Chapter 7
Novel Therapeutic for Systemic Inflammation: Role of MFG-E8

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Abstract Systemic inflammation associated with diverse clinical conditions is a vital problem in critical care medicine with significant morbidity and mortality. In this chapter, we describe exclusively on systemic inflammation caused by sepsis, ischemia and reperfusion injury, and trauma hemorrhagic shock. Despite all efforts in the clinical arena treatment for these indications remain limited. The only FDA approved drug as a treatment for sepsis, Xigris (drotrecogin alfa [activated]) has recently been voluntarily withdrawn by Eli Lily. There is an unmet and urgent clinical need exists for novel therapies for these conditions. There are pathological similarities as well as differences exist among these conditions. Even though all three pathologies are initiated by different means, all leads to exaggerated inflammatory response and multi-organ failure. Therefore, therapies developed to dampen the exaggerated systemic inflammation could be beneficial for all three pathologies. Milk fat globule-epidermal growth factor-factor 8 (MFG-E8) is first identified as a bridging molecule that accelerated the interaction between apoptotic cells and phagocytes and facilitates the engulfment of apoptotic cells. We then, demonstrated that MFG-E8 plays a significant role in sepsis, ischemia and reperfusion injury, and trauma hemorrhagic shock. In this chapter, we will briefly review the different systemic inflammatory conditions and describe the key evidence for the role of MFG-E8 and highlight the notion that MFG-E8 could be developed as a potential therapeutic for these indications.

Keywords Sepsis • Ischemia-reperfusion • Hemorrhagic shock • MFG-E8 • Systemic inflammation

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1 Introduction

Systemic inflammation caused by sepsis, ischemia/reperfusion injury and trauma/hemorrhage is a critical problem causing significant morbidity and mortality [1]. Sepsis and septic shock is the second leading cause of death in the non-coronary intensive care units and is in the top 10 leading causes of deaths overall in the United States [2]. The mortality rate for severe sepsis and septic shock is about 30 % annually, with over 200,000 deaths per year [3] which is similar to the number of people dying with acute myocardial infarction. Similar to sepsis, ischemia and reperfusion (I/R) injury also results in high morbidity and mortality. Gut or mesenteric ischemia remains a critical clinical condition, resulting in mortality as high as 60 % [4]. Hepatic ischemia-reperfusion (I/R) damage occurs in diverse clinical settings including liver transplantation and liver resection [5, 6]. Acute renal failure (ARF) caused by renal I/R injury is quite common in hospitalized patients, affecting 3–7 % of general admissions, and as much as 25–30 % of patients in intensive care units [7, 8]. Trauma is the fifth leading cause of death overall and the number one cause of death for patients between the ages of 1 and 40 [9, 10]. As such, development of therapies for such systemic inflammatory conditions is an unmet need exists for efficient patient care.

There are pathological similarities as well as differences exist among these conditions. Sepsis is defined as a systemic host response to an infectious origin which eventually leads to systemic inflammatory response and multi-organ failure (MOF) [11, 12]. Ischemia and reperfusion (I/R) injury is a pathological condition characterized by an initial restriction of blood supply to a specific organ followed by subsequent restoration of perfusion and reoxygenation. I/R injury is manifested as an initial tissue hypoxia due to occlusion of the arterial blood supply and a subsequent tissue injury and exacerbation of inflammatory response as a consequence of the reperfusion. The major organs that are affected by I/R injuries due to varied clinical conditions are the gut, the liver and the kidneys. I/R injury typically occurs in a sterile environment and eventually leads to exaggerated inflammation and MOF [13]. While exsanguinations and head injury continue to account for a large number of early trauma deaths, the majority of late trauma deaths occur as a result of infection and/or MOF [9, 10]. Even though all three pathologies are initiated by different means, all leads to exaggerated inflammatory response and MOF. Therefore, therapies developed to dampen the exaggerated systemic inflammation could be beneficial for all three pathologies.

