

Milk Fat Globule-EGF Factor VIII in Sepsis and Ischemia-Reperfusion Injury

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Sepsis and ischemia-reperfusion (I/R) injury are among the leading causes of death in critically ill patients at the surgical intensive care unit setting. Both conditions are marked by the excessive inflammatory response which leads to a lethal disease complex such as acute lung injury, systemic inflammatory response syndrome and multiple organ dysfunction syndrome. Despite the advances in the understanding of the pathophysiology of those conditions, very little progress has been made toward therapeutic interventions. One of the key aspects of these conditions is the accumulation of apoptotic cells that have the potential to release toxic and proinflammatory contents due to secondary necrosis without appropriate clearance by phagocytes. Along with the prevention of apoptosis, that is reported to be beneficial in sepsis and I/R injury, thwarting the development of secondary necrosis through the active removal of apoptotic cells via phagocytosis may offer a novel therapy. Milk fat globule-EGF factor VIII (MFG-E8), which is mainly produced by macrophages and dendritic cells, is an opsonin for apoptotic cells and acts as a bridging protein between apoptotic cells and phagocytes. Recently, we have shown that MFG-E8 expression is decreased in experimental sepsis and I/R injury models. Exogenous administration of MFG-E8 attenuated the inflammatory response as well as tissue injury and mortality through the promotion of phagocytosis of apoptotic cells. In this review, we describe novel information available about the involvement of MFG-E8 in the pathophysiology of sepsis and I/R injury, and the therapeutic potential of exogenous MFG-E8 treatment for those conditions.

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INTRODUCTION

The critically ill patient frequently develops a complex disease spectrum that may include acute lung injury (ALI), systemic inflammatory response syndrome (SIRS), sepsis and/or septic shock (1). It has been estimated that in the United States alone more than 750,000 patients per year develop sepsis and septic shock with an overall mortality of 28.6% (2). The incidence of sepsis and septic shock has increased significantly over the past two decades (3–5) and the economic burden of severe sepsis is becoming alarmingly high (2). Current wisdom implies that after severe injury or infectious chal-

lenge, some patients respond by overexpressing inflammatory mediators that lead to a systemic inflammatory response that culminates in severe shock, multiple organ dysfunction syndrome (MODS) and death (1).

Ischemia-reperfusion (I/R) injury is also one of the major clinical conditions that induces systemic inflammatory response. Ischemia causes tissue damage, which is exacerbated when reperfusion, restoration of blood flow, occurs (6,7). I/R injury happens commonly in cases of shock, tissue transplantation, myocardial infarction, stroke, certain infections and arterial disease and trauma. The intense

inflammation triggered by I/R injury may precipitate inflammatory damage in organs not involved in the initial ischemic insult. These remote effects of I/R injury are observed mostly in the lungs and cardiovascular system, and may result in the development of SIRS and MODS, both of which account for 30% to 40% mortality in tertiary referral intensive care unit (8). Despite extensive investigations, the cellular and molecular mechanisms that are involved in the initiation and propagation of sepsis and I/R injury have not been understood fully. This lack in the fundamental knowledge has made attempts at developing effective therapies for sepsis and I/R injury exceedingly difficult.

Milk fat globule-EGF factor VIII (MFG-E8, the lactadherin homolog in humans) is a membrane-associated glycoprotein originally found in milk and mammary epithelial cells (9). Previous investigators have shown that MFG-E8 participates in multi-

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ple physiological processes associated with tissue remodeling (10–15). Among these, its role for the clearance of apoptotic cells has made a huge impact on subsequent research. This review discusses the role of MFG-E8, focusing on the anti-inflammatory property that is derived from the enhancement of phagocytotic capacity, in the pathogenesis of sepsis and I/R injury and explores the therapeutic potential of MFG-E8 in those conditions.

