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Atg5 regulates formation of MyD88 condensed structures and MyD88-dependent signal transduction

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ABSTRACT

MyD88 is known as an essential adaptor protein for Toll-like receptors (TLRs). Previous studies have shown that transfected MyD88 forms condensed structures in the cytoplasm. However, upon TLR stimulation, there is little formation of endogenous MyD88 condensed structures. Thus, the formation of MyD88 condensed structures is tightly suppressed, but the mechanism and significance of this suppression are currently unknown. Here we show that Atg5, a key regulatory protein of autophagy, inhibits the formation of MyD88 condensed structures. We found that endogenous MyD88 had already formed condensed structures in Atg5-deficient cells and that the formation of condensed structures was further enhanced by TLR stimulation. This suppressive effect of Atg5 may not be associated with autophagic processes because MyD88 itself was not degraded and because TLR stimulation did not induce LC3 punctate formation and LC3 conversion. Immunoprecipitation analysis revealed that Atg5 could interact with MyD88. Furthermore, Atg5 deficiency increased formation of the MyD88–TRAF6 signaling complex induced by TLR stimulation, and it enhanced activation of NF-κB signaling but not MAPKs and Akt. These findings indicate that Atg5 regulates the formation of MyD88 condensed structures through association with MyD88 and eventually exerts a modulatory effect on MyD88-dependent signaling.

1. Introduction

MyD88 works as an important signaling adaptor molecule not only in Toll-like receptors (TLRs) but also in interleukin (IL)-1 receptor (IL-1R) [1–3]. After receptor ligation, MyD88 forms a signaling complex, so-called 'Myddosome', through recruitment of IL-1R-associated kinases (IRAK)-4 and IRAK-1/2 [4]. Myddosome activates downstream signaling through interaction with tumor necrosis factor receptor-associated factor 6 (TRAF6), which serves as both of an E3 ubiquitin ligase and a signaling scaffold to catalyze polyubiquitination of target proteins [5] or to synthesize free polyubiquitin chains [6]. The action of TRAF6ultimately activates transforming growth factor β-activated kinase 1 (TAK1)-mediated nuclear factor (NF)-κB signaling and mitogen-activated protein kinase (MAPK) signaling cascades, which leads to the transcription of a wide variety of genes involved in innate and adaptive immunity [7,8].

Many research groups have revealed the signaling function of MyD88 in TLRs/IL-1R, whereas several groups, including our group, have shown characteristic subcellular localization of MyD88. Transfected MyD88 results in formation of condensed structures in the cytoplasm [9–13]. Condensed structures of MyD88 have generally been regarded as signaling complexes or myddosomes because major signaling molecules are found within the structures [9,10]. However, upon TLR stimulation,endogenous MyD88 hardly forms such condensed structures [13]. Therefore, the formation of MyD88 condensed structures is strictly suppressed, but the mechanism and significance of this suppression have not yet been clarified.

Autophagy sequestrates intracellular constituents within autophagosomes, followed by delivery to lysosomes, in response to nutrient starvation and other metabolic stresses [14,15]. Autophagosome formation proceeds through a series of stages, including nucleation of the isolation membrane and elongation and closure of the membrane [14,15]. Several essential components of the autophagic machinery, so-called autophagy-related proteins (Atgs), have been identified by genetic screening in yeast [16,17].Most of the Atgs are highly conserved in mammalian cells [18]. Analysis of Atgs has revealed two ubiquitin-like conjugation systems required for autophagosome formation [19]. One of these systems mediates the conjugation of Atg5–Atg12. Formation of the Atg5–Atg12 conjugate facilitates activation of the other conjugation system, which induces lipidation of LC3 (mammalian homolog of Atg8). The protein LC3 is proteolytically cleaved by Atg4 to generate cytosolic LC3-I, which subsequently conjugates with phosphatidylethanolamine to form membrane-associated LC3-II.