Milk fat globule-epidermal growth factor-factor 8 (MFG-E8) was first identified by Hanayama et al. [14] as a bridging molecule that accelerated the interaction between apoptotic cells and phagocytes and facilitates the engulfment of apoptotic cells. We then, demonstrated that MFG-E8 is decreased in sepsis and the reduction in its expression leads to impairment of apoptotic cell clearance resulting in increased mortality. Administration of exogenous MFG-E8 in septic animals increased apoptotic cell clearance, reduction in inflammatory response, and improved survival [15–17]. We also showed beneficial effect of MFG-E8 in a number of other organ

injury conditions [18–21]. In this chapter, we will briefly review the different systemic inflammatory conditions and describe the key evidence for the role of MFG-E8 in these indications. With the available data, we highlight the notion that MFG-E8 could be developed as a potential therapeutic agent for sepsis, ischemia and reperfusion injury, and trauma hemorrhagic shock.

2 MFG-E8 and Sepsis

Sepsis is a critical problem causing significant morbidity and mortality [1]. It continues to be the second leading cause of death in non-coronary intensive care units, and is in the top 10 leading causes of deaths overall in the United States [2]. It is estimated that there are more than 1,000,000 cases of sepsis among hospitalized patients each year in the US. The incidence of sepsis among hospitalized patients is increasing by 8.7 % per year. Numerous reports have shown that the incidence of sepsis and severe sepsis is increasing in excess of the growth of the population [12]. The mortality rate for severe sepsis and septic shock is about 30 % annually, with over 200,000 deaths per year [3] which is similar to the number of people dying with acute myocardial infarction. Sepsis is defined as a systemic host response to an infection caused by bacteria, virus or fungi [11]. Sepsis tends to occur from specific and consistent sources such as respiratory infections, genitourinary and abdominal sources of infection with primary bacteremia, and other unknown sources. The occurrence of severe sepsis is related to the source of infection, as in the cases of patients with respiratory infection who are at high risk for developing respiratory related organ dysfunction. Regardless of the time and the organisms, the treatment of infection is the primary antisepsis therapy. From a clinical perspective, antimicrobial therapy is the chosen method of treatment. However, the choice of antibiotics, and the timing of their administration are extremely critical for successful outcome. Thus, there has been a substantial amount of work ranging from analyzing triage decisions made for intensive care unit admissions [22] to evaluating cortisol as a potential treatment against sepsis [23]. Despite these efforts, the treatment of sepsis however, has remained elusive. The only FDA approved drug as a treatment for sepsis, Xigris (drotrecogin alfa [activated]) [24, 25] has recently been voluntarily withdrawn by Eli Lily. There is an unmet and urgent clinical need exists for a sepsis therapy.

During inflammation and sepsis, systemic increases in pro-inflammatory cytokines have been shown to increase mortality [26]. During sepsis and other states with systemic inflammatory response, several cell types (e.g., B cells, CD4 T cells, dendritic cells (DCs), vascular endothelial cells and enteric epithelial cells) undergo apoptosis [27–31]. Apoptotic cells that are not cleared are likely to undergo secondary necrosis [32], thereby continuing to release harmful and toxic mediators and worsening sepsis. Studies have shown that phagocytic function of macrophages is impaired in late sepsis [33, 34]. Hanayama et al. [35] have discovered that lack of

clearance of apoptotic B cells in the spleen potentially leads to autoimmune diseases which underscores the importance of clearing apoptotic cells from organism [36]. MFG-E8, a 64-kDa secretory protein that is mainly produced by the spleen, was responsible for removal of apoptotic cells. Without MFG-E8, engulfment and removal of apoptotic cells were impaired which led to the release of autoantibodies [35]. MFG-E8 was originally identified as a component of milk-fat globules [37] but secreted by activated macrophages and immature dendritic cells [38]. The most remarkable function of MFG-E8 is its ability to promote the clearance of apoptotic cells by forming a tether between phagocytes and apoptotic cells [14, 39]. One unique characteristic of apoptotic cells is to expose their phosphatidylserine (PS) from its inner leaflet membrane to the outer surface. This is termed "eat me" signal which can allure distinct opsonins (i.e., MFG-E8), to recognize and bring apoptotic cells to the close vicinity of phagocytes [40]. MFG-E8 has a strong binding affinity to the exposed PS of apoptotic cells and facilitates phagocytic engulfment via $\alpha_V\beta_3$ or $\alpha_V\beta_5$ integrins. This triggers a conformational change in the integrin receptor that signals the recruitment of various signaling cascade proteins and transforms the macrophage into a phagocyte capable of engulfment [41, 42]. Thus, MFG-E8 promotes the engulfment of apoptotic cells by working as a bridging molecule between those cells and phagocytes [14].

