Loss of the Autophagy Protein Atg16L1 Enhances Endotoxin-induced IL-1beta Production
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LETTERS

Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production

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Systems for protein degradation are essential for tight control of the inflammatory immune response[1,2]. Autophagy, a bulk degradation system that delivers cytoplasmic constituents into autolysosomes, controls degradation of long-lived proteins, insoluble protein aggregates and invading microbes, and is suggested to be involved in the regulation of inflammation[3–5]. However, the mechanism underlying the regulation of inflammatory response by autophagy is poorly understood. Here we show that Atg16L1 (autophagy-related 16-like 1), which is implicated in Crohn's disease[6,7], regulates endotoxin-induced inflammasome activation in mice. Atg16L1-deficiency disrupts the recruitment of the Atg12-Atg5 conjugate to the isolation membrane, resulting in a loss of microtubule-associated protein 1 light chain 3 (LC3) conjugation to phosphatidylethanolamine. Consequently, both autophagosome formation and degradation of long-lived proteins are severely impaired in Atg16L1-deficient cells. Following stimulation with lipopolysaccharide, a ligand for Toll-like receptor 4 (refs 8, 9), Atg16L1-deficient macrophages produce high amounts of the inflammatory cytokines IL-1β and IL-18. In lipopolysaccharide-stimulated macrophages, Atg16L1-deficiency causes Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF)-dependent activation of caspase-1, leading to increased production of IL-1β. Mice lacking Atg16L1 in haematopoietic cells are highly susceptible to dextran sulphate sodium-induced acute colitis, which is alleviated by injection of anti-IL-1β and IL-18 antibodies, indicating the importance of Atg16L1 in the suppression of intestinal inflammation. These results demonstrate that Atg16L1 is an essential component of the autophagic machinery responsible for control of the endotoxin-induced inflammatory immune response.

Autophagy is a bulk degradation system, which controls the clearance and re-use of intracellular constituents, and is important for the maintenance of an amino acid pool essential for survival[3–5]. In addition, recent studies have disclosed multiple roles of autophagy in the regulation of cell death, differentiation and anti-microbial response in mammals[4,5]. Yeast genetic screening studies have identified a variety of essential components of autophagic machinery, called Atg proteins, which are phylogenetically highly conserved, and several mammalian counterparts, such as Atg5 and Atg7, have been reported[3–5]. Previously, we systematically characterized mammalian homologues of Atg proteins and identified Atg16L1 protein as an Atg5-binding protein[10]. Its coiled-coil domain, which mediates self-multimerization, is essentially required for starvation-induced autophagy in yeast, and this domain is conserved in mammalian Atg16L1 (refs 3, 10; Fig. 1a). We have proposed that the coiled-coil domain of Atg16L1 is required for the formation of an ~800 kDa high molecular weight protein complex with the Atg12-Atg5 conjugate and defines the site where LC3 (homologue of yeast Atg8) is conjugated to phosphatidylethanolamine (PE), an essential process for autophagy, by recruitment of an Atg3-LC3 intermediate to a source membrane of an autophagosome[10,11]. In addition, Atg16L1 has seven WD40 repeats at the carboxy terminus, which are absent in yeast Atg16 (ref. 10). Recent genome-wide association studies identified Atg16L1 as a candidate gene responsible for susceptibility to Crohn's disease[6,7]. However, the importance of Atg16L1 in autophagy and its role in inflammation have not been fully understood. Hence, we generated Atg16L1 mutant mice and examined the function of Atg16L1 in autophagosome formation as well as in the regulation of immune responses.

Atg16L1 mutant mice express deleted forms of Atg16L1 protein lacking the entire coiled-coil domain (Fig. 1a, b, and Supplementary Fig. 1a–c). However, such aberrant proteins do not act as dominant-negative molecules, because ectopic expression of truncated Atg16L1 protein lacking the coiled-coil domain (ΔCCD) in wild-type mouse embryonic fibroblasts (MEFs) did not interfere with autophagy (Supplementary Fig. 2a, b). Most Atg16L1-deficient mice died within 1 day of delivery, indicating that Atg16L1 is required for survival during neonatal starvation (Supplementary Fig. 1d, e). This phenotype is similar to that observed in Atg5- or Atg7-deficient mice[12,13]. Although Atg16L1 associates with Atg12-Atg5, Atg16L1 was dispensable for Atg12 conjugation to Atg5 (Fig. 1b). On the other hand, Atg16L1 was required for LC3 conjugation to PE (Fig. 1b). In Atg16L1-deficient MEFs, formation of the high molecular weight protein complex was disrupted and Atg12-Atg5 puncta were hardly observed (Fig. 1c, d, and Supplementary Fig. 3, 4a). On the other hand, GFP-Atg5 free from Atg12-conjugation formed puncta in Atg7-deficient MEFs or Atg5-deficient MEFs complemented with GFP-Atg5^{K130R}, although these puncta did not colocalize with LC3 (Fig. 1c, d, Supplementary Figs 4b, 5, data not shown). Formation of autophagosomes under the starved condition was not observed in Atg16L1-deficient MEFs, resulting in a decrease in the bulk degradation of long-lived proteins and the accumulation of p62/SQSTM1 (Fig. 1b–f). These results indicated that Atg16L1 is essentially required for autophagy by regulating the localization of the Atg12-Atg5 conjugate.