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The Atg5–Atg12 conjugate and LC3-II localize to the isolation membrane throughout its elongation process [19]. The Atg5–Atg12 conjugate finally dissociates from the membrane upon completion of autophagosome formation, while LC3-II remains on the isolation membrane and is regarded as a key marker for the autophagosome [14].

In this study, we investigated the mechanism involved in the suppression of MyD88 condensed structure formation. Here we show that Atg5 suppresses the formation of MyD88 condensed structures. Subsequently, this suppression leads to inhibition of formation of the MyD88–TRAF6 complex induced by TLR stimulation and control of MyD88-mediated signaling.

2. Materials and methods

2.1. Reagents, antibodies and DNA constructs

The synthetic bacterial triacylated lipopeptide Pam_3CSK_4 and highly purified *Escherichia coli* LPS were purchased from Invivogen. The *Streptomyces* product coumermycin A1 was purchased from Sigma–Aldrich. Anti-MyD88 rabbit polyclonal antibody (ab2064) was obtained from Abcam. Anti-Flag M2 monoclonal antibody (F3165) was purchased from Sigma–Aldrich. Phosphorylation-specific antibodies to p38 (4631), JNK (4671), ERK (4376), Akt Thr308 (4056) and NF-κB p65 (3033) and antibodies to p38 (9212), JNK (9258), ERK (9102), IκBα (9242) and Atg5 (8540) were obtained from Cell Signaling Technology. Rabbit polyclonal antibodies to TRAF6 (sc-7221), Akt1/2/3 (sc-8312), β-actin (sc-8432) and HA (sc-805) were purchased from Santa Cruz Biotechnology. Anti-LC3 monoclonal antibodies (M115-3 and M152-3) were obtained from MBL.

The expression plasmid for an *N*-terminal Flag epitope-tagged mouse MyD88 (Flag-MyD88) was previously described [20]. The plasmid encoding Flag-MyD88 *C*-terminally fused to *E. coli* DNA gyrase subunit B (Flag-MyD88-GyrB) was previously described [13]. The expression plasmid for hemagglutinin epitope (HA)-tagged Atg5 was a kind gift from N. Mizushima (Tokyo University, Tokyo, Japan). Transfection of plasmids into cells was performed using Lipofectamine 2000 reagent (Life Technologies).

2.2. Cell culture

Atg5-knockout (KO) mouse embryonic fibroblasts (MEFs) [21] and wild-type control MEFs were kindly provided by N. Mizushima. Human embryonic kidney (HEK) 293T cells were cultured under normal conditions as described previously [22]. Wild-type and Atg5 KO MEFs expressing Flag-MyD88-GyrB were generated by stable transfection of the plasmid and were maintained in media containing 1 mg/ml G418 (Roche).

2.3. Microscopy and image analysis

For immunofluorescence microscopy, cells were seeded on Lab-Tek chamber 4-well permanox slides (Nunc) and fixed at −20 °C with methanol for 30 min. Immunostaining was then carried out with a primary antibody and Alexa488-conjugated secondary antibody (Life Technologies). Fluorescent images were obtained as described previously [13]. The percentage of cells with more than 10 microscopy-visible condensed structures of MyD88 was quantified by counting cells in 10 different images of microscopic fields that included at least 15 cells. Results, shown as means ± standard deviation (SD), are representative of three independent experiments.

2.4. Immunoblotting and densitometry analysis

Cells seeded on 6-well plates were lysed with 250 μl of lysis buffer [13] at 4 °C for 15 min. The lysates were mixed with 2 × SDS sample buffer containing 2-mercaptoethanol, boiled, separated on 10–20% gradient SDS–PAGE, and then transferred to Immobilon-P transfer membranes (Millipore). The membranes were blocked in 5% skim milk in PBS. Immunoreactive bands were detected using the antibodies described above and visualized as described previously [13]. Densitometric quantification of the immunoblot bands was performed using an Epson ES-H7200 scanner and ImageJ densitometry software (Version 1.6, National Institutes of Health). Values of the bands of phosphorylated proteins were normalized to the total levels of respective proteins and expressed as relative values.