MILK-FAT GLOBULE-EGF FACTOR VIII (MFG-E8)

MFG-E8 Structure and Production

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 (9). It has since been isolated independently from the mammary gland of several other mammalian species such as bovine and human (16–19). Hanayama *et al.* (14) and Aziz *et al.* (20) investigated MFG-E8 gene expression in various tissues of normal mice and reported that MFG-E8 gene is expressed ubiquitously in almost all organs, and several organs, such as mammary glands, spleen, lymph nodes, brain and lung, express it abundantly. However, some vital organs including intestine and liver have lower expression of MFG-E8. MFG-E8 contains a signal sequence for secretion, two N-terminal epidermal growth factor (EGF) domains and two C-terminal discoidin domains with homology to the C1 and C2 domains found in blood-clotting factors V and VIII (9,16). The second EGF domain contains an arginine-glycine-aspartic (RGD) integrin-binding motif that engages $\alpha_v\beta_3/\alpha_v\beta_5$ integrins to facilitate cell adhesion as well as induce integrin-mediated signal transduction (21–23). Oshima *et al.* (24) found the presence of two mRNA variants for MFG-E8 in mouse mammary gland, which were formed by alternative splicing of the same premature mRNA. The 66 kDa long form of MFG-E8 (MFG-E8L) contains a 37 amino acid proline/threonine rich (P/T-rich) domain between the second EGF domain and the first discoidin domain

(24,25). The 53 kDa short form of MFG-E8 (MFG-E8S) lacks the P/T-rich domain (24,25). The expression of the two splice variants shows spatial and temporal specificity. MFG-E8S is distributed widely, whereas MFG-E8L has been found in activated mouse macrophages (14,26), as well as in immature dendritic cells (27), Langerhans cells of the skin (27) and in epidermal keratinocytes (28). Recently, MFG-E8L has been identified in a species other than the mice (24,25). It also is shown that both forms seem to have a similar biological activity (13,15).

The functional roles of MFG-E8-associated tissue remodeling have been investigated widely, they include promoting removal of apoptotic lymphocytes by macrophages (13,14), clearance of mammary epithelial cells in involution (12), mediating sperm-egg bindings (11), maintenance of intestinal epithelium (10) and facilitating neovascularization as a downstream effector of vascular endothelial growth factor (VEGF) signaling (15). The MFG-E8 production from macrophages is increased by granulocyte/monocyte colony-stimulating factor (27,29) and fractalkine (CX₃CL1) (30, 31). Furthermore, it is reported that MFG-E8 expression, which is evaluated in human and animal models, is downregulated in some disease conditions such as autoimmune disease (14), Alzheimer disease (32), atherosclerosis (33), acute colitis (20), sepsis (10,31,34–36) and I/R injury (37,38). However, in contrast, MFG-E8 is highly expressed in systemic lupus erythematosus (39), lung fibrosis (40), breast cancer (19) and melanoma (41).

MFG-E8 Contributes to Phagocytic Removal of Apoptotic Cells

Phagocytic removal of apoptotic cells occurs quite efficiently *in vivo* such that, even in tissues with significant apoptosis, very few apoptotic cells are detectable (42). This is thought to be due to the release of so called “eat-me” signals by apoptotic cells that recruit motile phagocytes such as monocytes, macrophages and dendritic cells, leading to the prompt clearance of the dying cells

(43,44). So far, several “eat-me” signals have been discovered. The redistribution of phosphatidylserine (PS) to the external surface of the plasma membrane is a key element of apoptotic cell recognition and is a molecular cue that dying cells should be engulfed (45). Some receptor molecules, including PS receptor (46), LDL receptor (47) and scavenger receptors (48), directly recognize PS on apoptotic cells. In addition, soluble proteins that bind to PS on apoptotic cells for phagocytosis also were reported (49). How these receptors are involved in the recognition and engulfment of apoptotic cells has not been fully understood. Hanayama *et al.* (13) were first to show that activated peritoneal macrophage strongly produces MFG-E8 and the C-terminal discoidin domains mediate attachment to PS on apoptotic lymphocytes and the RGD motif of N-terminal domains engages $\alpha_v\beta_3/\alpha_v\beta_5$ integrins expressed on the advancing phagocytes. Through this process, MFG-E8 plays a role as “bridging molecule” and enhances the engulfment of apoptotic cells by phagocytes. Additionally, MFG-E8 deficient mice showed various characteristics of autoimmunity that are specially due to defects in apoptotic cell engulfment by tingible-body macrophages in germinal centers (14). Analogously, the human homolog of MFG-E8 (hMFG-E8) also has a role for “bridging molecule” and low levels of hMFG-E8 enhance phagocytosis, however, at high concentrations, engulfment is inhibited in a dose-dependent manner (39). This biphasic function of hMFG-E8 indicates the complicated involvement of MFG-E8 for disease pathogenesis and progression.

