学 位 論 文 の 要 旨

氏名　李　永宇

学　位　論　文　名　Autophagy Is Required for Toll-Like Receptor-Mediated Interleukin-8 Production in Intestinal Epithelial Cells

発　表　雑　誌　名　International Journal of Molecular Medicine
巻，初頁～終頁，年　（**27**: 337~344, 2011）

著　　者　　名　Yong-Yu Li, Shunji Ishihara, Md. Monowar Aziz, Akihiko Oka, Ryusaku Kusunoki, Yasumasa Tada, Takafumi Yuki, Yuji Amano, Mesbah Uddin Ansary, Yoshikazu Kinoshita

論 文 内 容 の 要 旨

INTRODUCTION

Autophagy is an evolutionarily conserved process that maintains cellular homeostasis via synthesis, degradation, and subsequent recycling of cellular products under various physiologic conditions. Recent studies have also revealed a variety of roles for autophagy in the regulation of cell death, differentiation, and anti-microbial responses. Innate immunity is triggered by pattern recognition receptors (PRRs) that sense pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS), flagellin, peptidoglycans, and bacterial DNA. The Toll-like receptor (TLR) family, an important class of PRRs, is well known to induce expressions of various inflammatory genes in response to microbial components, which regulate the balance between activation and inhibition of the innate immune system. Although TLR-dependent induction of autophagy has been noted in numerous studies, the role of autophagy in the gut innate immune system remains largely unknown. A link between autophagy and the innate immune system was revealed by the discovery that intracellular pathogens can be eliminated from cells via a TLR-induced autophagy pathway, which may help to maintain normal homeostasis during pathogen infection. To understand the pathogenesis of innate immune-related gut disorders, it is considered important to clarify the crosstalk that occurs between the TLR-mediated pathway and autophagy in intestinal epithelial cells (IECs), which respond directly to luminal microbial components. In the present study, we evaluated the induction of TLR-mediated

autophagy in IECs and compared it to that in macrophages, as well as its relationship to the production of interleukin (IL)-8.

MATERIALS AND METHODS

The human colorectal cancer cell line HCT-15, human monocytic leukemia cell line THP-1, rat small intestine epithelial cell line IEC-6, and mouse macrophage cell line RAW264.7 were cultured with or without various TLR ligands, then the expressions of pro-inflammatory cytokines (IL-8, CINC-2β, MIP-2) were determined using real-time PCR and ELISA. To reveal the status of autophagy in IECs and macrophages, LC3-II expression was examined using western blotting and immunofluorescence with confocal microscopy, with rapamycin used as a positive control for autophagy induction. Also, to evaluate the influence of TLR ligands on autophagy-mediated innate-immune response, ATG7 specific siRNA was transfected into intestinal epithelial cells and the efficiency of target gene knockdown was assessed using real-time PCR. In addition, the efficacy of Atg7 gene knockdown on autophagy induction and IL-8 secretion were assessed by western blotting for the detection of LC3-II and p62, for autophagy, and EIA for IL-8. Furthermore, a non-radioactive cell proliferation assay kit was used to assess cell viability after treatment with Atg7 siRNA. Values were analyzed using Student's *t* test with SPSS software version 10.1. For comparisons of multiple values, ANOVA was used. *P* values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Macrophage cells (RAW264.7, THP1) treated with TLR ligands significantly induced the expressions of MIP-2 and IL-8. On the other hand, though stimulation with flagellin and Pam2csk4 markedly induced CINC-2β and IL-8 in IEC-6 and HCT-15 cells, respectively, those cells showed relatively lower levels in response to stimulation with LPS. Stimulation with LPS and Pam2CSK, but not flagellin, significantly increased cellular LC3-II protein levels in RAW264.7 and THP1 cells, showing that TLR4 and TLR2 are essential sensors for autophagy induction in macrophages. In contrast, HCT-15 and IEC-6 cells had high basal levels of LC3-II protein in the absence of TLR ligand incubation, which were not changed following stimulation with the TLR ligands. Furthermore, confocal microscopy revealed significant LC3-II immunoreactive signals, indicating the presence of autophagosomes in the

cytoplasm. Thus, the basal levels of LC3-II were markedly higher in IECs as compared to those in macrophages. Our findings indicated that autophagy induction following TLR ligand stimulation was not significantly evident in IECs as compared to that in macrophages. We also found that Pam2CSK and flagellin markedly stimulated proinflammatory cytokines in HCT-15 and IEC-6 cells. However, those TLR ligands did not have an influence on autophagy induction in those cells. Together, these results suggest that basal autophagy and that induced by TLR-mediated induction occur in a cell type- and TLR ligand-dependent manner.

Gene knockdown of Atg7 did not influence cell viability, however, TLR ligand-induced IL-8 secretion by Atg7 siRNA-treated cells was significantly lower than that by control siRNA-treated HCT-15 cells. Furthermore, though rapamycin had no influence on cell viability, after stimulation with TLR ligands, IL-8 mRNA expression and IL-8 contents in the culture supernatants of rapamycin-treated cells were significantly increased as compared to the control cells. Those findings support our results and suggest the possibility that autophagy is required for production of inflammatory cytokines in response to a variety of stimuli, including TLR ligands, in epithelial cells. Mucosal neutrophil response is induced when bacteria stimulate epithelial cells in various organs to secrete several chemokines such as IL-8. Thus, we consider that autophagy has various essential roles in gut immunity, which may be dependent on cell type and the type of autophagy-regulatory genes involved. Taken together, autophagy may have an essential role to maintain innate immune response by regulating inflammatory cytokine production in IECs.

CONCLUSION

In summary, we investigated the induction of TLR-mediated autophagy in IECs and the role of autophagy in IL-8 production by TLR-activated IECs. Our results showed that IECs have a high basal level of autophagy, which essentially regulates TLR-mediated IL-8 production in those cells. Autophagy may be an important intracellular machinery for inducing the innate immune system in IECs. These findings provide new insight into the crosstalk that occurs between autophagy and TLR signaling in IECs.