学 位 論 文 の 要 旨

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学　位　論　文　名	Down-regulation of Single Immunoglobulin Interleukin-1R-related molecule (SIGIRR)/TIR8 Expression in Intestinal Epithelial Cells during Inflammation
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INTRODUCTION

The monolayer of intestinal epithelial cells (IECs) acts as the first line of defense against gut luminal microbial pathogens. IECs can recognize conserved pathogen-associated molecular patterns (PAMPs) via several kinds of pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). TLR signaling in IECs induces innate immune responses, which regulate the gut under physiological and pathological conditions. On the other hand, several negative regulatory mechanisms control TLR-mediated inflammatory responses, and maintain a balance between activation and inhibition of the innate immune system. Single immunoglobulin (Ig) interleukin (IL)-1-related receptor/Toll IL-1 receptor 8 (SIGIRR/TIR8), one of the transmembrane negative regulators of TLR4 signaling, has a single extracellular Ig domain and intracellular TIR domain, and is expressed in a variety of cells, such as epithelial cells, dendritic cells, macrophages, fibroblasts, and endothelial cells. Overexpression of SIGIRR inhibits TLR4-induced NF-κB activation and attenuates the production of inflammatory cytokines in

vitro. Although the role of SIGIRR was recently demonstrated in mice experimental colitis models, little is known regarding the regulation mechanism of SIGIRR expression in IECs during inflammation. In the present study, we investigated SIGIRR expression in colonic mucosa with or without inflammation, as well as its regulation mechanism in IECs.

MATERIALS AND METHODS

First, we examined SIGIRR expression in human colonic mucosa with and without inflammation. Endoscopic biopsy specimens were taken from active and inactive colonic mucosa of patients with ulcerative colitis (UC), and SIGIRR expression was examined by real-time PCR and immunohistochemistry (IH). Next, an experimental colitis mouse model was established by administrations of trinitrobenzene sulfonic acid (TNBS) and dextran sodium sulphate (DSS), and colonic expression time-course changes of SIGIRR, tumor necrosis factor (TNF)-α, TLR-4, and IL-1-receptor-associated kinase (IRAK)-M were examined using real-time PCR. We also determind the localization of SIGIRR expression in the colonic tissues of mice with or without DSS colitis using IH. Furthermore, we investigated SIGIRR expression in epithelial cells isolated from normal and DSS colitis mice using flow cytometry.

We also employed several in vitro experiments to evaluate SIGIRR expression and functions in the colon epithelial cell line SW480 following treatments with LPS and TNF-α.

Changes in SIGIRR expression after stimulation with LPS and TNF-α in SW480 cells were evaluated using real-time PCR. In addition, we transiently silenced endogenous SIGIRR expression in SW480 cells using siRNA, and then confirmed the responsiveness of the cells to LPS and TNF-α in terms of elevated production of IL-8 and NF-κB, by using enzyme immunoassay and luciferase assays. Finally, to elucidate SIGIRR expression regulation in IECs, the binding ability of the potent transcription factor SP1 at the proximal-end responsive element of the SIGIRR promoter was examined using gel-shift and ChIP assays.

RESULTS AND DISCUSSION

In human colonic samples, SIGIRR was mainly expressed in IECs at levels significantly

higher in inactive as compared to active mucosa. In the model mice, SIGIRR colonic expression rapidly decreased after colitis development and gradually returned to basal levels. Experimental colitis-mediated down-regulation of SIGIRR in IECs was also clearly confirmed by results of IH and flow cytometry. In contrast to the expression of SIGIRR, that of IRAK-M, an intracellular negative regulator, was significantly up-regulated in inflamed colonic epithelial cells. These findings indicate that SIGIRR expression and its role may be different from those of inducible intracellular negative regulators in the gut innate immune system. Constitutive expression of SIGIRR in gut surface epithelium may regulate the gut innate immune balance under physiological conditions, whereas intracellular negative regulators may be induced after breakdown of the first-line defense system that is regulated by SIGIRR.

Our *in vitro* results demonstrated that gene knockdown using siRNA for SIGIRR accelerated IL-8 production in LPS-stimulated SW480 cells via activation of NF-κB. Furthermore, inflammatory conditions induced by LPS and TNF-α caused significant down-regulation of SIGIRR expression in SW480 cells. These findings clearly support the results of our *in vivo* experiments indicating that SIGIRR expression in colonic epithelial cells functions as a negative regulator, and is down-regulated during inflammation. Although LPS- and TNF-α-dependent decreased expression of SIGIRR has been noted in several studies, the underlying mechanism of that downregulation has not been established. Interestingly, we noted the presence of an SP1 binding site at the proximal end of the human SIGIRR promoter. Also, results of gel-shift and luciferase assays clearly revealed decreased SP1 binding at the responsive element of the SIGIRR promoter in LPS-treated SW480 cells, which may regulate the expression of SIGIRR under inflammatory conditions.

CONCLUSION

SIGIRR is constitutively expressed in IECs and serves as a negative regulator to maintain gut innate immunity, which is down-regulated during inflammation by inhibition of an SP1-mediated pathway.