—Review—

Novel Therapeutic Targets for Sepsis: Regulation of Exaggerated Inflammatory Responses

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Abstract

Sepsis is a devastating and complex syndrome and continues to be a major cause of morbidity and mortality among critically ill patients at the surgical intensive care unit setting in the United States. The occurrence of sepsis and septic shock has increased significantly over the past two decades. Despite of highly dedicated basic research and numerous clinical trials, remarkable progress has not been made in the development of novel and effective therapeutics. The sepsis-induced physiologic derangements are due largely to the host responses to the invading microorganism in contrast to the direct effects of the microorganism itself. Sepsis, the systemic inflammatory response to infection, is marked by dysregulated production of pro-inflammatory cytokines. Although pro-inflammatory cytokine production is normally indispensable to protect against pathogens and promote tissue repair, the dysregulated and prolonged production of these cytokines can trigger a systemic inflammatory cascade mediated by chemokines, vasoactive amines, the complement and coagulation system, and reactive oxygen species (ROS), amongst others. These mediators collectively lead to multiple organ failure, and ultimately to death. In this regard, the role of inflammation in the pathophysiology of sepsis, although still incompletely understood, is clearly critical. Recent findings resulting from vigorous investigations have contributed to delineate various novel directions of sepsis therapeutics. Among these, this review article is focused on new promising mechanisms and concepts that could have a key role in anti-inflammatory strategies against sepsis, including 1) "inflammasome": a multiprotein complex that activates caspase-1; 2) "the cholinergic anti-inflammatory pathway": the efferent arm of the vagus nerve-mediated, brain-to-immune reflex; 3) "stem cells": unspecialized and undifferentiated precursor cells with the capacity for self-renewal and potential to change into cells of multiple lineages; 4) "milk fat globule-EGF factor VIII (MFG-E8)": a bridging molecule between apoptotic cells and phagocytes, which promotes phagocytosis of apoptotic cells.
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Key words: sepsis, inflammation, inflammasome, cholinergic pathway, stem cells, MFG-E8

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Introduction

Sepsis is the tenth leading cause of death in the United States and, from 1999 to 2005, over one million deaths, 6% of overall death, were attributed to sepsis[1,2]. The mortality rates of severe sepsis and septic shock ranges from 25% to 70% when complicated by shock and multiple organ failure[3–6]. The incidence of sepsis and septic shock has increased significantly over the past two decades[7–9] and the economic burden of severe sepsis is becoming alarmingly high[3]. Sepsis is characterized by infection with systemic inflammatory state which represents clinical manifestations of the following: altered body temperature of >38℃ or <36℃, tachycardia, tachypnea, and abnormalities of white blood cell count. Systemic inflammatory response syndrome (SIRS) can be diagnosed when two or more of these criteria are present[10]. "Severe sepsis" is defined as sepsis associated with organ dysfunction, hypoperfusion, or hypotension. The manifestations of hypoperfusion may include, but are not limited to, lactic acidosis, oliguria, or an acute alteration in mental status. "Septic shock" is a subset of severe sepsis and is defined as sepsis-induced hypotension despite adequate fluid resuscitation. It includes perfusion abnormalities such as lactic acidosis, oliguria, or an acute alteration in mental status. Patients receiving inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured[11,12]. Although recent treatment modalities and interventions including broad-spectrum antibiotics, intravenous fluids resuscitation, artificial ventilation, glucocorticoids, intensive insulin therapy, and recombinant human activated protein C, have contributed to the modest improvement of patient outcome[5,13,14], the high mortality rate of severe sepsis suggests the necessity for additional therapies. So far, vigorous experimental basic studies have been undergone to identify the effective therapy for sepsis and these novel therapies may be tested in clinical situation in the future. Herein, we review the therapeutic potentials of new concepts and modalities for sepsis, which is under experimental investigation, and the plausible mechanisms, focusing the regulation of exaggerated inflammatory responses in sepsis.

Systemic Inflammatory Response in Sepsis

The physiologic derangements elicited by sepsis in large part are due to the host responses to the invading microorganism as opposed to the direct effects of the microorganism itself. Sepsis is characterized by a systemic inflammatory response, mediated by innate immune cells, including neutrophils, monocytes, and macrophages. A production of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-8, normally triggers beneficial host innate immune responses to confine the infection and tissue damage. However, in sepsis, the excessive and prolonged production of these cytokines can produce overwhelming inflammatory responses, even more deadly than the original infection. This theory is especially notable in severe sepsis, where the excessive production of pro-inflammatory cytokines causes capillary leakage, tissue injury, and lethal multiple organ failure[15–17]. It is also reported that elevated pro-inflammatory cytokine levels directly correlate with severity and mortality in human sepsis[18,19]. However, despite undeniable successes in a huge number of preclinical studies, the clinical trials using direct anti-inflammatory strategies, such as anti-TNF-α, IL-1-based therapies, and high-dose corticosteroids, have failed with no establishment of a clear or consistent benefit in sepsis[16]. In this regard, experts agree that grouping patients with heterogeneous conditions under the same diagnosis of "severe sepsis" has led to a lack of significance and, failure to reproduce results in clinical trials.

