61] In particular, treatment with flutamide in male mice subjected to heatstroke significantly attenuated hypothermia, reduced the

number of apoptotic cells in the hypothalamus, the spleen, the liver, the kidney, and attenuated the plasma index of toxic oxidizing radicals (e.g., nitric oxide metabolites and hydroxyl radicals), diminished the plasma index of the organ injury index (e.g., lactate dehydrogenase), attenuated plasma systemic inflammation response molecules (e.g., TNF-α and IL-6), and reduced the index of infiltration of polymorphonuclear neutrophils in the lung (e.g., myeloperoxidase activity). Since therapeutic use of flutamide in patients with testicular cancer for longer periods of time did not exhibit major adverse effects, the short-term use of this androgen receptor antagonist in male patients with a septic constellation may be a safe and useful therapeutic option for the treatment of immune and cardiovascular depression.

The steroid hormone DHEA

Dehydroepiandrosterone (DHEA) is the major circulating steroid hormone in humans. As an intermediate in the sex steroid synthesis it can be metabolized to both testosterone and estrogen. DHEA administered at a dose of 100 μg per animal in a mouse model prevented the depression of cell-mediated immune responses following trauma-hemorrhage and resuscitation, as evidenced by maintained splenic and peritoneal macrophage cytokine release and normalized splenocyte lymphokine release under those conditions.[62,63] In addition, administration of DHEA following trauma-hemorrhage significantly improved the survival rate of animals subjected to subsequent sepsis compared with vehicle-treated animals.[62] DHEA has been demonstrated to maintain TNF-α mRNA expression in the lung of a murine model of polymicrobial sepsis and trauma.[64] In particular, natural killer (NK) cells may be one of the effector cells of the protective mechanisms of DHEA in polymicrobial sepsis.[65] Furthermore, DHEA treatment in an experimental sepsis model restored splenocyte proliferation and delayed type hypersensitivity reaction, decreased cellular apoptosis rate of splenocytes, and attenuated cytokine releases.[66] DHEA has been shown to increase expression of Toll-like receptors in polymicrobial sepsis, which in turn preserves innate immunity and confers a longer survival in mice.[67]

These studies are in accordance with the observation that DHEA normalized the depressed pro-inflammatory cytokine release capacities of human PBMC in vitro following major abdominal surgery.[68] The effect of DHEA on PBMC function followed a dose-dependent manner. Studies demonstrated that DHEA can be administered intravenously or subcutaneously in septic male mice to achieve improvement of immune functions.[63-65] In the United States, DHEA is widely used as an over-the-counter drug without any serious side effects. Therefore, DHEA might be a promising treatment option in male patients with sepsis by preventing the depression of cell-mediated immune responses. Finally, it is noteworthy that a DHEA metabolite, androstenediol, when administered in experimental sepsis and trauma-hemorrhage, reduces organ damage, improves cardiac function, attenuates inflammation, reduces apoptosis, and extends survival.[66-71]

Treatment with female mesenchymal stem cells

Recent studies have demonstrated the capability of mesenchymal stem cells (MSCs) to augment the immune system when faced with various challenges. MSCs attenuate the excessive inflammation occurring in sepsis by specific signal pathways thereby antagonizing disorders of the organ systems.[72,73] As a potential mechanism, these cells may increase the antibacterial defense as well as the expression of anti-inflammatory substances. Furthermore, MSCs have been shown to reduce an overwhelming expression of inflammatory cytokines, as well as decreasing apoptosis under septic conditions.[74-76] In mice, MSCs have further been shown to improve survival in an experimental model of pulmonary sepsis.[76] In other models (i.e., endotoxemia or cecal ligation and puncture-induced sepsis) MSCs have been shown to increase survival which was mediated by reducing organ dysfunction as well as influencing the immune response.[73,77] Interestingly, MSCs possess gender-specific characteristics. Following stimulation, an enhanced expression of vascular endothelial growth factor, lower TNF-α production, as well as a reduced inflammatory response has been observed in female-derived murine MSCs compared with male-derived MSCs.[78] Moreover, female MSCs may be more effective than male MSCs in the treatment of myocardial recovery following cardiac I/R.[79] Gender-specific expression of receptors and intracellular signaling enzymes have been discussed as potential mechanisms for these differences. In a knockout model of TNF receptor 1 (TNFR1) female MSCs stimulated with LPS show greater resistance to the increased pro-inflammatory cytokine and a decreased growth factor production compared with male MSCs.[80] However, gender discrepancies exist in MSC function after injury, and injury-induced TNF signaling via TNFR1 may have disparate effects in males and females.

Summary

Despite the fact that gender differences in the morbidity and mortality from trauma, shock, and sepsis have been observed in several clinical studies, alterations in the immune functions following shock have been investigated primarily using young male laboratory animals. Numerous studies have recently been initiated investigating the effect of gender, age, and sex hormones on immune responses following sepsis. The findings of those studies suggest that low DHT and/or high estradiol appear to be protective for the host following adverse circulatory conditions, i.e., septic shock (**Fig. 1**). Although the exact underlying mechanism(s) for the immunomodulatory properties of sex hormones on cell-mediated immune responses and cardiovascular functions following sepsis remain unknown, there is evidence that both direct and indirect effects of sex steroids synergistically modulate the immune and cardiovascular responses (**Table 1**). In this respect, sex hormone receptors have been identified on various immune and non-immune cells suggesting receptor-mediated processes. Other studies suggest the release of secondary mediators which alter immune responses following adverse circulatory conditions. In view of these findings, clinically relevant therapeutic strategies should use the androgen receptor antagonist flutamide and/or estrogen or agents with estrogenic effects, i.e., DHEA, which might yield safe and useful therapeutic adjunct approaches for the treatment of immune and cardiovascular depression in sepsis. Moreover, gender should be taken into account when studying the immune or cardiovascular responses following sepsis.