2.5. Immunoprecipitation

HEK293T cells seeded on 6-well plates were transfected with the plasmids encoding Flag-MyD88 together with the plasmids encoding HA-Atg5. Cells were lysed with 250 μl of lysis buffer [13] at 4 °C for 15 min. After clarification by centrifugation at 15,000×*g* for 10 min, cell lysates were immunoprecipitated using 25 μl anti-Flag M2 agarose (Sigma–Aldrich) for 1 h at 4 °C on a rotating platform. The beads were washed four times with 1 ml of lysis buffer, boiled with SDS sample buffer containing 2-mercaptoethanol, and subjected to immunoblotting using the indicated antibodies. Immunoprecipitation of Flag-MyD88-GyrB was performed as described previously [13]. Results are representative of three separate experiments.

2.6. Statistical analysis

Data are expressed as means ± SD ($n = 3$). *P* values were calculated by Student's *t* test and considered significant at a value of 0.01.

3. Results

3.1. Atg5 is involved in suppression of MyD88 condensed structure formation

We previously found that there was little formation of MyD88 condensed structures after TLR stimulation and that such structures are related to cytoplasmic aggregation of sequestosome 1 (SQSTM1; also known as p62) and histone deacetylase (HDAC) 6 [13]. Both SQSTM1 and HDAC6 are autophagy-related ubiquitin-binding proteins, which mediate aggregation of polyubiquitinated proteins, ultimately leading to degradation of them by autophagy [23]. However, the correlation between autophagy and MyD88 condensed structures has not been clarified. Therefore, we first examined the role of the autophagy-related protein Atg5, a key regulator of autophagy, in formation of MyD88 condensed structures. In wild-type MEFs, LPS and Pam_3CSK_4, both of which activate MyD88-dependent signaling, only weakly induced formation of MyD88 condensed structures (Fig. 1A). In contrast, surprisingly, in Atg5 KO MEFs, MyD88 was already present as condensed structures (Fig. 1A). Cells containing MyD88 condensed structures were increased by approximately 15% (Fig. 1B). Furthermore, in Atg5 KO MEFs, LPS and Pam_3CSK_4 increased MyD88 condensed structures (Fig. 1A). Cells containing MyD88 condensed structures were increased by approximately 30% after 60 min of stimulation (Fig. 1B). These results suggest that Atg5 is involved in suppression of MyD88 condensed structure formation.