The critical role of pro-inflammatory cytokines have been examined and proved in the pathophysiology of sepsis. These cytokines functionally contribute to the development of an acute phase response in the host manifested as fever, leukocytosis, alterations in glucose and muscle metabolism, and activation of the complement and coagulation cascades[20]. Subsequently, persisted elevation of these cytokines result in a variety of

pathologic phenomena, including priming of the vascular endothelium by synthesis of adhesion molecules, activation of neutrophils, synthesis of cyclooxygenase products, generation of nitrous oxide, reactive oxygen species (ROS), apoptotic cell death, and induction of hypotension and shock-like state[21–25]. Furthermore, pro-inflammatory cytokines can up-regulate inflammatory mediator expression via positive feedback loop and, consequently, induce further detrimental phenomena[26].

Pathogen Recognition System in Sepsis

During infection, innate immune cells are activated by foreign molecular products, named pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), flagellin, double-stranded RNA, and CpG DNA[27]. The initiation of the host response by recognition of PAMPs during sepsis or tissue injury involves three families of pattern recognition receptors (PRRs): 1) Toll-like receptors (TLRs); 2) NOD-like receptors (NLRs); and 3) RIG-I-like receptors (RLRs)[28,29]. The interplay between these families of PRRs ensures the efficient co-ordination of innate immune responses, through either synergistic or co-operative signaling. Members of the TLR family recognize bacteria, viruses, fungi and protozoa at either the cell surface or in lysosomes or endosomes. Pathogens that invade the cytosol are recognized by various cytoplasmic PRRs. The NLRs recognize a wide range of ligands within the cytoplasm. The RLRs recognize the RNA from RNA viruses in the cytosol. Among these, TLR signaling and associated downstream regulations of immune cell functions play a crucial role in the innate system as a first line of defense against pathogens. However, signaling is sometimes conflicting and a sustained inflammatory response can result in tissue damage[30]. In addition to the numerous exogenous pathogen-derived ligands that activate the different TLRs, endogenous TLR ligands have been identified in recent years. This includes hyaluronic acid, high-mobility group box 1 (HMGB1), and heat-shock proteins (HSPs), termed as damaged-associated molecular patterns (DAMPs). During tissue injury or proteolysis, extracellular matrix components undergo cleavage, exposing moieties that can act as ligands for TLRs and therefore initiating TLR-induced signal transduction[31]. Multiple positive feedback loops between DAMPs and PAMPs, and their overlapping receptors temporally and spatially drive these processes and may represent the molecular basis for the observation that infections, as well as nonspecific stress factors, can trigger flares in systemic inflammatory response[32].

Inflammasome: A Multiprotein Complex That Activates Caspase-1

The NLR family contains 23 members in humans and approximately 34 in mice. Among them, functions of several NLR members are well characterized. The NLR family has a unique structure composed of three domains: 1) the C-terminal domain consists of several leucine-rich repeat (LRR) that is involved in the recognition of microbial PAMPs or DAMPs; 2) the N-terminal domain consists of a death effector domain (DED), a pyrin domain (PYD), a caspase-recruitment domain (CARD), and Baculovirus IAP repeat (BIR) domain; and finally 3) an intermediate domain consisting of nucleotide-binding and oligomerization (NACHT) domain, which are required for ligand-induced, ATP-dependent oligomerization of PRRs and formation of active receptor complexes for activation of downstream signaling. The NLRs can be grouped based on their effector domains and are named NLR with a suffix of P or C referring to the N terminal moiety, PYD or CARD, respectively, followed by a number. NODs and NLRCs contain CARD effector domains, NLRPs contain PYD domains, and NAIPs contain BIR domains[33,34]. The mechanism by which the NLRs are able to recognize bacterial components is not well understood. The NOD proteins NOD1 and NOD2, the discovered NLRs, contribute to the detection of common fragments of peptidoglycan (ie, diamino-pimelate for NOD1, and muramyl dipeptide for NOD2) in the cytosol. When NODs recognize PAMPs, they rapidly oligomerize, which leads to the recruitment of the receptor-interacting protein 2 (RIP2) kinase, and activate NF-κB and MAP kinases

to induce the transcription of inflammatory cytokines[35,36]. A number of the NLR molecules have been shown to form a complex with caspase-1 and the adaptor molecule apoptosis associated speck-like protein containing a CARD (ASC) termed an "inflammasome", with the specific name stemming from the individual NLR molecules. The central effector molecule of the inflammasome is the cysteine protease caspase-1 that, upon activation cleaves cytosolic pro-IL-1β, pro-IL-18, and pro-IL-33 to their active forms IL-1β, IL-18, and IL-33, respectively, enabling them to be secreted into the extracellular/systemic compartments[37,38].