INAPPROPRIATE CLEARANCE OF APOPTOTIC CELLS INDUCES INFLAMMATORY RESPONSES

Historically, apoptosis has been seen as an ordinary process of cell suicide that, unlike necrosis, does not elicit inflammation (50). Recently, it has been shown that if the removal process of apoptotic cells fails, apoptotic cells undergo post-apoptotic secondary necrosis and release

potentially cytotoxic and antigenic intracellular contents such as heat shock proteins and high mobility group box 1 protein (HMGB1), which can elicit an inflammatory response (51–54). In a study by Hotchkiss *et al.* (55), pretreatment of animals with apoptotic splenocytes worsens the outcome in a mouse model of sepsis, pointing out the detrimental effect of accumulated apoptotic cells in the body. Furthermore, it is well known that one of the key biological features of apoptotic cell clearance itself is the non-inflammatory and non-immunogenic nature of this uptake process (56). In contrast to the uptake of pathogens or FcR-mediated phagocytosis, the engulfment of apoptotic cells does not lead to proinflammatory cytokine production by macrophages (56). In this context, the ability of apoptotic cells to induce the secretion of antiinflammatory cytokines may be of relevance. Macrophages that engulf apoptotic cells have been shown to secrete antiinflammatory cytokines, such as transforming growth factor- β (TGF- β) and interleukin-10 (IL-10), which could potentially dampen inflammation (57,58). In another study, lipopolysaccharide (LPS)-stimulated macrophages inhibited the production of inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), IL-1 and IL-12 after phagocytosis of apoptotic cells as macrophages that did not phagocytose before (58,59). We have shown that mature resident peritoneal macrophages (RPMs) which are major sources of inflammatory cytokines, while losing their efficacy for phagocytosis of apoptotic cells, express lower MFG-E8 levels and show higher TNF- α response to LPS compared with immature thioglycolate-elicited peritoneal macrophages (TGPM) (60). After LPS-stimulation, the capability of RPMs to phagocytose is decreased along with further downregulation of MFG-E8 and RPMs also lack phagocytosis-induced inhibition of TNF- α release (60). Furthermore, MFG-E8-mediated apoptotic cell phagocytosis results in an inhibition of mitogen-activated protein kinase (MAPK) and nuclear factor (NF)- κ B sig-

naling pathways (60). Asano *et al.* (26) reported that a dominant-negative form of MFG-E8, designated as D89E, carrying a point mutation in an RGD motif, inhibited not only the phagocytosis of apoptotic cells by a wide variety of phagocytes, but also inhibited the enhanced production of IL-10 by TGPM phagocytizing apoptotic cells.

Taken together, these results indicate that MFG-E8 plays a pivotal role in controlling excess inflammatory responses through the inhibition of apoptotic cell accumulation, which otherwise results in subsequent secondary necrosis, the anti-inflammatory cytokine production and the inhibition of inflammatory cytokine production by activated phagocytes.

MFG-E8 MEDIATES SEPSIS-INDUCED SYSTEMIC INFLAMMATION

MFG-E8 Expression and Apoptotic Cell Clearance Are Suppressed in Sepsis

Under septic conditions, it has been shown that widespread and profound apoptosis of crucial immune cells occurs and this excessive apoptosis induces the impairment of immune function and proinflammatory cytokine upregulation (61–63). Hence, focusing on MFG-E8, which promotes appropriate clearance of apoptotic cells by phagocytes before the secondary necrotic cell development, is considered to be promising therapeutic approach for sepsis to avoid further tissue injury along with the antiapoptotic strategies (57,62).

