学 位 論 文 の 要 旨

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学 位 論 文 名　Intrarectal Administration of Milk Fat Globule Epidermal Growth Factor-8 Protein Ameliorates Murine Experimental Colitis

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論 文 内 容 の 要 旨

INTRODUCTION

The gut is a multi-layered organ in which various cell types contribute to maintain homeostasis mechanisms. Among those cell types, intestinal epithelial cells (IECs) function as the first line of defense against microbial pathogens. IECs can sense a variety of pathogen-associated molecular patterns (PAMPs) via a limited number of pattern recognition receptors (PRRs) to preserve the gut under physiological conditions. On the other hand, prolonged and uncontrolled immune activation via PRRs in IECs induce several gut immune-mediated disorders including inflammatory bowel disease (IBD). Experimental approaches regarding regulation of the innate immune function of IECs may lead to development of novel therapeutic options for IBD. Milk fat globule-epidermal growth factor 8 (MFG-E8), a glycoprotein secreted by mammary epithelial cells, activated macrophages, and immature dendritic cells (DCs), enhances engulfment of dying cells by forming a bridge between phosphatidylserine (PS) on apoptotic cells and $\alpha_v\beta_3$-integrin on phagocytes. This scavenging function avoids the release of numerous inflammatory mediators from apoptotic cells and physiologically contributes to maintain the host immune system. We recently reported that recombinant MFG-E8 protein inhibits production of inflammatory cytokines in LPS-treated macrophages, which contributed to attenuating intestinal inflammation in murine experimental colitis by modulating $\alpha_v\beta_3$-integrin signaling. We speculated that MFG-E8 directly regulates

IECs in an innate immune activated condition. In the present study, we aimed to examine the effects of MFG-E8 on IECs to determine whether it has a protective role in murine experimental colitis.

MATERIALS AND METHODS

First, we examined the gene expressions of α_v-and β_3-integrins in the mouse colonic epithelial cell line Colon-26 using RT-PCR, and evaluated their cellular localization with immunofluorescence staining. Next, we examined the direct effects of MFG-E8 on production of inflammatory cytokines in IECs. Flagellin was used as a ligand for induction of inflammatory cytokines in cultured Colon-26 cells, then IL-6 and MIP-2 levels were determined after stimulation with or without flagellin and recombinant MFG-E8 proteins in culture supernatants by EIA. Furthermore, we investigated the effects of recombinant wild-type and mutant MFG-E8 proteins (w-MFG and m-MFG) on NF-κB activity in flagellin-stimulated Colon-26 cells using a dual-luciferase assay system. Finally, we established an experimental colitis model by administrations of trinitrobenzene sulfonic acid (TNBS). Using this colitis model, we evaluated the time course changes of MFG-E8 expression, and assessed the direct effects of MFG-E8 on IECs by intrarectally injecting w- or m-MFG into mice before TNBS administration. We then measured body weight (BW), examined the appearance, weight, and length of dissected colon specimens, determined the amounts of IL-6 and MIP-2 in colonic tissues, and compared histological findings of the w-MFG-treated TNBS group with those of the m-MFG- and PBS-treated TNBS groups.

RESULTS AND DISCUSSION

In Colon 26 cells, α_v-and β_3-integrins were expressed on the cellular surface. Our stimulation study results indicated that flagellin markedly induced IL-6 and MIP-2 in the culture supernatants. While cells treated with w-MFG showed significantly decreased cytokine production, those treated with m-MFG showed no changes. Furthermore, cells treated with w-MFG showed significant down-regulation of NF-κB activity, whereas m-MFG had no significant effects on that activity. These *in vitro* findings revealed that MFG-E8 inhibits flagellin-mediated production of inflammatory cytokines in cultured IECs by modulating NF-κB

activation via $\alpha_v\beta_3$-integrin. Furthermore, no protective effect was found in mutant protein-treated cells. These results also suggest that the anti-inflammatory function of MFG-E8 is dependent on $\alpha_v\beta_3$-integrin expression in IECs, because the mutant protein targeting the RGD domain of MFG-E8 decreased its binding to $\alpha_v\beta_3$-integrin on the cells. Prior to performing our *in vivo* examinations, we evaluated the time course changes of MFG-E8 expression during TNBS-induced colitis. MFG-E8 was down-regulated during the acute and severe inflammatory phases of colitis, while it became up-regulated during the healing phase. Based on these results we designed the *in vivo* study protocol. To assess the direct effects of MFG-E8 on IECs, w- or m-MFG was intrarectally injected into mice before TNBS administration, while other mice were injected with PBS instead of those proteins. In mice that received TNBS with PBS or m-MFG, BW loss was clearly observed, while those injected with w-MFG showed significantly lower amounts of BW loss. Colon specimens dissected from the w-MFG-treated group had increased length and decreased weight as compared to those dissected from the PBS- and m-MFG-treated TNBS groups. Histological examinations of inflammation, extent of injury, and crypt damage also revealed significantly decreased levels in the w-MFG-treated colitis model mice. In addition, our evaluation of colonic tissues showed that treatment with w-MFG down-regulated the tissue contents of IL-6 and MIP-2 as compared to those in PBS- and m-MFG-treated colitis model mice. Together, there results indicate a therapeutic benefit of intrarectal administration of w-MFG for intestinal inflammation. In the present study, we focused on $\alpha_v\beta_3$-integrin expression in IECs and confirmed its association with the anti-inflammatory role of MFG-E8.

CONCLUSION

MFG-E8 inhibits flagellin-mediated production of inflammatory cytokines in cultured IECs by modulating NF-κB activation via $\alpha_v\beta_3$-integrin, while intrarectal administration of recombinant MFG-E8 protein ameliorates TNBS colitis mice. Our results show an anti-inflammatory role of MFG-E8 in IECs as well as its therapeutic potential for intestinal inflammation.