The important fact is that the NLRs and TLRs may synergize. TLRs activation by different ligands, induce pro-IL-1β and pro-IL-18 production; and to become biologically active, these cytokines need to be cleaved by caspase-1, its post-translational activation is tightly regulated by inflammasome[39]. The NLRP3 inflammasome is the most widely studied inflammasome and, to date, numerous PAMPs and host DAMPs have been reported, which activate the NLRP3 inflammasome. Although NLRP3 inflammasome biology has rapidly expanded, it is unclear whether NLRP3 senses ligands directly or indirectly. The NLRP3 inflammasome is composed of NLRP3, ASC, and pro-caspase-1 (**Fig. 1**). Stimulation of cells with appropriate ligands induces oligomerization of NLRP3, which promotes the clustering of ASC with NLRP3 via a PYD-PYD interaction. The CARD of ASC and the CARD of pro-caspase-1 then interact to induce catalysis of pro-caspase-1 to yield caspase-1, consisting of a p10/p20 tetramer, which catalyzes the proteolysis of pro-IL-1β and pro-IL-18 to IL-1β and IL-18, respectively. Several models of NLRP3 activation have been proposed including ATP-induced efflux of potassium ions via P2X7 ion channels and pannexin-1, and ROS induction, which leads to disruption of lysosomal membranes and the release of lysosomal proteins that activates NLRP3 inflammasome[40,41] (**Fig. 1**). In addition, a recent study has shown that IL-33 production is also mediated by TLR (TLR3 and TLR5)-NF-κB innate signaling pathways in corneal epithelial cells[42]. This suggests the critical role of synergetic action between TLRs and inflammasome in IL-33 production.

The role of inflammasome in the pathogenesis of sepsis is revealing. Caspase-1-deficient mice are protected from sepsis, while IL-1β-and IL-1β/IL-18-deficient mice are not[43]. However, inhibition of caspase-1 in IL-1β/IL-18-deficient mice confers protection against sepsis-associated mortality than caspase-1-intact IL-1β/IL-18-deficient mice[43]. This finding indicates the existence of another caspase-1-dependent mediator of sepsis. Recently, it was unveiled that reduced serum HMGB1 levels in caspase-1-deficient mice correlated with their resistance to LPS, and HMGB1 release requires the inflammasome components of ASC, caspase-1, and NLRP3. They concluded that HMGB1 secretion, which is a critical cytokine mediator of lethality in sepsis, occurs downstream of inflammasome assembly and caspase-1 activation[44]. In addition, NLRP3-deficiency was associated with partial protection against the lethal effect of endotoxin, and ASC-deficient mice were also resistant to LPS-induced sepsis[45,46]. However, caspase-12, which negatively regulates pro-inflammatory action of caspase-1, -deficient mice exhibit increased survival after sepsis[47]. In a clinical study, the mRNA levels of monocyte inflammasome components, such as ASC, NLRP1, and caspase-1, were found to be decreased in early stage septic patients. This suggests that this monocyte deactivation, characterized by lower expression of inflammasome components, may be maladaptive in the later phases of sepsis[48]. Great strides have been made over the past few years into understanding the crucial role of NLRs and inflammasome for the immune response to intracellular pathogens and cellular perturbations. However, further investigations are required to clarify the wider significance and the contribution to the pathogenesis of sepsis.

The Cholinergic Anti-inflammatory Pathway in Sepsis

With the accumulation of the knowledge regarding pro-inflammatory cytokines produced by the immune system causing numerous diseases, investigators focused on the physiological control