We were the first to show that MFG-E8 protein expression dramatically decreased (by 48% in the spleen and even by 70% in the liver) at 20 hours after septic insult induced by rat cecal ligation and puncture (CLP) model (35). The blood level also was decreased by 45%, indicating the systemic scale of MFG-E8 depletion under septic condition (36). We also showed that LPS from Gram-negative bacteria, which play a pivotal role for the pathogenesis of CLP sepsis, downregulate this protein production from cultured RAW 264.7 macrophages

(murine macrophage cell line) and peritoneal macrophages *in vitro* (31,35). We pursued this MFG-E8 inhibition by LPS and showed that *in vivo* LPS injection in mice also reduced splenic MFG-E8 mRNA expression in a dose-dependent manner and the downregulation of splenic MFG-E8 mRNA expression in mice CLP sepsis was attenuated by polymyxin B administration, which neutralizes LPS activity (34). Furthermore, this CLP-induced MFG-E8 inhibition was not observed in either CD14^{-/-} or Toll-like receptor (TLR)4^{-/-} mice, and we concluded that sepsis-induced downregulation of MFG-E8 production is mainly LPS-CD14-TLR4 pathway dependent (34). Besides LPS-CD14-TLR4 pathway, sepsis-derived apoptosis of immune cells as a source of MFG-E8 is suggested to be responsible for the depletion of MFG-E8 in sepsis (36). It also is reported that MFG-E8 is expressed constitutively in intestinal lamina propria macrophages of mice and CLP sepsis downregulates MFG-E8 production (10).

We have demonstrated a decreased phagocytic activity of macrophages after CLP sepsis which was evaluated by coinubation with isolated peritoneal macrophages and autologous dexamethasone-induced apoptotic thymocytes *ex vivo* (35). Then we developed a novel phagocytosis assay using pHrodo succinimidyl ester-labeled apoptotic lymphocytes as targets and tissue macrophages as phagocytes, which is quick and reliable to evaluate the internalization of apoptotic cells, and is able to clearly distinguish the engulfment, rather than the attachment, of apoptotic cells by phagocytes (64). Using this method, the decreased phagocytic activity of isolated splenic and peritoneal macrophages was reconfirmed in CLP sepsis models (34,36).

We have demonstrated extensively in this model that exogenous administration of MFG-E8 with recombinant murine protein (rmMFG-E8) and immature dendritic cell-derived exosome that contains MFG-E8 robustly promotes the engulfment of apoptotic cells and a de-

crease in apoptotic cell number. In this regard, MFG-E8 deficient mice showed a decreased phagocytic activity and the promotion for phagocytic activity by MFG-E8-containing exosomes administration was abrogated in MFG-E8 inhibition study (35,36). Hence, the sepsis-associated decrease of MFG-E8 expression contributes to the impairment of clearance of apoptotic cells even under septic conditions. Additionally, from the evidence of *in vitro* study, we have shown also that MFG-E8 did not inhibit apoptosis directly; MFG-E8-containing exosomes do not decrease the presence of apoptotic cells in sepsis through the direct modulation of apoptotic pathways, but rather through the increased clearance of apoptotic cells (35,36).

Exogenous Administration of MFG-E8 Attenuates Sepsis-Induced Inflammation and Improves Survival

Sepsis is marked by a systemic inflammatory response caused by an overwhelming infection. Although this inflammatory response is helpful in minor infections, it becomes overzealous in sepsis, causing more harm than good to the organism. Among a number of sepsis models that have been used to study the pathophysiology of sepsis, the model of CLP mimics many features, such as cytokine profiles, of clinical sepsis-peritonitis (65–67). Sepsis-induced overstimulation of inflammatory cytokines such as TNF- α , IL-1 β , IL-6 and HMGB1, which act as a late proinflammatory cytokine by a substantial neuroendocrine and immune activation, plays a central role in the morbidity and mortality in experimental sepsis as well as in septic patients (68–72). Studies using inhibitors of these cytokines demonstrated increased survival of septic mice treated with TNF- α or HMGB1 blocking antibodies (71,73). Therefore, we investigated the alterations of inflammatory response after exogenous administration of MFG-E8 in a rat model of CLP-sepsis. MFG-E8-containing exosome administration dramatically suppressed CLP-induced systemic TNF- α , IL-6 and HMGB1 responses (35,36). Inter-