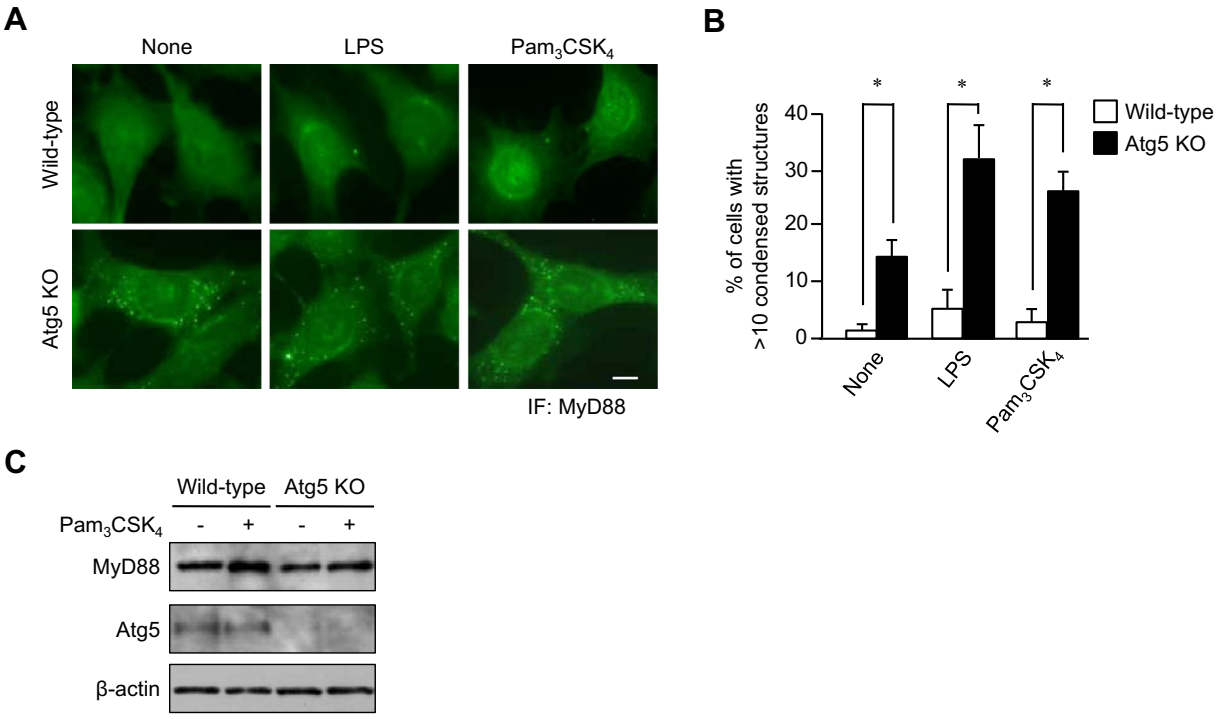

Fig. 1. Loss of Atg5 increases MyD88 condensed structures. (A) Wild-type and Atg5 KO MEFs were stimulated with LPS (1 μg/ml) or Pam_3CSK_4 (1 μg/ml) for 60 min. The cells were fixed and immunostained with anti-MyD88. Scale bar, 10 μm. (B) The percentage of cells with more than 10 microscopy-visible condensed structures of MyD88 was quantified by counting cells in 10 different images of microscopic fields that included at least 15 cells. Results, shown as means ± SD ($n = 3$), are representative of three independent experiments. ($^*P < 0.01$). (C) Wild-type and Atg5 KO MEFs were stimulated with Pam_3CSK_4 (1 μg/ml) for 60 min and then subjected to immunoblot analysis using the indicated antibodies.

To determine whether Atg5 deficiency-induced MyD88 condensed structure formation is due to impairment of Atg5-mediated degradation of MyD88, we examined MyD88 protein expression in Atg5 KO MEFs. The level of MyD88 in Atg5 KO MEFs was the same as that in wild-type MEFs (Fig. 1C). Pam_3CSK_4 stimulation for 60 min slightly induced expression of MyD88 protein in wild-type and Atg5 KO MEFs (Fig. 1C). These results suggest that Atg5 suppresses the formation of MyD88 condensed structures, but it does not lead to degradation of MyD88.

3.2. MyD88-dependent signaling is not associated with LC3-dependent autophagic processes

Since the formation of MyD88 condensed structures is induced by LPS and Pam_3CSK_4, we investigated whether MyD88-dependent signaling is associated with autophagic processes. Generally, induction of LC3 puncta formation and conversion of LC3-I to LC3-II are known as a hallmark feature of autophagic flux [15]. We observed LC3 puncta structures and conversion of LC3-I to LC3-II induced by nutrient deprivation in wild-type MEFs but not in Atg5 KO MEFs (Fig. 2A and B). However, LPS stimulation did not induce LC3 puncta formation and LC3 conversion in wild-type and Atg5 KO MEFs (Fig. 2A and B). These results indicate that MyD88-dependent signaling is not correlated with LC3-dependent autophagic processes.

3.3. Atg5 interacts with MyD88

To examine the association between MyD88 and Atg5, coimmunoprecipitation analysis was performed. We found that Atg5 could interact with MyD88 (Fig. 3). This observation suggests that Atg5 interacts with MyD88 and then mediates the suppressive effect on formation of MyD88 condensed structures.

