Roles of Milk Fat Globule-Epidermal Growth Factor 8 in Intestinal Inflammation

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Introduction

Apoptotic cells are rapidly engulfed by phagocytes to avoid the release of numerous inflammatory mediators from dying cells. This function is essential for maintaining immune homeostasis and highly regulated by various unique molecular mechanisms. The glycoprotein milk fat globule-epidermal growth factor 8 (MFG-E8) was originally discovered as a mammalian milk fat globule membrane component. Later, it was shown that MFG-E8 binds to apoptotic cells and bridges them to phagocytes for accelerating engulfment [1]. Severe inflammatory and autoimmune consequences with abnormal homeostasis in MFG-E8 null mice are due to infiltration by apoptotic cells [2]. Recent studies have also revealed that MFG-E8 is functionally involved in the pathogenesis of sepsis, ischemia, atherosclerosis, and neurodegenerative disorders [3–6].

In addition to its scavenging function, MFG-E8 was shown to be effective in attenuating inflammation, by controlling epithelial integrity and healing of injured mucosa in the intestinal tract [7, 8]. Those functions have been suggested to be dependent not only on enhanced clearance of apoptotic cells, but also on various novel molecular mechanisms. We recently reported that MFG-E8 attenuated in-
Recent reports revealed that MFG-E8 is also ubiquitously expressed in the brain, heart, lungs, intestines, liver, and kidneys under normal physiological conditions [10–12]. We examined MFG-E8 expression in different tissues of normal BALB/c mice and observed high levels in the colon, spleen, lungs, and kidneys [9]. In each organ, MFG-E8 is expressed in a variety of cell types, including mammary epithelial cells, macrophages, splenocytes, dendritic cells, fibroblasts, vascular smooth muscle cells, glial cells, and astrocytes [13–18].

In various tissues and cells, MFG-E8 expression is tightly regulated by several factors and stimuli. Prolactin (PRL), a growth hormone, as well as insulin and steroid hormones are potent stimulators of MFG-E8 expression in their target cells [11, 19, 20]. We recently investigated the effects of PRL on MFG-E8 expression in macrophages by evaluating its promoter function [21]. Following treatment with PRL, significant up-regulation of MFG-E8 was observed in macrophages, while its effect was mediated by the presence of a responsive element of the transcription factor C/EBPβ in the MFG-E8 promoter. In addition, hormone-related regulation of MFG-E8 production, fractalkine (a CX3C chemokine), peroxisome proliferator-activated receptor (PPAR)-δ ligand, and granulocyte macrophage colony-stimulating factor (GM-CSF) have also been reported to induce MFG-E8 expression [16, 22, 23]. These factors are up-regulated in sites of inflammation in organs, suggesting that MFG-E8 may play essential roles for attenuating inflammation and regenerating injured tissues.

In contrast to the above findings, LPS is known to down-regulate MFG-E8 expression in macrophages. Komura et al. [3] used LPS-induced septic mice and found that endotoxemia decreased the endogenous levels of MFG-E8 in serum and several organs. Their findings also indicated that LPS-induced down-regulation of MFG-E8 expression in macrophages is mediated via the Toll-like receptor 4 (TLR4)/CD14 pathways.

**MFG-E8 Expression in Intestinal Tissues with Normal and Pathophysiological Stress**

As in other tissues and organs, basal levels of MFG-E8 expression have been observed in different compartments of mice gut tissues, e.g. the stomach, and small and large intestines, while that expression level in the colon was shown to be relatively higher as compared to the stomach and small intestine [9]. Furthermore, an immunohistochemical study detected MFG-E8 expression in lamina propria mononuclear cells in mice colonic sections.
On the other hand, altered MFG-E8 expression has been found during intestinal inflammation. We recently examined changes of MFG-E8 expression during dextran sulfate sodium (DSS)-induced colitis in mice [9]. In that model, MFG-E8 expression was dramatically reduced during the acute phase of the disease, while it gradually became elevated during the regeneration phase after DSS in water intake was stopped and finally returned to a normal level when the disease was abrogated. Similar time-course changes of MFG-E8 expression were also found in a trinitrobenzene sulfonic acid (TNBS)-induced colitis model [24]. In an experimental model of sepsis established by cecal ligation and puncture, MFG-E8 levels in small intestinal tissues were markedly decreased [7]. Moreover, severe injury and inflammation were induced in small intestines of mice after intestinal ischemia and reperfusion (I/R), which decreased MFG-E8 levels in the spleen and other affected tissues [25]. Thus, MFG-E8 expression is down-regulated during the acute and severe inflammatory phases of intestinal disorders.