estingly, MFG-E8-containing exosomes failed to suppress TNF- α release from LPS-stimulated macrophage *in vitro* in the absence of apoptotic cells, suggesting that the antiinflammatory effect of MFG-E8 is derived from an indirect immunosuppressive effect (36). In this regard, we demonstrated that administration of fractalkine (CX₃CL1) in mice, reduced the CLP sepsis-induced increases in blood lactate levels, liver injury, and proinflammatory markers—which was associated with a complete reversal of decreased plasma MFG-E8 levels (31).

We also showed the protective effect of exogenous MFG-E8 administration for sepsis-associated mortality using a consistent septic model with an approximate lethality of LD₅₀, which is provided by CLP, and excision of necrotic cecum after 20 hours after CLP (35,36). Both MFG-E8-containing exosomes and rmMFG-E8 administration dramatically increased the survival rate to approximately 80% in a rat model (35,36). In addition, MFG-E8 deficient mice showed a reduced survival rate compared with wild-type mice (36). As mentioned above, although only a limited amount of research has been done to study the role of MFG-E8 in sepsis, MFG-E8 has been reported to be of crucial importance in this phagocytic and secondary immunosuppressive effect, which finally leads to a survival benefit, even under septic conditions.

MFG-E8 IN ISCHEMIA-REPERFUSION INJURY

The hallmarks of I/R injury are the formation of reactive oxygen species (74), cytokine release (75), complement activation (6,76), the production of eicosanoids (77,78), recruitment of activated leukocytes (79,80), mitochondrial dysfunction (81) and a combination of cell necrosis and apoptosis (82,83). These I/R injury-induced events can trigger intense and detrimental inflammatory responses which lead to organ damages. Hence, similar to sepsis, mediating inflammatory responses after I/R injury is considered to be a possible therapeutic approach (75,84–86).

It is recognized that I/R injury induces the increased occurrence of apoptotic cell death of not only targeting but also remote organs (87–89), and deficient clearance of apoptotic cells potentially leads to increased inflammation and impaired tissue repair (90,91). Recently, we have shown the clinical importance of MFG-E8 in the pathogenesis and the therapeutic potentials of ALI after intestinal I/R injury (37). Mesenteric ischemia remains a critical problem, resulting in mortality as high as 60% to 80% (92). Multiple organ failure, including ALI, is a common complication of intestinal I/R injuries and contribute to its high mortality rate (93). Using a mouse intestinal I/R injury model, which consists of a mesenteric artery occlusion for 90 min followed by reperfusion for 4 h, we showed dramatic decrease of MFG-E8 mRNA and protein levels in the spleen and lungs by 50% to 60% (37). Administration of rmMFG-E8 markedly suppressed the intestinal I/R injury-induced local and systemic hyper-inflammation (gut TNF- α and IL-1 β ; lung IL-1 β , IL-6 and myeloperoxidase [MPO] activity; plasma TNF- α , IL-1 β and IL-6) and organ injury (histological injury scores of intestine and lung; plasma lactate, LDH, ALT, AST, creatinine), and finally improved the survival rate from 0% to 47% during 24-hour observation (37). In addition, apoptosis of lung epithelium and endothelium, as well as the accumulation of neutrophils, play a major role in the development and progression of ALI (94,95). We showed that treatment with rmMFG-E8 improved intestinal I/R injury-induced apoptotic cells accumulations in the lungs (37). Although the direct data showing the alterations of clearance of apoptotic cells after intestinal I/R injury was not demonstrated in our work, previous studies (35,36) strongly support the scenario that the decreased MFG-E8 level is associated with impaired phagocytosis which result in accumulation of apoptotic cells. Additionally, the MFG-E8's effect on decreasing apoptosis is not mediated by a direct antiapoptotic effect but through the stim-

ulation of apoptotic cell clearance. These studies collectively suggest that MFG-E8 is indeed beneficial in downregulating local and remote detrimental inflammatory response through the stimulation of apoptotic cell clearance even under I/R injury.