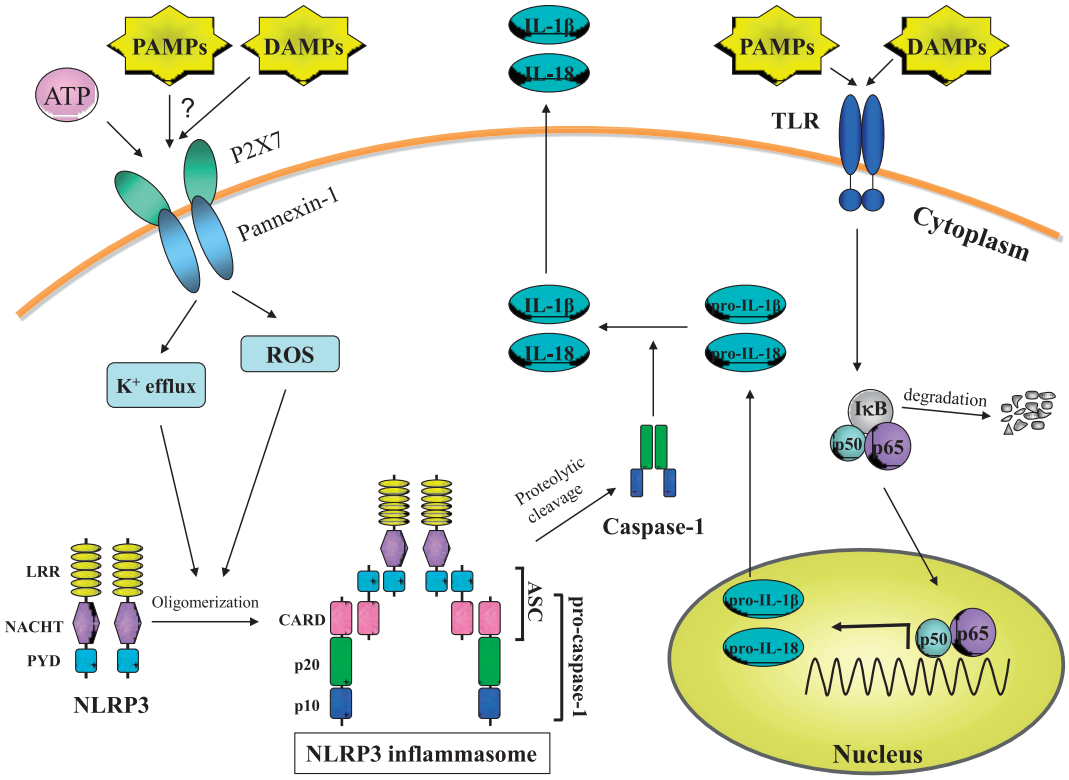

Fig. 1 NLRP3 inflammasome activation and the synergetic action with TLR signaling.
To date, numerous stimuli such as pathogen-associated molecular patterns (PAMPs), ATP, and damaged-associated molecular patterns (DAMPs) have been shown to activate the NLRP3 inflammasome. However, it is still unclear whether NLRP3 senses these ligands directly or indirectly. NLRP3 activating PAMPs and DAMPs induce K^+ efflux and reactive oxygen species (ROS) generation that play a role in the NLRP3 inflammasome activation. The NLRP3 inflammasome comprises NLRP3, apoptosis associated speck-like protein containing a CARD (ASC), and pro-caspase-1. It is well accepted that maturation and secretion of interleukin (IL)-1β and IL-18 requires two distinct signals. The initial signal is transcriptional and translational up-regulation of inactive form of cytokines (pro-IL-1β and pro-IL-18) in response to Toll-like receptor (TLR) ligands such as PAMPs through activation of NF-κB. The second signal, which originates from appropriate ligands, results in the assembly of the NLRP3 inflammasome, that induce catalysis of pro-caspase-1 to yield to caspase-1, and caspase-1 in turn, catalyzes the proteolysis of pro-IL-1β and pro-IL-18 to active form of IL-1β and IL-18, respectively, which is then released to extracellular space. *LRR*, leucine-rich repeat; *NACHT*, nucleotide-binding and oligomerization domain; *PYD* a pyrin domain; *CARD*, a caspase-recruitment domain.

mechanisms that maintain homeostasis by counter-regulation of cytokine release. So far, various endogenous anti-inflammatory mediators and mechanisms are discovered and proved to have a capacity to prevent pro-inflammatory cytokine-mediated diseases[49–51]. Recently, Tracey, *et al.* described an alternative cytokine control mechanism based on the structure of the nervous system. The central nervous system (CNS), through the vagus nerve, can reflexively modulate innate immune responses and control systemic inflammation; a mechanism termed "the cholinergic anti-inflammatory pathway"[50]. Stimulation of the efferent vagus nerve either by intracerebroventicular administration of pharmacological agents such as CNI-1493, muscarine, McN-A-343 (selective M1 muscarinic receptor agonist), methoctramine (M2 receptor antagonist) or by direct electrical stimulation, restrains the production of TNF-α from the organs of the reticuloendothelial system, including the liver and spleen, and in the systemic levels after endotoxemia[52–55]. These data indicates that cholinergic signaling through central muscarinic acetylcholine receptors modulates peripheral cytokine production by activation of the cholinergic anti-inflammatory pathway **(Fig. 2)**. In the