In an animal model of cecal ligation and puncture (CLP)-induced sepsis, we showed that MFG-E8 levels were decreased by 45 % in the blood during late sepsis (i.e., 20 h after CLP) indicating the systemic scale of its depletion under septic conditions [16]. A 48–70 % reduction was observed in the spleen and liver tissues [15]. This decrease in the MFG-E8 expression in late sepsis was associated with impaired phagocytosis of apoptotic cells or apoptotic cell clearance [15]. Splenic macrophages from MFG-E8 deficient (*Mfge8*$^{-/-}$) mice showed a dramatically decreased ability to phagocytose apoptotic cells under normal conditions as compared to wild type mice, suggesting a critical role for MFG-E8 in this process. Interestingly, *Mfge8*$^{-/-}$ mice accumulated higher amounts of apoptotic cells as compared to the WT mice during late sepsis. These data clearly demonstrated that the clearance of apoptotic cells is directly regulated by MFG-E8 [16]. Endotoxemia also reduced splenic MFG-E8 expression in a dose dependent manner and the downregulation of MFG-E8 expression in CLP-induced sepsis was attenuated by the LPS inhibitor, polymyxin B. The CLP-induced suppression was not observed in either CD14$^{-/-}$ or TLR4-mutated mice. These studies indicated that MFG-E8 production is downregulated in sepsis by LPS-CD14 dependent fashion, leading to a reduction of phagocytosis of apoptotic cells [43].

MFG-E8 is secreted from DCs in exosomes that resemble milk fat globules in size and membrane lipid composition [38, 44]. These tiny vesicles (50–100 nm in diameter) are derived from multivesicular bodies, intermediates in the endosome maturation between endosome and endolysosome [44, 45]. Fusion of these multivesicular bodies with the plasma membrane leads to the release of MFG-E8 containing exosomes. In this regard, we isolated MFG-E8 containing exosomes from rat bone marrow immature DCs. Treatment of rats with MFG-E8 containing exosomes at the time of CLP, reduced the presence of apoptotic cells by 33 %. Peritoneal

macrophages from exosome-treated rats displayed a 2.8-fold increased ability to phagocytose apoptotic thymocytes [16]. Thus, the reduced presence of apoptotic cells in exosome-treated septic rats could have been due to the increase in apoptotic clearance. Treatment also reduced plasma tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) levels and improved survival from 44 % in the saline treated animals to 81 % in the treatment ones [15]. Similarly, treatment of septic rats with recombinant murine MFG-E8 (rmMFG-E8) attenuated the inflammatory response during sepsis, increased apoptotic cell clearance, and improved survival [16]. To develop MFG-E8 as a therapeutic agent against sepsis, recombinant human MFG-E8 (rhMFG-E8) was expressed in bacterial system, purified and confirmed of having biological activity similar to the mouse counterpart in abilities of mediating the phagocytosis of apoptotic cells by macrophages [46]. Treatment with the purified rhMFG-E8 in septic rats significantly reduced, organ injury indicators (AST, ALT, creatinine, lactate), serum IL-6 and TNF-α, and plasma HMGB-1 levels [17]. In a 10-day survival study in septic rats, vehicle-treated rats produced 36 % survival rate, while rhMFG-E8 treatment significantly improved survival rate to 68–72 %. Treatment with rhMFG-E8 significantly reduced the number of apoptotic cells detected suggesting increased apoptotic cell clearance. In addition to its role in apoptotic cell clearance, a recent study showed that MFG-E8-mediated potential therapeutic benefits in sepsis and intestinal injury were not solely dependent on the enhanced clearance of apoptotic cells, but also due to diverse cellular events to maintain epithelial integrity and healing of the injured mucosa [47]. In this regard, we have shown that the pre-treatment with rmMFG-E8 followed by endotoxemia showed significant attenuation of TNF-α levels in circulation and in splenic tissues suggesting an anti-inflammatory role of MFG-E8. In contrast, endotoxemia in the *Mfge8*$^{-/-}$ mice caused greater increase in TNF-α than those in WT mice [48]. Aziz et al. [48] further demonstrated that MFG-E8-mediated decrease in TNF-α is regulated by pSTAT3/SOCS3 leading to downregulation of NF-kB and subsequent decrease in TNF-α. Nevertheless, these findings taken together clearly provided evidence to develop rhMFG-E8 as a therapy for patients suffering from sepsis (Fig. 7.1).