Although the underlying mechanism of this decreased production of MFG-E8 has not been clearly revealed, the abundance of pro-inflammatory mediators and involvement of LPS/TLR4 signaling may play important roles. One recent speculation states that the increased expression of MFG-E8 in injured intestinal mucosa during the acute phase of DSS-induced colitis may be due to the extent of inflammation and/or variations in mouse strains [26]. Consistent with these findings, in the majority of stress-induced disease conditions, e.g. renal I/R, alcohol-intoxicated septic animals, and human atherosclerosis plaques, MFG-E8 expression has been found to be abruptly decreased [4, 8, 27].

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Anti-Inflammatory Effects

Based on our findings of down-regulation of MFG-E8 during DSS-induced colitis, we treated mice with recombinant MFG-E8 (rMFG) and observed its beneficial effect to inhibit intestinal inflammation (fig. 2) [9]. In addition, an anti-inflammatory effect of rMFG was shown in a mouse model of I/R-induced intestinal injuries [25]. A recent study also revealed that DSS-induced colonic inflammation in MFG-E8 null mice was more severe than that in wild-type mice [26], indicating that MFG-E8 plays a crucial role in inhibiting intestinal inflammation (fig 2). In injured intestinal mucosa of MFG-E8 null mice, infiltration of apoptotic cells was not clearly evident, suggesting that the protective role of MFG-E8 is not only due to efficient clearance of apoptotic cells, but also that this glycoprotein directly modulates innate immune functions [7]. Mice treated with rMFG-E8 at the onset of acute colitis showed significant down-regulation of the tissue contents of pro-inflammatory cytokines from inhibition of NF-κB activation [9]. During activation of the innate immune system, integrin signaling pathways are up-regulated, which then recognize potent ligands to boost the intracellular inflammatory cascade. Osteopontin (OPN) is an extracellular matrix phosphoprotein that contains the RGD domain, which is predominantly expressed in macrophages and induces the production of NF-κB-mediated inflammatory cytokines after binding to α3β3-integrin. We employed several in vitro experiments and observed that MFG-E8 reduced LPS-induced NF-κB activation by blocking OPN binding, while it also modulated α3β3-integrin-dependent downstream signaling (fig. 3). After OPN binding, activation of α3β3-integrin also results in recruitment of phosphorylated focal adhesion kinase (FAK), leading to NF-κB activation. Moreover, stimulation with LPS increases phosphorylation of FAK to enhance binding of exogenous OPN to α3β1-integrin. By targeting this pathway, MFG-E8 can reduce LPS-induced NF-κB activation by blocking OPN binding, as well as modulation of α3β3-integrin-dependent and FAK-mediated downstream sig-
MFG-E8 also competitively inhibits HMGB1-mediated intestinal tissue injury. Bu et al. [7] reported that MFG-E8 plays a crucial role in tissue regeneration during the healing process of injured colonic mucosa. They investigated whether MFG-E8 stimulates IEC migration in an in vitro wound-healing model and observed that treatment with rMFG-E8 promoted the migration of IECs by activating intracellular protein kinase C (PKC). In addition, administration of rMFG-E8 to experimental septic mice accelerated mucosal healing by binding to the transiently exposed PS receptor of the injured IECs. In DSS- and TNBS-mediated mouse colitis models, increased levels of colonic MFG-E8 were detected during the regenerating phase of colitis [9, 24], which may contribute to healing of injured colonic mucosa by promoting IEC migration. On the other hand, angiogenesis is also a crucial event for intestinal tissue regeneration. MFG-E8 binds to α3β1-integrin on endothelial cells and accelerates vascular endothelial growth factor-induced angiogenesis under physiological and pathological conditions [30], which may contribute to colonic tissue regeneration during inflammation.

Future Perspective

The crucial roles of MFG-E8 have been delineated in several animal models as well as knock-out mice studies that mimicked human intestinal disorders. Notably, MFG-E8 has been shown to have both anti-inflammatory and regenerating roles during colitis. However, most of those findings were obtained in experiments that used acute and severe intestinal inflammation models, and the precise roles of MFG-E8 in chronic gut immune disorders remain largely unknown. Moreover, there have been no studies of the expression and functions of MFG-E8 in human intestinal mucosa. On the other hand, recent findings have revealed direct roles of MFG-E8 in innate immune functions by activating regulatory T cells, and subsequent production of IL-10 and transforming growth factor-β, which may further promote immunoregulatory functions within the tissue microenvironment [31, 32]. Collectively, these results will provide direction for future investigations of MFG-E8 administration for ameliorating gut inflammatory disorders, including inflammatory bowel diseases.

Conclusion

In this review, findings regarding the various roles of MFG-E8 in intestinal tissues are presented, indicating the glycoprotein to be an essential factor for maintaining intestinal homeostasis.

Disclosure Statement

The authors declare that no financial or other conflicts of interest exist in relation to the content of the article.
References