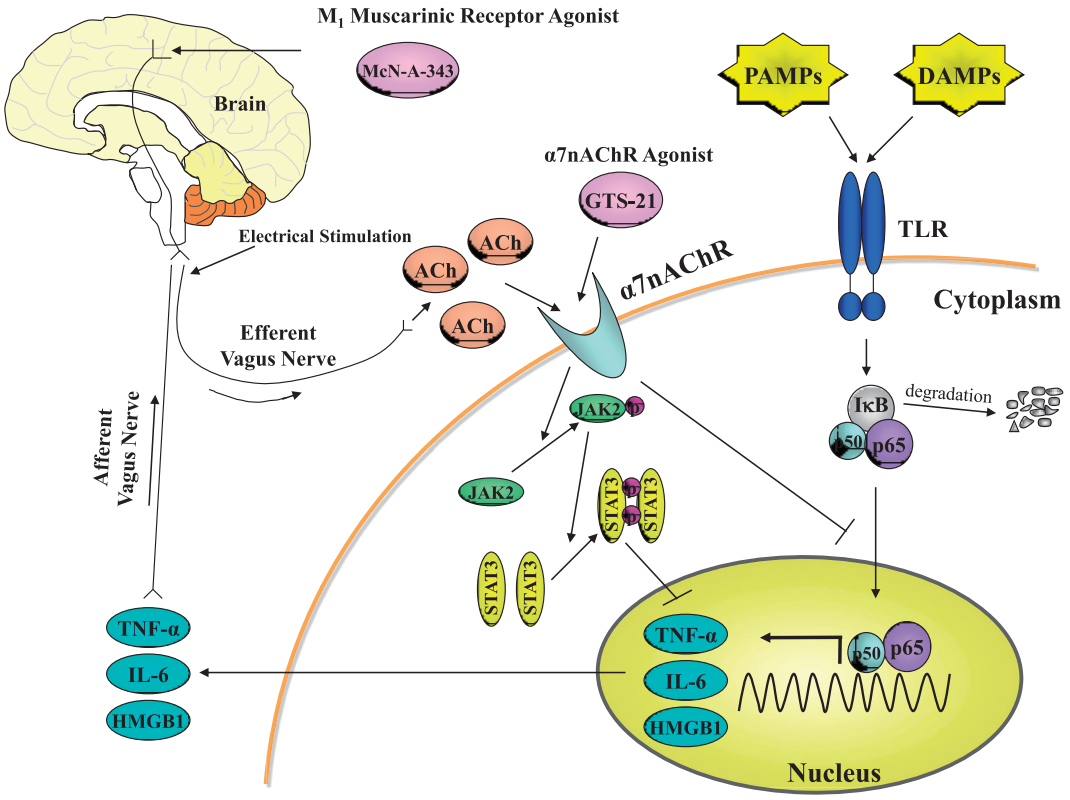

Fig. 2 The cholinergic anti-inflammatory pathway and the modulation.
If infection occurs, innate immune cells induce an inflammatory response characterized by the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-6, and high-mobility group box 1 (HMGB1). These inflammatory mediators stimulate the afferent vagus nerve and the signals are relayed to the central nervous system (CNS). The CNS coordinates major physiological responses to maintain homeostasis for parameters as varied as heart rate, blood pressure, body temperature, and organ perfusion via the efferent vagus nerve. Furthermore, efferent vagus nerve signals can control pro-inflammatory production via pathways dependent on the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR) on macrophages; a mechanism termed "the cholinergic anti-inflammatory pathway". Intracerebroventricular administration of cholinergic agonist, such as McN-A-343 (M1 muscarinic receptor agonist), can activate efferent vagus nerve. The activation of efferent vagus nerve by pharmacological and electrical simulation, and the systemic administration of cholinergic agonists, such as GTS-21 (selective α7nAChR agonist) inhibit the production of pro-inflammatory cytokines in endotoxemia and sepsis. The plausible molecular mechanisms related the anti-inflammatory effect of α7nAChR activation with ligands on macrophages suppress the nuclear translocation of NF-κB as well as activation of STAT3 via phosphorylation by JAK2, which is recruited to α7nAChR. *Ach*, acetylcholine.

cholinergic anti-inflammatory pathway, enhanced efferent activity of parasympathetic nerve endings results in the release of acetylcholine, which suppresses pro-inflammatory cytokine production by a specific action on the α7 subunit of the nicotinic acetylcholine receptor (α7nAChR) on macrophages[56]. Mice exposed to endotoxin showed excessive pro-inflammatory cytokine response if they are deficient in either α7nAChR or vagus nerve activity, which is induced by vagotomy[53,56]. Furthermore, vagus nerve stimulation in α7nAChR deficient mice fails to restrain cytokine release after endotoxemia[56]. Additionally, vagus nerve stimulation or α7nAChR agonists inhibit not only TNF-α but also IL-1, IL-6, IL-8, and HMGB1 after sepsis[57]. The activation of α7nAChR with ligands on macrophages suppresses the nuclear translocation of NF-κB as well as activation of STAT3 via phosphorylation by JAK2, which is recruited to α7nAChR and, as a consequence, result in the down-regulation of pro-inflammatory cytokine release[57,58] **(Fig. 2)**. Recently, a critical role for the spleen in inducing lethal

excessive cytokine response after endotoxemia and sepsis has been identified[54]. This study also suggested that the principal components for cytokine suppression via the cholinergic anti-inflammatory pathway converge in the spleen, by the fact that splenectomy significantly suppresses TNF-α levels in the liver and blood, and vagus nerve stimulation fails to suppress these levels further[54]. Unlike glucocorticoids or other humoral anti-inflammatory factors that function via systemic diffusion-dependent mechanisms, the cholinergic anti-inflammatory pathway is a direct physical link between the CNS and the immune system that precisely regulates immune responses in "real time"[50,59]. Considering that a deficient response to infection can lead to a failure to clear infection, whereas an excessive response can be more injurious than the initial insult, the prompt and precise regulation of the inflammatory response by the cholinergic anti-inflammatory pathway could be a promising therapeutic target for sepsis intervention. Several preclinical studies have shown the efficacy of modulating the cholinergic anti-inflammatory pathway not only for the cytokine response but also for the following deteriorating scenarios of sepsis. Serum TNF-α was increased by bilateral cervical vagotomy, whereas electrical stimulation of the vagus nerve attenuated it and prevented hypotension in rat polymicrobial peritonitis[60]. The hallmark of sepsis is organ dysfunction associated with abnormalities in the coagulatory system, which can result in disseminated intravascular coagulation. Vagus nerve stimulation regulates coagulation activation and fibrinolysis and thus can alter haemostatic responses[61]. The selective α7nAChR agonist GTS-21 improved survival and decreased neutrophil recruitment into peritoneal cavity in endotoxemia[62], attenuated serum HMGB1 levels, and improved survival in sepsis[63].