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Introduction

Autophagy is a bulk degradation system, which controls the clearance and re-use of intracellular constituents, and is important for the maintenance of an amino acid pool essential for survival[1-2]. In addition, recent studies have disclosed multiple roles of autophagy in the regulation of cell death, differentiation and anti-microbial response in mammals[2]. Yeast genetic screening studies have identified a variety of essential components of autophagic machinery, called Atg proteins, which are phylogenetically highly conserved, and several mammalian counterparts, such as Atg5 and Atg12, have been reported[1-2]. Previously, Yoshimori and colleague identified Atg16-like 1 (Atg16L1) protein as an Atg12-Atg5 conjugate-binding protein[3]. Coiled-coil domain of Atg16 is essentially required for starvation-induced autophagy in yeast, and this domain is conserved in mammalian Atg16L1. In addition, Atg16L1 has seven WD40 repeats at the C-terminus, which are absent in yeast Atg16[3]. Recent genome-wide association studies identified Atg16L1 as a candidate gene responsible for susceptibility to Crohn's disease[4]. However, role of Atg16L1 in inflammation have not been fully understood. Hence, we generated Atg16L1 mutant mice and examined the function of Atg16L1 in autophagosome formation as well as in the regulation of immune responses.

Essential role of Atg16L1 for autophagy

Most Atg16L1-deficient mice died within 1 day of delivery, indicating that Atg16L1 is required for survival during neonatal starvation. In Atg16L1-deficient MEFs, LC3 conjugation to PE was hardly observed. Formation of autophagosomes under the starved condition was not observed in Atg16L1-deficient MEFs, resulting in a decrease in the bulk degradation of long-lived proteins and the accumulation of p62 (Fig.1 and Fig.2). These results indicated that Atg16L1 is essential for autophagy.

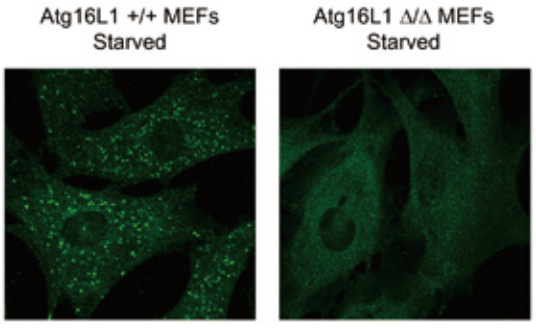

Fig. 1 Absence of LC3 puncta in Atg16L1-deficient MEFs.

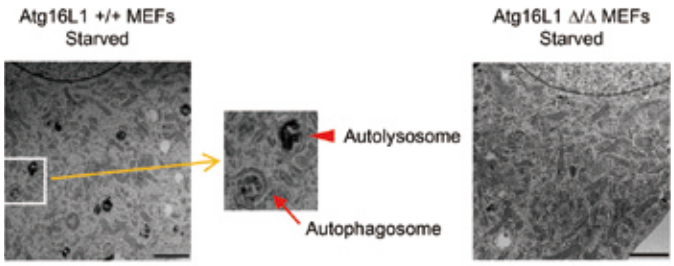

Fig. 2 Atg16L1 is required for the starvation-induced formation of autophagosomes.

Enhanced endotoxin-induced IL-1β production by Atg16L1-deficiency

We examined the role of Atg16L1 in the production of inflammatory cytokines induced by LPS, a major component of bacterial endotoxin. Although production of TNFα and IL-6 were almost normal in Atg16L1-deficient macrophages, IL-1β production was highly elevated compared with that in wild-type macrophages (Fig.3). The expression levels of immature IL-1β protein following LPS stimulation in Atg16L1-deficient macrophages were almost comparable with those in wild-type cells, indicating an abnormality of post-translational regulation. Cleaved capase-1, an activated form that mediates processing of IL-1β, was detected in the culture supernatants of Atg16L1-deficient macrophages following LPS stimulation, and was responsible for the production of IL-1β. Among TLR family members, TLR2, TLR4 and TLR5 recognize bacterial components and play important roles in the anti-bacterial response. Importantly, TLR4 ligand, but not ligands for TLR2 or TLR5, induced potent IL-1β production from Atg16L1-deficient macrophages. These findings prompted us to assess the

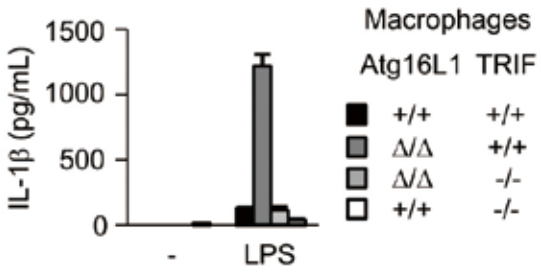

Fig. 3 Loss of Atg16L1 enhances TRIF-dependent IL-1β production by LPS.