3.4. Atg5 regulates MyD88-dependent signaling

We have shown that the loss of Atg5 increases the formation of MyD88 condensed structures (Fig. 1A). Since condensed structures of MyD88 have been regarded as signaling complexes [9,10], we speculated that Atg5 exerts some physiological effects on formation of MyD88–TRAF6 signaling complex induced by TLR stimulation. To examine the effect of Atg5 on formation of MyD88–TRAF6 complex, we applied a biochemical approach utilizing MyD88 *C*-terminally fused to GyrB, which is known to dimerize upon binding with the *Streptomyces* product coumermycin [24]. In cells stably expressing MyD88–GyrB, coumermycin can activate signaling pathways similar to TLR signaling pathways through recruitment of TRAF6 to MyD88–GyrB [25]. Consistent with the results of a previous study [25], TRAF6 was co-immunoprecipitated with MyD88–GyrB after coumermycin treatment in wild-type and Atg5 KO MEFs (Fig. 4A). Interestingly, compared with wild-type MEFs, co-immunoprecipitated TRAF6 upon coumermycin treatment was obviously increased in Atg5 KO MEFs (Fig. 4A). These results indicate that Atg5 suppresses the formation of MyD88–TRAF6 signaling complex induced by TLR stimulation.

We next investigated whether Atg5 exerts downregulatory effects on the MyD88-mediated signaling pathway, since Atg5 inhibits formation of the MyD88–TRAF6 signaling complex. Unexpectedly, Pam_3CSK_4-induced activation of p38, JNK, ERK and Akt was decreased in Atg5 KO MEFs compared with that in wild-type MEFs (Fig. 4B). On the other hand, Pam_3CSK_4-induced activation of NF-κB p65 and degradation of IκBα were enhanced in Atg5 KO MEFs compared with those in wild-type MEFs (Fig. 4B). The distinct regulatory effect of Atg5 on activation of MAPKs and NF-κB was also observed in wild-type and Atg5 KO MEFs after stimulation with LPS (Fig. S1). These results suggest that Atg5 has a downregulatory effect on activation of NF-κB signaling, whereas it has an upregulatory effect on MAPKs and Akt.

