学 位 論 文 の 要 旨

氏名　　大　嶋　直　樹

学　位　論　文　名	A20 Is an Early Responding Negative Regulator of Toll-Like Receptor 5 Signaling in Intestinal Epithelial Cells During Inflammation
発　表　雑　誌　名 （巻，初頁～終頁，年）	Clinical & Experimental Immunology（**159**: 185~198, 2010）
著　者　名	Naoki Oshima, Shunji Ishihara, MAK Rumi, MM Aziz, Yoshiyuki Mishima, Chikara Kadota, Ichiro Moriyama, Norihisa Ishimura, Yuji Amano, Yoshikazu Kinoshita

INTRODUCTION

The nnate immune system recognizes conserved pathogen-associated molecular patterns (PAMPs) via a limited number of pattern recognition receptors (PRRs). The family of Toll-like receptors (TLRs) is an important PRR group and TLR signaling in epithelial cells immediately induces innate immune responses, which function to preserve the gut under physiological and pathological conditions. Furthermore, TLR-mediated nuclear factor kappa B (NF-κB) activation induces inflammatory responses. To maintain a balance between activation and inhibition of the innate immune system, a variety of negative regulatory mechanisms control TLR-mediated cellular signaling. Some of these are present constitutively to control TLR activation at a physiological level, whereas others are up-regulated by TLR signaling during inflammation to attenuate TLR responses in a negative feedback loop. The zinc-finger protein A20, a ubiquitin-modifying enzyme, is an inducible and broadly expressed cytoplasmic protein that inhibits tumor necrosis factor (TNF)-α-induced NF-κB activity. A20 overexpression inhibits

TLR-induced NF-κB activation and attenuates production of inflammatory cytokines *in vitro*, thus it has been suggested to be an intracellular negative regulator for downstream signalling of TNF receptor-associated factor (TRAF) 6 and NF-κB translocation in TLR-signalling pathways. However, though TLR-dependent activation of NF-κB may play a vital role in maintaining epithelial homeostasis, as well as regulating infection and inflammation in the gut, little is known regarding the expression of A20 in intestinal epithelial cells (IECs) during inflammation. In the present study, we investigated TLR-mediated A20 expression and its role in IECs, and compared the results to those of other TLR negative regulators, including IL-1-receptor-associated kinase (IRAK)-M and Toll-interacting protein (Tollip).

MATERIALS AND METHODS

Two human colorectal cancer cell lines, HCT15 and HT29 cells, were stimulated with flagellin and lipopolysaccharide (LPS), then the expressions of A20, IRAK-M, and Tollip were evaluated using RNase protection assays. In addition, flagellin-induced CXCL2 and A20 expressions in colonic epithelial cells from BALB/c mice were examined using a primary culture system and real-time PCR. For this, TLR-5 blocking peptide was injected into BALB/c mice at the onset of DSS-induced inflammation, then A20 expression in colonic tissue was evaluated by real-time PCR at 5 and 7 days after beginning DSS administration. Furthermore, experimental colitis was induced in *tlr4*-deficient CH3/HeJ mice by administration of DSS, then flagellin was anally injected, and the colonic expression of A20 was examined by real-time PCR and immunohistochemistry. To confirm flagellin-induced expression of A20, we employed an organ culture system using colonic tissues dissected from C3H/HeJ mice. Cultured tissues were stimulated with flagellin, after which the gene expressions of A20 and TNF-α were examined using real-time PCR. The role of A20 in flagellin-induced tolerance induction was evaluated *in vitro*, using a gene knockdown method targeting A20.

RESULTS AND DISCUSSION

The expressions of A20, IRAK-M, and Tollip were increased in ligand-stimulated IECs, which were clearly related to the levels of cellular NF-κB activity and IL-8 production. The gene expression of A20 rapidly increased and peaked at 1 hour after ligand stimulation, then gradually declined thereafter, whereas the expressions of IRAK-M and Tollip gradually increased. The flagellin-induced expression of A20 in mouse primary cultured epithelial cells also rapidly increased and peaked at 1 hour after flagellin stimulation, after which the time-course change of expression was similar to that found in previous experiments with human cell lines. In our mouse experimental colitis model, A20 expression was significantly increased from days 5 to 9 after administration of DSS, and clearly inhibited by intraperitoneal injection of TLR-5-blocking peptide. Anal injection of flagellin rapidly induced the expression of A20 as well as TNF-α in DSS-treated injured colonic tissues, which was confirmed *in vitro* using an organ culture system. In addition, immunohistochemistry results clearly revealed abundant flagellin-induced expression of A20 in epithelial cells, as the level rapidly increased and peaked at 1 hour after flagellin stimulation in colonic tissues injured by DSS administration, whereas there was no significant increase of A20 expression in non-injured normal tissues. The time-course changes of flagellin-induced TNF-α expression showed a pattern similar to that of A20. Although prior treatment with flagellin significantly decreased IL-8 production by IECs, A20 gene knockdown did not influence tolerance induced by pre-stimulation with flagellin.

CONCLUSION

Our examination of the expression of A20 in IECs stimulated by TLR ligands showed that A20 is an early response negative regulator of TLR5 signaling in IECs that functions in the early phase of intestinal inflammation. These results provide new insights into the negative feedback regulation of TLR signaling that maintains the innate immune system in the gut.