3 MFG-E8 and Ischemia/Reperfusion Injury

3.1 MFG-E8 and Gut I/R

Gut or mesenteric ischemia remains a critical clinical condition, resulting in mortality as high as 60 % [4]. Intestinal ischemia and subsequent reperfusion are encountered in a variety of clinical conditions, including acute mesenteric ischemia, intestinal obstruction, incarcerated hernia, small intestine volvulus and necrotizing colitis. The consequences of mestenteric ischemia are devastating to the patient and usually results in severe diarrhea, malabsorption, short bowel syndrome, and death.

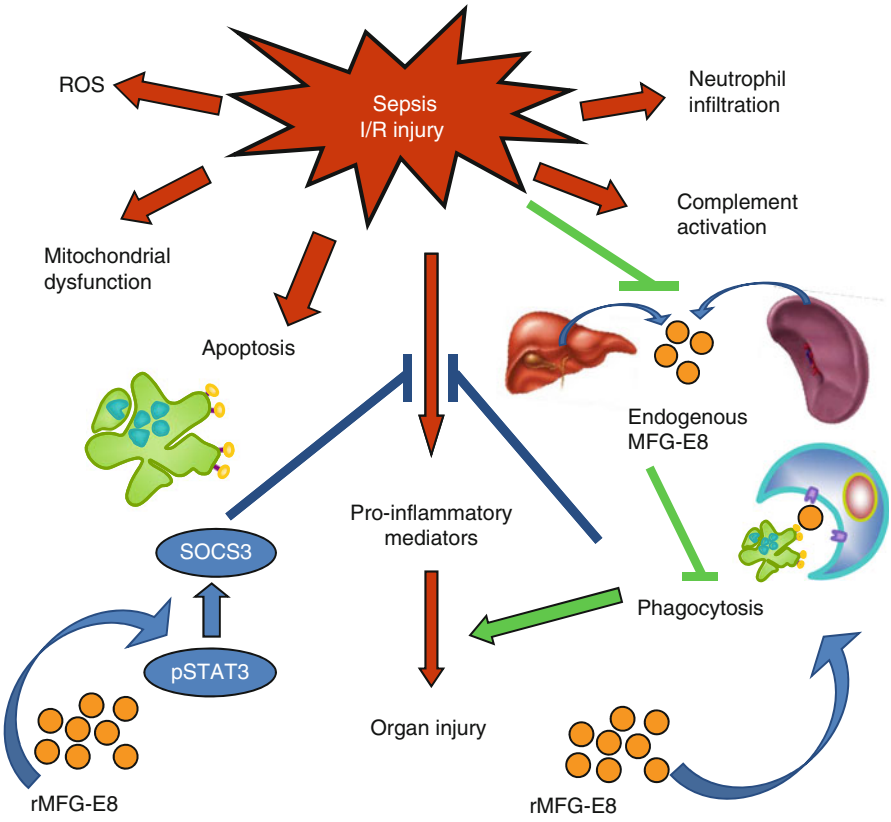

Fig. 7.1 Potential mechanism of MFG-E8 in sepsis and I/R injury. Sepsis and I/R injury is caused by a number of factors including neutrophil infiltration, complement activation, ROS, mitochondrial dysfunction and apoptosis leading to increase in proinflammatory mediators and organ injury. During sepsis, downregulation of the endogenous MFG-E8 in the tissues, i.e., spleen, liver and kidneys, attenuates apoptotic cell clearance (phagocytosis) and exacerbates organ injury. Administration of recombinant MFG-E8 (rMFG-E8) enhances the phagocytic activity and attenuates inflammation and decreases organ injury. In macrophages, rMFG-E8 upregulates pSTAT3/SOC3 signaling pathway and attenuates proinflammatory cytokines and decrease organ injury (Schematic illustration of data compiled from references [15, 16, 18–20, 48])