As mentioned above, the vagus nerve serves the critical interface between involuntary nervous system and immune cells for the counter-regulation of cytokine release. Interestingly, recent studies have unveiled that the anti-inflammatory property of a novel peptide ghrelin is also mediated through the vagus nerve activation[64–70]. Ghrelin, an orexigenic hormone, was first identified in the stomach as an endogenous ligand for the growth hormone secretagogue receptor type 1a (GHSR-1a, i.e., ghrelin receptor)[71]. Although various functions of ghrelin, including food intake, growth hormone production, adipogenesis, and neurogenesis, have been established, recently, ghrelin has emerged as a potent immune-regulatory and anti-inflammatory agent[72,73]. We have clearly shown that intravenous ghrelin administration significantly reduced systemic TNF-α, IL-6, and HMGB1 levels after cecal ligation and puncture (CLP)-induced sepsis, and vagotomy abrogated the ghrelin's down-regulatory effect on pro-inflammatory cytokine production[68,70]. Ghrelin can cross the blood-brain barrier, and ghrelin receptors are expressed at a high density in the brain[74–76]. Intravenous administration of ghrelin restores the sepsis-induced decrease of brain ghrelin levels and, moreover, intracerebroventricular ghrelin injection inhibits HMGB1 release[70]. Taken together, ghrelin can suppress the pro-inflammatory cytokine release in sepsis by the vagus nerve activation via central ghrelin receptors. From these evidences, targeting the vagus nerve activation appears to be a potential therapeutic candidate for sepsis.

Stem Cells in Sepsis

Stem cells are unspecialized or undifferentiated precursor cells with the capacity for self-renewal and the power to differentiate into multiple cell types[77]. Since the discovery[78], vigorous efforts have been made by investigators to be applied for the clinical situations based on its multiple potencies. So far, various stem cells have been identified and tried to utilize as cell-based therapy for a variety of disease conditions experimentally[79–83]. Until recently, the beneficial effects of stem cells were attributed to their ability to incorporate into tissue (engraftment), differentiate into the appropriate cell type, and repair injured areas. Although engraftment may still occur with some stem cells, recent investigations propose that other mechanisms, especially paracrine effects, may be involved[84–86]. Stem cells are broadly classified into adult tissue-derived vs. embryonic

stem cells. Although adult stem cells do not possess the full plasticity of embryonic stem cells (ESCs), which is derived from the inner cell mass of a developing blastocyst, they offer practical advantages including ease of isolation and propagation. More significantly, they have a limited risk of tumor formation and are not associated with the ethical controversy that surrounds ESC research. Among these, mesenchymal stem cells (MSCs) (also known as bone marrow-derived stromal cells) are the most widely studied adult stem cells. The current enthusiasm surrounding the potential use of MSCs for therapeutic purposes is derived from their low immunogenicity, their immunomodulatory properties, and their ability to secrete endothelial and epithelial growth factors and, more recently, antimicrobial peptides[87–91]. The unique surface markers of MSCs have not been identified, however, minimal criteria which is in consensus to define human MSCs include 1) selection for a plastic-adherent cell population in standard culture conditions; 2) expression of CD105, CD73, and CD90 and lack of expression of CD45, CD34, CD14, or CD11b; and 3) ability to differentiate into mesenchymal lineages including osteoblasts, adipocytes, and chondrocytes *in vitro*[92].

Stem cells, recently, have demonstrated prominent potentials to both directly regenerate tissue by virtue of their pluripotency and attenuate injury and inflammation via paracrine mechanisms in sepsis and sepsis-induced acute lung injury. Stem cells have anti-inflammatory properties, which counter-regulate pro-inflammatory cytokines, such as TNF-α, IL-1, and IL-6, by producing anti-inflammatory cytokines TGF-β, IL-10, and IL-13[93]. In a mice model of sepsis, bone marrow-derived MSCs administration at the time of surgery for CLP, between 24 h before to 1 h after surgery, improved survival than untreated animals, mitigated liver and renal dysfunction, decreased TNF-α and IL-6 levels by increasing IL-10, and attenuated vascular permeability compared to untreated animals. Furthermore, the beneficial effects of MSCs were eliminated by macrophages depletion, or blocking of IL-10 or its receptor, suggesting that MSCs may interact with immune cells, especially macrophages, to reprogram the host responses[94]. In a model of ALI induced by intratracheal *E. coli* endotoxin in mice, it was shown that syngeneic MSCs improved survival and lung injury which was associated with a decrease in MIP-2 and TNF-α levels in bronchoalveolar lavage (BAL) fluid and elevated levels of IL-10 in both the plasma and BAL fluid[87]. Another group showed that intravenous administration of syngeneic MSCs following intraperitoneal LPS injection prevented endotoxin-induced pulmonary injury, and edema as well as the influx of neutrophil into the injured alveoli. In this study, MSCs regulated the increase in systemic pro-inflammatory cytokines but did not alter the levels of anti-inflammatory cytokine, IL-10[95]. Moreover, a recent evidence demonstrated that human allogeneic MSCs ameliorated lung edema, improved lung endothelial barrier integrity, normalized alveolar epithelial fluid transport, and decreased inflammatory cell infiltration in an *ex vivo* perfused human lung injured by *E. coli* endotoxin. The authors suggested that this beneficial effect of human MSCs was derived from the secreted keratinocyte growth factor from MSCs[96]. In these studies, the therapeutic effect could not be accounted for by the level of lung MSCs engraftment, suggesting the importance of paracrine soluble factors or direct interaction with host cells. Besides, MSCs treatment decreases bacterial load in sepsis[89,94,97]. The plausible mechanisms of improved bacterial clearance by MSCs are enhanced phagocytic activity of splenic monocytes and macrophages, and/or a direct inhibition of bacterial growth via the secretion of an anti-microbial peptide LL-37[88,89]. As mentioned above, stem cells have shown significant promise as potential novel therapies for sepsis. However, there are still many complexities, such as an accurate definition of stem cells, a risk of formation of neoplasm, an optimal method of delivery. Initial findings suggest that the unique abilities of stem cells may offer benefit in the setting of sepsis and ongoing investigation to precisely characterize their role in the treatment of this condition is warranted.