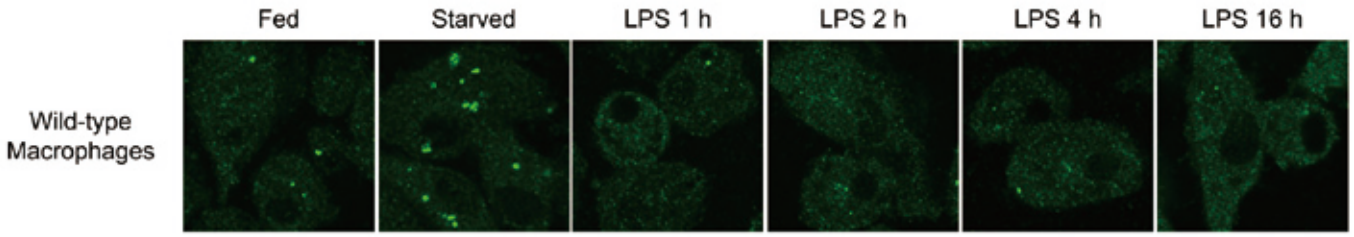

Fig. 4 LPS does not induce formation of autophagosomes.

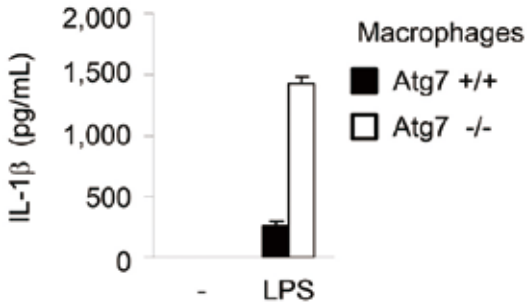

Fig. 5 Enhanced LPS-induced IL-1β production by Atg7-deficient macrophages.

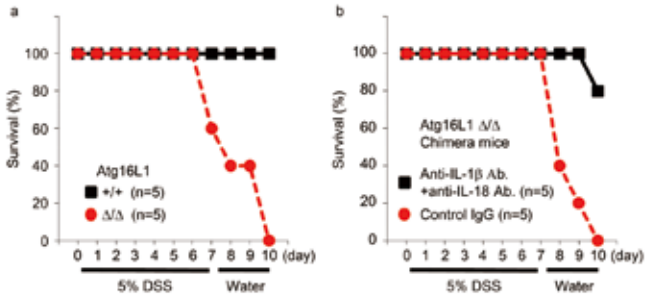

Fig. 6 Severe DSS-induced colitis in Atg16L1-deficient chimeric mice.

involvement of the TRIF signaling, which is strongly triggered by the engagement of TLR4 in macrophages. Consistent with the hypothesis, Atg16L1/TRIF double-deficient macrophages failed to produce IL-1β due to a lack of caspase-1 activation in response to LPS (Fig.3).

Loss of basal autophagy enhances endotoxin-induced IL-1β production

Increasing evidence has revealed that basal autophagy plays critical roles under both physiological and pathological conditions, including neurodegeneration, hepatic dysfunction and the immune response[2]. In Atg16L1-deficient macrophages, autophagosomes were hardly detected and p62 protein was accumulated under nutrient-rich conditions, indicating that basal autophagy is almost completely inhibited. LPS stimulation did not induce formation of autophagosomes in macrophages (Fig.4). Atg7-deficient macrophages also produced high levels of IL-1β in response to LPS, but produced normal levels of IL-6 (Fig.5). These results indicate that inhibition of basal autophagy induces IL-1β overproduction.

Severe DSS-induced colitis in Atg16L1-deficient chimeric mice

Aberrant expression of inflammatory cytokines, including IL-1β and IL-18, has been shown to be involved in the development of colitis[5-6]. We next assessed if Atg16L1-deficiency exacerbates inflammation in a DSS-induced experimental model of colitis. Chimeric mice with Atg16L1-deficient hematopoietic cells died together with severe body weight loss following seven days of DSS exposure, whereas chimeric mice expressing wild-type Atg16L-1 survived (Fig.6). The levels of the proinflammatory cytokines IL-1β and IL-18 were significantly elevated in the sera of DSS-treated Atg16L1-deficient chimeric mice relative to the levels in wild-type counterparts. Mortality and loss of body weight after DSS-exposure in Atg16L1-deficient chimeric mice were improved by the injection of neutralizing antibodies for IL-1β and IL-18, showing the involvement of excessive production of these cytokines in the development of severe colitis.

Conclusion

Our present study highlights a novel role of autophagy in the regulation of the inflammatory response. Loss of autophagy enhances endotoxin-induced inflammasome activation leading to production of the inflammatory cytokines IL-1β and IL-18. Given the importance of elevated expression of IL-1β and IL-18 caused by Atg16L1 deficiency in the pathology of chemical-induced colitis, it would be of interest to examine the involvement of autophagy in the pathogenesis of Crohn's disease.

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