The pathophysiology of gut ischemia/reperfusion (I/R) involves tissue ischemia followed by cellular damage due to resumption of blood (reperfusion). Tissue ischemia initiates a series of events that can ultimately lead to cellular dysfunction and necrosis, and subsequent reperfusion causes more tissue damage including remote organ injury and subsequent death [13, 49–58]. A common complication of gut I/R is acute lung injury (ALI) and it contributes to the high mortality rate observed in gut I/R injuries. ALI is caused by a systemic inflammatory response due to the release of proinflammatory cytokines and bacteria-derived endotoxins from reperfused ischemic tissue [59, 60]. The mechanism of ALI involves a complex cross-talk among various cellular components of the alveolar microenvironment, their

secretory products, and leukocyte recruitment from the vascular bed in regions of inflammation. Activated neutrophils release proteolytic enzymes, such as elastase and myeloperoxidase (MPO) and reactive oxygen species, including hydrogen peroxide and superoxide. Excessive production of these factors not only destroys invaded pathogens, but also engages in the disruption of the endothelial barrier and promotes tissue damage. These events lead to ALI that is clinically manifested as acute respiratory distress syndrome followed by multiple organ dysfunction syndrome [61–64]. Even though numerous treatment modalities have been implicated in reducing ALI-induced mortality, none have been successful [65]. Thus, the development of novel and effective therapies for ALI is crucial for the improvement of patient outcome.

The gut is one of the most sensitive organs to I/R injury [55, 66]. Ischemia initiates a series of events that can ultimately lead to cell dysfunction and necrosis, and resumption of blood (reperfusion) causes more tissue damage [49–58]. The lungs are among the organs that are most severely affected by gut I/R-induced injury [67]. Ischemia or I/R induces apoptosis in various organs [68–70]. Apoptosis has been considered as the principal mode of cell death during I/R [66, 71–73]. Apoptotic cells stimulate inflammatory responses if they are not removed by phagocytes [74]. Deficient clearance of apoptotic cells leads to inflammation and tissue injury [39, 75]. MFG-E8 plays a crucial role for the engulfment of apoptotic cells by phagocytes [14, 35]. In this regard, we have shown that in a mouse model of gut I/R induced by superior mesenteric artery occlusion followed by reperfusion, as compared to the WT mice, *Mfge8*$^{-/-}$ mice produced much severe ALI after gut I/R [18]. MFG-E8 levels were markedly reduced in the spleen, gut and lungs by 50–70 %, suggesting impaired apoptotic cell clearance [76]. Treatment with rmMFG-E8 in gut I/R-induced WT mice significantly decreased lung apoptosis, improved lung morphology, and reduced neutrophil infiltration into the lungs. Treatment also suppressed tissue injury and inflammation as evidenced by reduction in liver enzymes (AST, ALT), lactate and creatinine, decreased proinflammatory cytokines (TNF-α, IL-6, IL-1β), and improved survival. Thus, MFG-E8 may serve as a novel treatment option for gut I/R-induced ALI.

3.2 MFG-E8 and Hepatic I/R

Hepatic ischemia-reperfusion (I/R) damage, which occurs in diverse clinical settings including liver transplantation, trauma, hemorrhagic shock, or liver surgery, is a serious clinical complication that may compromise liver function because of extensive hepatocellular loss. I/R injury represents a complex series of events that result in cellular and tissue damage. It involves the transient deprivation of blood flow and oxygen, and the return of blood flow during reperfusion with concomitant release of reactive oxygen species (ROS), inflammatory mediators, adhesion molecules, adenosine triphosphate (ATP) depletion, and derangements in calcium homeostasis. Finally, these functional changes induce cell death due to apoptosis as

well as necrosis [5, 6]. Despite the fact that hepatic injury is a major clinical problem, no reliable therapies have been established. The development of a therapy for hepatic I/R would indeed benefit patients undergoing liver surgery and liver transplantation.