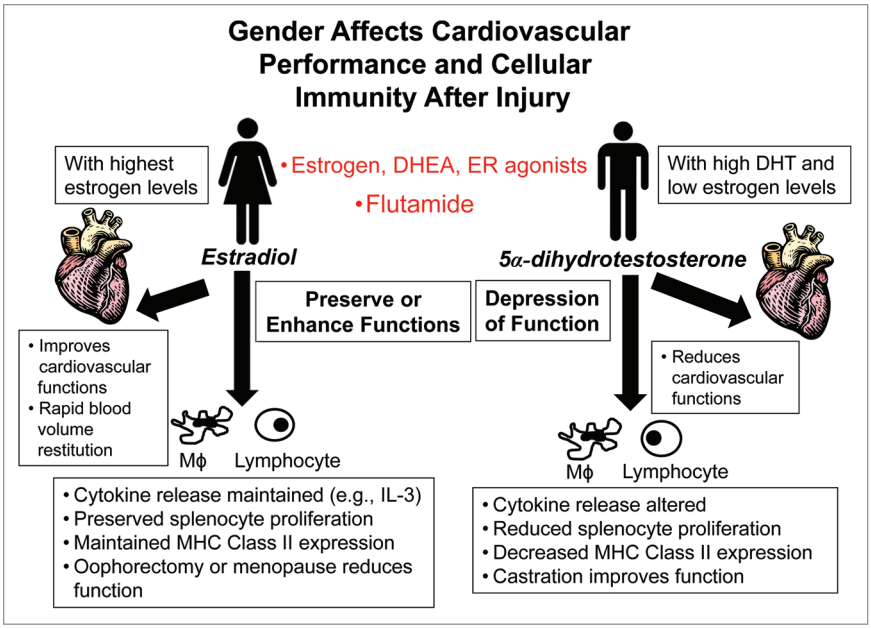

Figure 1. Schematic illustration of the effect of gender on cardiovascular performance and cellular immunity following trauma and severe blood loss.

Clinical studies, however, have been unable to consistently reproduce the experimental findings following low flow conditions. There continues to be a gap between the "bench and bedside" in regard to our understanding of gender-based differences following sepsis.[81] Relative to controlled animal experiments, predisposing comorbidities, injury characteristics, additional medication (analgesics, pain killers, inotropic agents, antibiotics, allogeneic blood transfusions, etc.), and a lack of information about the hormone milieu of the septic patient disallow reproducible results from clinical analyses. Continued clinical research into potential sex hormone-based differences, genetic differences, and the cellular and molecular mechanisms responsible for these gender-based differential responses is required to close this gap. There is hope that this research may ultimately promote sex-based therapeutic interventions, which will allow for improved outcomes for male and female trauma victims in the near future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Table 1. Effect of sex steroids/agents on immune/cardiovascular responses following injury/sepsis in murine models and humans

Author	Reference	Species	Model	Agent	Effect
Angele et al.,1997	59	Murine male C3H/HeN mice	Hemorrhage, sepsis	Testosterone receptor blockade (flutamide)	Splenocyte proliferation ↑, IL-2 ↑, splenic macrophage IL-1,6 ↑, survival ↑
Yu et al., 2005	34	Murine male Sprague–Dawley rats	Trauma, hemorrhage, cardiac function	Testosterone receptor blockade (flutamide)	Cardiac output ↑, estrogen receptors in cardiomyocytes ↑
Lin et al., 2012	61	Murine inbred male mice	Heatstroke	Testosterone receptor blockade (flutamide)	Systemic inflammation ↓, survival ↑, apoptosis ↓
Barkhausen et al., 2009	64	Murine male NMRI mice	Hemorrhage, sepsis	DHEA	Survival ↑, restoration of TNF-α mRNA expression in lung and liver
Frantz et al., 2005	68	Human	PBMCs from patients undergoing abdominal surgery	DHEA	Proinflammatory cytokine release ↑ (TNF-α, IL-6, IL-1β)
Shimizu et al., 2004	70	Murine Sprague–Dawley rats	Trauma, hemorrhage	Androstenediol/ DHEA	Cardiac function ↑, splanchnic perfusion ↑, inflammatory response (IL-6) ↓
Kiang et al., 2007	71	Murine Sprague–Dawley rats	Trauma, hemorrhage	Androstenediol/ DHEA	Apoptosis ↓ via caspase 3 ↓, iNOS ↓
Gonzalez-Rey et al., 2009	74	Human/murine	Experimental colitis	Female human MSCs	Restoration of immune functions, overwhelming inflammatory response ↓ (TNF-α, IFN-γ, IL-6, IL-1β, IL-12)
Nemeth et al., 2009	77	Human/murine	Experimental sepsis	Female human MSCs	Survival and organ function ↑, inflammation ↓ via macrophage derived IL-10

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