Recently, we have shown the involvement of MFG-E8 in the pathophysiology not only of intestinal but also of renal I/R injury (38). This study revealed that renal I/R injury reduced MFG-E8 expressions in the spleen and kidneys. Exogenous administration of rmMFG-E8 improved deteriorated renal functions (BUN, creatinine) and histological tubular injuries, and increased inflammatory markers (kidney IL-6, IL-1 β , macrophage inflammatory protein-2 [MIP-2] and MPO) after renal I/R injury. These findings suggested to us a novel potential of MFG-E8 as treatment for broad-spectrum I/R injuries.

POTENTIAL MECHANISM OF MFG-E8 IN SEPSIS AND I/R INJURY

Sepsis and I/R injury share some similar pathophysiological features, which induce a detrimental inflammatory response that causes tissue injury. Among these, increased accumulation of apoptotic cells and inappropriate phagocytotic removal of these cells, have the potential to release toxic and proinflammatory contents owing to secondary necrosis (51–54). The synergistic effect of increased apoptotic cell death, and decreased clearance of apoptotic cells by phagocytes presumably due to the downregulation of MFG-E8 emphasize the importance of mediating apoptosis in sepsis and I/R injury. Administration of MFG-E8 enhances the clearance of apoptotic cells and inhibits the detrimental proinflammatory cascade which is induced by apoptotic cells (Figure 1).

FUTURE STUDIES AND PERSPECTIVES

The data described above provide a rationale for continued investigation of MFG-E8 as an antiinflammatory agent that serves to ameliorate sepsis or I/R injury. MFG-E8 is crucial in enhancing en-