It has been shown that programmed cell death or apoptosis of liver sinusoidal cells and hepatocytes is a prominent feature of liver I/R injury, in both experimental models and clinical transplantation [5, 77, 78]. Historically, apoptosis has been seen as an ordinary process of cell suicide that, unlike necrosis, does not elicit inflammation [32]. Studies have shown that if the removal process of apoptotic cells fails, apoptotic cells undergo secondary necrosis, which enables to release potentially cytotoxic intracellular contents, followed by inflammation and impaired tissue repair [79, 80]. In a rat model of hepatic I/R, liver and plasma levels of MFG-E8 were significantly decreased. Administration of rhMFG-E8 significantly improved liver injury, suppressed apoptosis, attenuated inflammation and oxidative stress, and downregulated the NF-κB signaling pathway. In a survival study conducted using *Mfge8*$^{-/-}$ mice and WT mice, the survival rate of the *Mfge8*$^{-/-}$ mice was markedly reduced as compared to that of the WT mice indicating that the *Mfge8*$^{-/-}$ mice were more susceptible to hepatic I/R-mediated mortality than the WT mice. In contrast, exogenous administration of rhMFG-E8 in WT mice improved the survival rate after hepatic I/R from 31 % in the saline treated animals to 70 % in the treatment ones [19]. Furthermore, it has been demonstrated that MFG-E8-mediated therapeutic potential is not only dependent on enhancement of phagocytosis, but also on multiple cellular events associated with tissue remodeling [47, 81, 82]. MFG-E8-mediated multiple physiological events may represent an effective therapeutic option in tissue injury following an episode of hepatic I/R.

3.3 MFG-E8 and Renal I/R

Acute renal failure (ARF) is a critical clinical problem posing significant economic and financial burden on the society. ARF is quite common in hospitalized patients, affecting 3–7 % of general admissions, and as much as 25–30 % of patients in intensive care units. Renal ischemia-reperfusion (I/R) injury causes ARF in various clinical settings, including kidney transplantation and cardiopulmonary and aortic bypass surgery. Renal I/R injury is associated with high mortality and morbidity [7, 8]. Current strategies used to prevent ARF consist mainly of fluid resuscitation and diuretics, and/or prevention of the insinuating factor. Despite these efforts, the mortality remains unacceptably high and has not improved in several decades. There is an urgent need to develop therapeutics to fight this pathological condition.

During renal I/R injury, renal damage begins immediately from the onset of ischemia. Upon restoration of perfusion, however, the tissues undergo further injury. Reperfusion injury involves the accumulation of neutrophils, generation of free oxygen radicals, and cytokine activation. These changes may also be seen

histopathologically, as demonstrated by the loss of the brush border, tubular disruption, and cast formation [83]. Renal damage due to I/R injury occurs as early as 5 h following injury as evidenced by a rising serum lactate, TNF-α, IL-6 and TGF-β levels, as well as decreasing systemic venous oxygen levels [84]. In the clinical setting, serum markers such as blood urea nitrogen and creatinine, are regarded as gold standards for renal compromise, but these markers may not become elevated until 24 h after the initial injury. Studies looking at early biomarkers, such as keratinocyte-derived chemokine (KC) and neutrophil gelatinase-associated lipocalin (NGAL), demonstrate that increases in these markers are associated with the development of ARF [85, 86]. With a better understanding of the pathophysiology of ARF as well as the identification of new biomarkers, one is able to determine the actual time point in the evolution of renal compromise pharmacological or hormonal therapy would be beneficial.