MFG-E8: Novel Immunomodulatory Opsonin in Sepsis

Apoptosis, historically, has been seen as an ordinary process of cell suicide that, unlike necrosis, does not induce inflammation[98]. Recently, it has been demonstrated that not only necrotic cells but also apoptotic cells that were not engulfed appropriately by phagocytes, a termed "secondary necrosis," release potentially cytotoxic and antigenic intracellular contents, such as HSPs and HMGB1, that can promote an inflammatory response[88,89,99–101]. Furthermore, it is reported that pretreatment of animals with apoptotic splenocytes worsens the outcome in sepsis, suggesting that the detrimental effect of accumulated apoptotic cells in the body[102]. It has been shown that widespread and profound apoptosis of immune cells and component cells of respective organs are induced under septic conditions. This excessive apoptosis, which is an overload for professional phagocytes, elicits the impairment of immune function and increase in pro-inflammatory cytokine responses, which lead to organ injury[103–105].

Milk fat globule-EGF factor VIII (MFG-E8), a 66 kDa glycoprotein, initially was identified as one of the major protein components associated with milk fat globule membrane in the mouse[106]. MFG-E8 is known to be expressed in various cells, mainly in macrophages and dendritic cells, and expressed ubiquitously in almost all organs[107,108]. MFG-E8 contains a signal sequence for secretion, two N-terminal EGF domains and two C-terminal discoidin domains with homology to C1 and C2 domains found in blood-clotting factors V and VIII[106,109]. The second EGF domain contains a highly conserved arginine-glycine-aspartate (RGD) motif, by which it recognizes $\alpha_v\beta_3/\alpha_v\beta_5$-integrin of phagocytes, while the C-terminal discoidin domains enable it to bind to the apoptotic cells via phosphatidylserine (PS), which is redistributed to the external surface of the plasma membrane after the induction of apoptosis[110,111]. Through this process, MFG-E8 plays a role as a "bridging molecule" and facilitates the engulfment of apoptotic cells by phagocytes[108] **(Fig. 3)**. The engulfment of apoptotic cells by phagocytes is known to be non-inflammatory and non-immunogenic processes[112]. In fact, macrophages that engulf apoptotic cells have been shown to produce anti-inflammatory cytokines, including TGF-β, IL-10, which could potentially dampen inflammation[113,114]. Collectively, MFG-E8 functions as an immunomodulatory molecule via the enhancement of phagocytosis of apoptotic cells. We have shown that MFG-E8-mediated apoptotic cell phagocytosis in primary macrophages results in an inhibition of MAPK and NF-κB signaling pathways, which lead to the inhibition of TNF-α release[115]. MFG-E8-deficient mice showed various characteristics of inflammation and autoimmunity due to the accumulated apoptotic cells without appropriate engulfment by phagocytes[108].

MFG-E8 is reported to be differentially expressed from its normal condition under various pathophysiological stresses. Under sepsis or endotoxemia conditions, MFG-E8 expression was significantly suppressed in various organs, including spleen, liver, and intestine[116–119]. In addition, we have unveiled that not only infectious state, but also ischemia/reperfusion stress elicits significant down-regulation of MFG-E8[120,121]. Mechanistically, sepsis-induced suppression of MFG-E8 expression has been proved to be LPS-TLR4 pathway dependent[122]. One of the hallmarks of sepsis is accumulated systemic apoptotic cells, which is partially derived from a decreased phagocytic activity of professional phagocytes. The suppressed MFG-E8 expression is a plausible contributor for the decreased phagocytotic activity in sepsis[117,119,123]. Taken together, these evidences indicate that MFG-E8 serve a critical role in controlling excess inflammatory responses through the inhibition of apoptotic cell accumulation, which otherwise results in subsequent secondary necrosis **(Fig. 3)**. Hence, focusing on MFG-E8 could be a promising therapeutic approach for sepsis. To prove the therapeutic potential, exogenous administration of MFG-E8 conferred beneficial effects in CLP-sepsis model. We have shown that exogenous administration of immature dendritic cell-derived exosome (IDCE), that contains MFG-E8 robustly, attenuated the release of pro-inflammatory