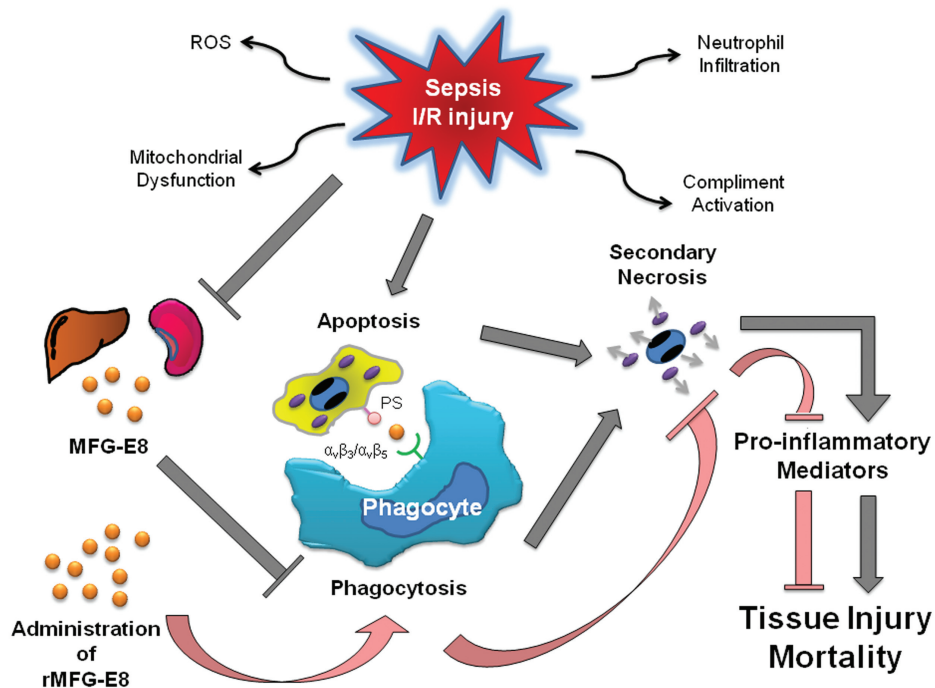


Figure 1. Schematic representation of pathophysiology of sepsis and I/R injury. Sepsis and I/R injury induce the increased apoptotic cell death in various cells and tissues and decrease apoptotic cell clearance by phagocytes, such as macrophages and dendritic cells, through the downregulation of milk fat globule-EGF factor VIII (MFG-E8). Apoptotic cells, without appropriate phagocytotic removal, have the potential to release toxic and proinflammatory contents due to secondary necrosis, and potentiate tissue injury and mortality. Administration of MFG-E8 enhances phagocytotic activity and therefore attenuates detrimental inflammation, thereby decreasing tissue injury and mortality.

gulfment of apoptotic cells by phagocytes. Phagocytosis prevents the release of potentially harmful or immunogenic materials from dying cells. We have shown that MFG-E8 expressions are downregulated in sepsis and I/R injury, and that this downregulation causes the impairment of clearance of apoptotic cells. Recently, however, Swan *et al.* (96) reported that during mice CLP-sepsis the capacity of splenic macrophages to engulf apoptotic cells was enhanced in the later stage of sepsis (at over 24 hours after CLP). The author suggests that this could lead to an antiinflammatory macrophage phenotype shift, thus potentially contributing to the immunosuppression observed in late sepsis. The divergence of phagocytotic activity of macrophages in sepsis between our results and Swan *et al.* (96) may be derived from the difference of the phase of sepsis (inflammatory and antiinflamma-

tory) and this may be associated with the pathophysiological complexity and the therapeutic difficulty of sepsis. We have made further significant progress toward understanding the molecular mechanisms associated with the downregulation of MFG-E8 in sepsis, which involves the LPS-CD14-TLR4 pathway. However, the corresponding mechanism of the downregulation of MFG-E8 in I/R injury has not been elucidated. It is reported that diverse TLR agonists, besides LPS, and necrotic cells downregulated the expression of MFG-E8 on antigen presenting cells *in vitro* (29). Future studies are required to investigate such issues.

On the basis of the available animal studies and clinical patients findings observed, cell death caused by apoptosis, necrosis and pyroptosis plays a significant role in the pathogenesis of sepsis and I/R injury. Direct or indirect inhibi-

tion of apoptotic cell death and/or enhancement of apoptotic cell clearance seem to be promising and interesting therapeutic strategies to prevent death of immune cells and cells that make up living organisms. However, plenty of factors including the severity, timing and types of damaged organs affect the contribution of cell death for the pathophysiology of these disease conditions. Although MFG-E8 has no direct effect on altering apoptosis, its role in the enhancement of phagocytosis of apoptotic cells and the subsequent antiinflammatory property, could be promising strategies for sepsis and I/R injury treatment in the clinical settings, but various optimizations or combinations with other molecules should be considered for the clinical applications.

Since its discovery, MFG-E8 has proven to be an essential factor for multiple physiologic systems. In the past decade, major emphasis has focused on its role for clearance of apoptotic cells in various organ systems to maintain homeostatic balance. More recently, however, MFG-E8-mediated findings of a variety of clinical, immunological, physiological and pharmacological events have been reported, where it may serve as a major player for various cell signaling events. Therefore, further studies are needed to explore other mechanisms involving the beneficial effect of MFG-E8 treatment in sepsis and I/R injury.

In conclusion, these data taken together strongly indicate that treatment with MFG-E8 reduces the morbidity and mortality associated with sepsis and I/R injury and would enrich our view for the MFG-E8-mediated therapeutic potential in several life-threatening disease conditions.

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DISCLOSURE

One of the authors (P Wang) is an inventor of the pending PCT application #WO/2006/122327: "Milk fat globule

epidermal growth factor-factor VIII and sepsis" and PCT application #WO/2009/064448: "Prevention and treatment of inflammation and organ injury after ischemia/reperfusion using MFG-E8." These patent applications cover the fundamental concept of using MFG-E8 for the treatment of sepsis and ischemia/reperfusion injury. All other authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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