Ischemia typically damages renal tubular epithelial cells and also glomerular cells and is characterized by several hallmark features at the cellular level: Profound intracellular ATP depletion and a fall in tissue oxygen and glucose content with a concomitant rise in intracellular calcium [87, 88]. Although ischemic events alone may lead to necrosis and apoptosis in the kidneys, reperfusion occurs upon restoration of blood flow and is associated with increased apoptosis and necrosis in addition to the production of reactive oxygen species (ROS) and inflammatory mediators [89, 90]. Renal I/R injury can be ameliorated by inhibiting molecules involved in apoptosis, necrosis, or inflammation, suggesting that multiple injury and death mechanism may be involved in renal I/R injury [91]. Among these, the coexistence of apoptosis and necrosis in renal tissues is a characteristic feature of renal I/R injury. Both types of cell death have been implicated significantly in the pathogenesis of ARF, which is marked by a loss of tubular epithelial cells and subsequent renal dysfunction [92, 93]. Moreover, additional mechanisms which contribute to the ongoing pathogenesis of I/R injury-induced ARF has been reported. For instance, renal vascular endothelial injury and dysfunction, due to increases in renal vascular resistance and persistent reductions in renal blood flow, exacerbates hypoxia and play an important part in extending renal tubular epithelial injury and subsequent cell death [94]. In a rat model of bilateral renal ischemia followed by reperfusion (renal I/R) [20], MFG-E8 mRNA and protein expressions were significantly decreased in the kidneys and spleen. Treatment with rmMFG-E8 recovered renal dysfunction, significantly suppressed inflammatory responses, reduced apoptosis and necrosis, and improved capillary functions in the kidneys. In a 60 h survival study, survival rate after renal I/R injury decreased significantly from 44 % in the WT mice to 11 % in the *Mfge8*$^{-/-}$ mice. Interestingly, the exogenous treatment with rmMFG-E8 in the WT mice showed significant improvement in survival rate to 73 %. These data collectively demonstrated that the protective effect of MFG-E8 is mediated through the enhancement of apoptotic cell clearance and improvement of capillary functions in the kidneys. Thus, MFG-E8 could be developed as a novel treatment for renal I/R injury.

3.4 MFG-E8 and Hemorrhagic Shock

Trauma is the fifth leading cause of death overall and the number one cause of death for patients between the ages of 1 and 40 [9, 10]. In the US, about 90,000 people die annually due to traumatic injuries and complications. It is estimated that 10–20 % of the deaths are potentially preventable and nearly 80 % of these occur due to hemorrhage and it occurs within the first 24 h after injury [9, 95–97]. Immediate hemorrhage control and adequate fluid resuscitation are the key components of early trauma care. While fluid resuscitation decreases the risk of death in severe hemorrhage, it increases the risk of death in less severe hemorrhage. Despite the fact that fluid infusion at a pre-determined rate has shown to reduce organ injury and reduce mortality, the best approach recommended is to avoid unnecessary field interventions and focus on fast and efficient transport of the patient to hospital [98]. Advances in trauma care systems and emergency medical services have resulted in a significantly large percentage of patients who survive to hospital admission [96]. Another strategy implemented is the hypotensive resuscitation that showed some reduction in the risk of death. Hypotensive resuscitation at a fixed rate of 60–80 cc/kg/h generally maintains the systolic blood pressure of 80–90 mmHg and mean arterial pressure of 40–60 mmHg. Although the data suggest this strategy of infusion rates is beneficial in hemorrhagic shock, it requires monitoring of hemodynamic changes which would be difficult to accomplish in the field. Another strategy for resuscitation is the use of hypertonic saline. An number of pre-clinical studies have demonstrated that hypertonic saline modulate the immune response and leads to attenuation of immune mediated cellular injury [99–108]. However, in two recent multicenter clinical trials, hypertonic saline treated patients experienced early high mortality in comparison to normal saline treatment and thus, hypertonic saline is not recommended for resuscitation in trauma patients [109].

Observational data from trauma centers and the battlefield suggest that early administration of component therapy containing fresh frozen plasma and platelets may be beneficial [110, 111]. Based on battlefield experience, US Army instituted a policy of using a 1:1:1 ratio of packed red blood cells:fresh frozen plasma:platelets in the battlefield for those who meet the criteria for massive resuscitation. However, no study has identified the optimal ratios of blood components to be used for resuscitation [109]. In addition, although advance in viral screening have markedly decreased the risk of infectious transmissions, blood transfusion remains to be associated with numerous side effects. Blood transfusion has shown to cause early immune activation resulting in systemic inflammatory response syndrome and immune suppression which predisposes the patients to infection [112–116]. In addition to fluid resuscitation, a wide range of pharmacological agents including neuroendocrine agents, calcium channel blockers, prostaglandins, sex steroids, immune modulators, and histone deacetylase inhibitors have been tested in pre-clinical trials. Although majority of these agents show beneficial effects in animal models, none have been in clinical use as resuscitative agents. Thus, there is an urgent unmet need in the development of novel therapies for hemorrhagic shock.