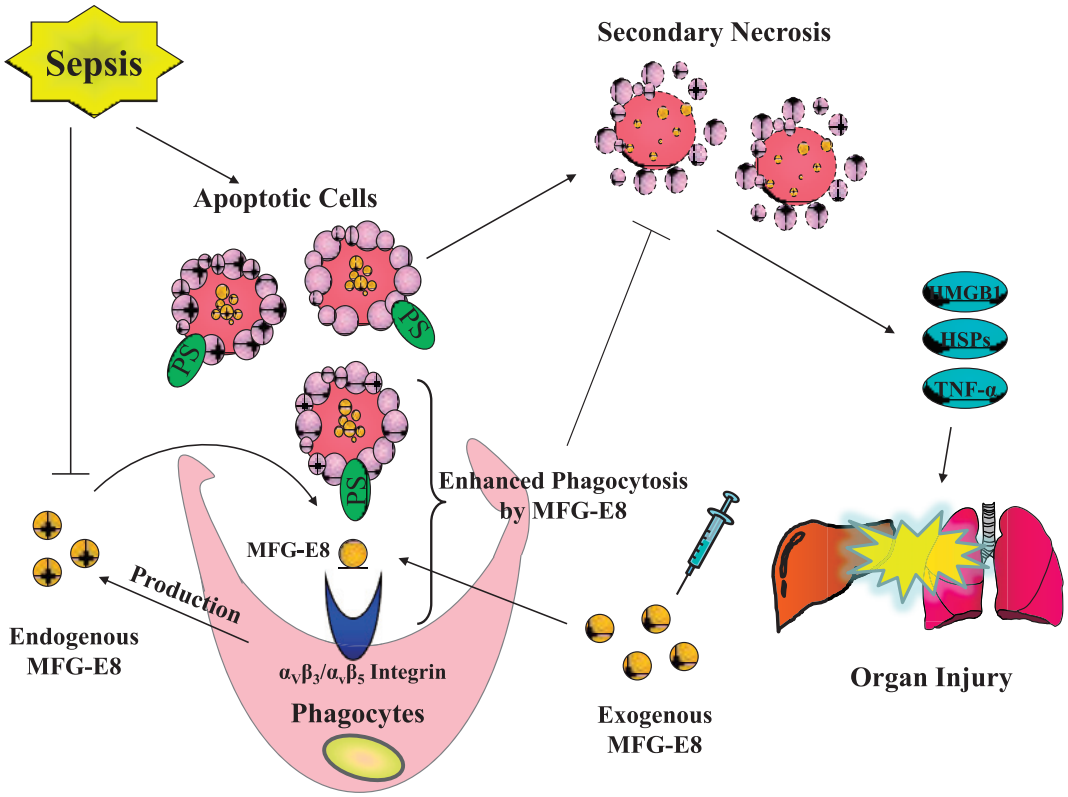

Fig. 3 The immunomodulatory effect of MFG-E8 through the enhancement of phagocytosis of apoptotic cells.
The accumulation of apoptotic cells is one of the major hallmarks of sepsis. Sepsis decreases apoptotic clearance by phagocytes through the down-regulation of milk fat globule-EGF factor VIII (MFG-E8). MFG-E8, a membrane-associated glycoprotein, plays a role as bridging molecule between phosphatidylserine (PS) on apoptotic cells and $\alpha_v\beta_3/\alpha_v\beta_5$-integrin of phagocytes, and facilitates the phagocytosis of apoptotic cells. If apoptotic cells were not engulfed appropriately by phagocytes, apoptotic cells have the potential to release toxic and pro-inflammatory contents due to secondary necrosis, and potentiate organ injury. MFG-E8 is known to be differentially expressed under various pathophysiological conditions. In sepsis or endotoxemia, MFG-E8 expression was significantly suppressed and which leads to the impairment of phagocytic capacity against apoptotic cells. Exogenous administration of MFG-E8 enhances phagocytotic activity and therefore attenuates detrimental inflammation thereby decrease tissue injury and mortality. *HSPs*, heat shock proteins.

cytokines, including TNF-α, IL-6, and HMGB-1[119,123]. IDCE and recombinant MFG-E8 treatment facilitated the clearance of apoptotic cells and consequently, decreased apoptotic cells. Furthermore, these treatments also dramatically improved the survival rate using CLPE model, which is provided by CLP and excision of necrotic cecum after 20 hrs CLP[123]. Besides, we have also demonstrated MFG-E8's direct anti-inflammatory property that MFG-E8 suppresses pro-inflammatory cytokine production from macrophages after LPS stimulation via the activation of negative regulators, such as SOCS3[124].

Conclusion

Considerable progress has been made in the last decade regarding management strategies in sepsis. Despite advances in supportive care, sepsis still claims a high death toll among affected patients due to cardiovascular shock and multiple organ failure. This is partly due to the fact that sepsis is a poorly defined clinical syndrome that encompasses heterogeneous patient populations with potentially diverse disease etiologies. The role of exaggerated inflammatory responses against infection in the

pathophysiology of sepsis, although quite complex and still incompletely understood, is clearly critical. Therefore, prompt and efficient anti-inflammatory strategies can provide therapeutic benefits for septic clinical settings. In this concise review, we described novel potential concepts targeting regulation of inflammatory responses in sepsis, however these are still in infancy state with many key questions unresolved and others as yet unasked. It is anticipated that continuous dedication in answering these questions will greatly improve our understanding, and may shed light on novel therapeutic targets for the treatment of sepsis.

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