Exsanguination and head injury continue to account for a large number of early trauma deaths, the majority of late trauma deaths occur as a result of infection and/or multi-organ failure. The clinical association of late trauma deaths and the development of multi-organ failure have been established as early as in the 1970s. However, only within the past few decades that the focus has been directed towards inflammatory response and how it may predisposes the body to infection and multi-organ failure. Hemorrhagic shock induces a surge of inflammatory cytokines including IL-6 and TNF-α which is associated with increased mortality [26, 117]. Prolonged and severe hemorrhagic state leads to tissue hypoxia and the presence of apoptotic cells [32]. If these apoptotic cells are not cleared, they will likely undergo secondary necrosis and release harmful agents and worsens hemorrhagic shock.

Apoptotic cell death is prevalent in gastrointestinal associated intestinal epithelial cells [118–120]. These cells are already prone to apoptosis after noxious stimuli exposure because these cell types normally undergo a rapid physiological turnover that is believed to be a result of apoptosis. Since bowel is the primary organ responsible for inflammatory response in trauma and shock, if accelerated cell death occurs in the intestine of patients with trauma and shock, important pathologic consequences could result. During trauma and shock, the intestinal wall loses its barrier function which results in the leakage of endotoxin and bacteria into the circulation causing a systemic inflammatory response. Apoptosis of intestinal epithelial tissues occur as rapidly as 2–3 h after initial injury and it compromises bowel wall integrity and becomes the primary mode for bacterial or endotoxin translocation into the systemic circulation [121]. In contrast, increased apoptosis of peripheral blood neutrophils is associated with reduced incidence of infection in trauma patients with hemorrhagic shock [122]. Clearance of apoptotic peripheral blood neutrophils by the liver and spleen inhibit inflammatory response thereby sparing the other organs such as the lung, which is among the most common sites of infection following serious trauma that leads to multi-organ failure and death.

Ingestion of apoptotic cells by macrophages results in the release of anti-inflammatory mediators, including TGF-β1 and PGE_2 and suppresses the production of pro-inflammatory cytokines such as IL-8, TNF-α and thrombaxane A_2 [123, 124]. In this regard, MFG-E8 has been identified as a bridging molecule between professional phagocytes via the $\alpha_V\beta_3$ or $\alpha_V\beta_5$ integrins and apoptotic cells via PS, which accelerates the engulfment of apoptotic cells [41, 42]. In a mice model of pressure-controlled (25 ± 5 mmHg) hemorrhagic shock [21], MFG-E8 levels in the plasma, lungs and spleen were significantly decreased at 4 h after hemorrhage. Resuscitation with rhMFG-E8 significantly improved apoptosis at 4 h as evidenced by a reduction in TUNEL positive cells and cleaved caspase-3 expression. Neutrophil infiltration into the lungs and spleen were also blunted. Pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) were reduced significantly in plasma (64–73 %), lungs (24–58 %) and spleen (49–76 %). In a 7 day survival study, a significant improvement (83 % vs. 43 %) with one-time dose of rhMFG-E8 as compared to normal saline treated mice after hemorrhage was observed. These data taken together suggest that rhMFG-E8 could be developed as a treatment for hemorrhagic shock.

4 Future Perspectives

In this chapter, we clearly demonstrated that MFG-E8 could be developed as a novel therapy for sepsis, ischemia and reperfusion injury, and trauma hemorrhagic shock. The data described further indicates that MFG-E8 could be functioning as a tether between apoptotic cells and phagocytes for efficient engulfment of apoptotic cells and thereby reduce inflammation and improve survival. It is also implicated that MFG-E8 could function directly by binding to $\alpha_V\beta_3$ or $\alpha_V\beta_5$ integrins and upregulate or downregulate signaling components causing the reduction in inflammation. Regardless of its mechanism of action, it is clear that administration of MFG-E8 is beneficial in attenuating the exaggerated inflammatory response associated with systemic inflammation caused by sepsis, ischemia and reperfusion injury, and trauma hemorrhagic shock. Thus, MFG-E8 treatment could be a potential therapy for patients suffering from complications associated with such conditions.

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