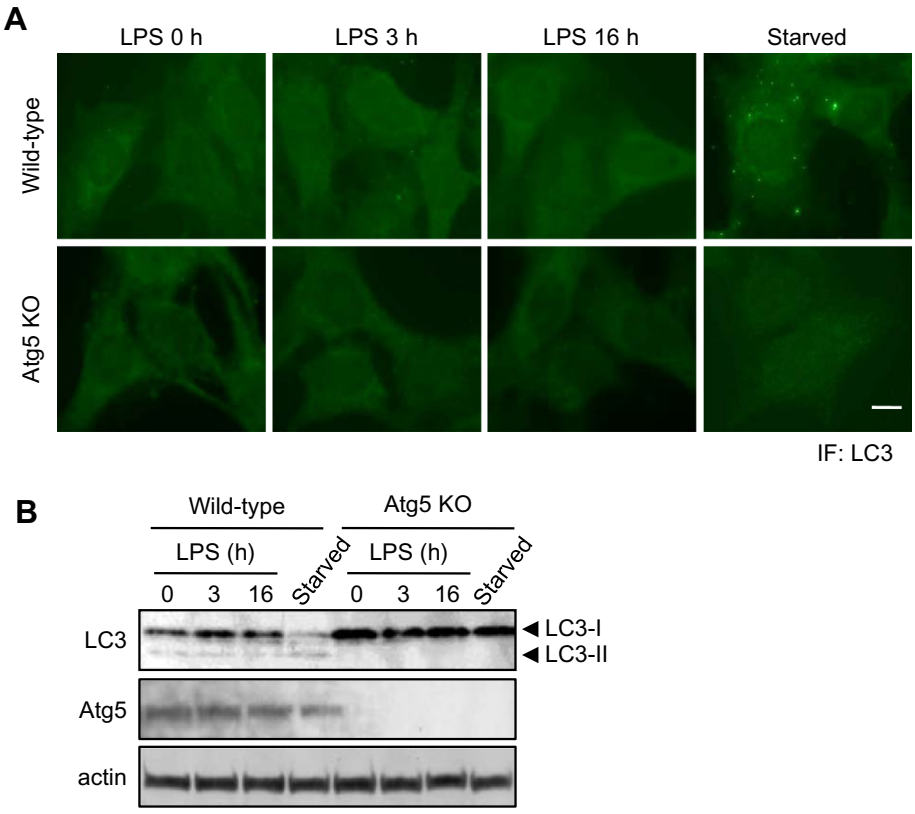

Fig. 2. TLR stimulation does not induce LC3-dependent autophagy. (A) Wild-type and Atg5 KO MEFs were stimulated with LPS (1 μg/ml) for the indicated periods. Alternatively, cells were cultured in DMEM without amino acids and serum for 16 h (starved). The cells were fixed and immunostained with anti-LC3. Scale bar, 10 μm. (B) Wild-type and Atg5 KO MEFs were stimulated with LPS (1 μg/ml) for the indicated periods. Alternatively, cells were cultured in DMEM without amino acids and serum for 3 h (starved). Cell lysates were analyzed by immunoblotting with indicated antibodies.

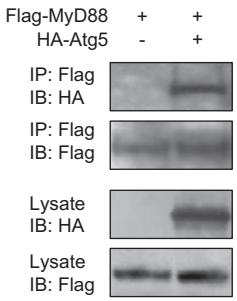

Fig. 3. Atg5 interacts with MyD88. HEK293T cells were transfected with Flag-MyD88 together with HA-Atg5. Cell lysates were immunoprecipitated with anti-Flag antibody and then subjected to immunoblotting analysis using anti-HA or anti-Flag antibodies.

4. Discussion

In the present study, we revealed that the autophagy-related protein Atg5 suppresses the formation of MyD88 condensed structures in the cytoplasm. We also obtained evidence that Atg5 inhibits formation of the MyD88–TRAF6 signaling complex induced by TLR stimulation, which ultimately limits the activation of NF-κB p65 and degradation of IκBα. Interestingly, Atg5 does not inhibit the activation of p38, JNK, ERK and Akt. These findings suggest that Atg5 may more preferentially inhibit activation of TAK1, which mediates the NF-κB signaling downstream of TRAF6. It is currently understood that TRAF6-generated unanchored polyubiquitin chains directly activate TAK1 but not MAPKs and Akt [6]. Therefore, Atg5 may suppress the formation of TRAF6-synthesized free polyubiquitin chains as well as the MyD88–TRAF6 signaling complex and exert a modulatory effect on MyD88-mediated signal transduction.

We found that endogenous MyD88 was already present as condensed structures in Atg5 KO MEFs. Although MyD88 condensed structures have been regarded as signaling complexes or myddosomes [9,10], activation of several signaling events, such as phosphorylation of NF-κB p65, was not observed. Therefore, it is possible that MyD88 is normally present as inactive condensed structures in the cytoplasm, but the formation of the structures is tightly regulated by Atg5.

Recent studies have indicated that each Atg controls certain types of cellular response through association with signaling molecules. Atg5 and the conjugate of Atg5–Atg12 associate with IFN-β promoter stimulator 1 (IPS-1) and retinoic acid-inducible gene I (RIG-I), essential molecules for immunostimulatory RNA (isRNA)-mediated signaling [26]. This association suppresses the interaction between IPS-1 and RIG-I, leading to inhibition of isRNA-mediated innate immune response [26]. In the case of Atg9a, it co-localizes with cytoplasmic punctate structures of stimulator of interferon genes (STING) and TANK-binding kinase 1 (TBK1), which are critical signaling molecules for double-stranded DNA (dsDNA)-induced signaling [27]. Subsequent characterization by electron microscopy revealed that punctate structures of STING induced by dsDNA do not have the morphological characteristics of autophagosomes, suggesting that Atg9a does not lead to autophagic degradation of STING [27]. However, interestingly, the loss of Atg9a greatly enhanced dsDNA-induced formation of punctate structures of STING and TBK1, resulting in activation of the innate immune response [27]. Therefore, it is possible that Atg9a regulates dsDNA-induced innate immune response independently of

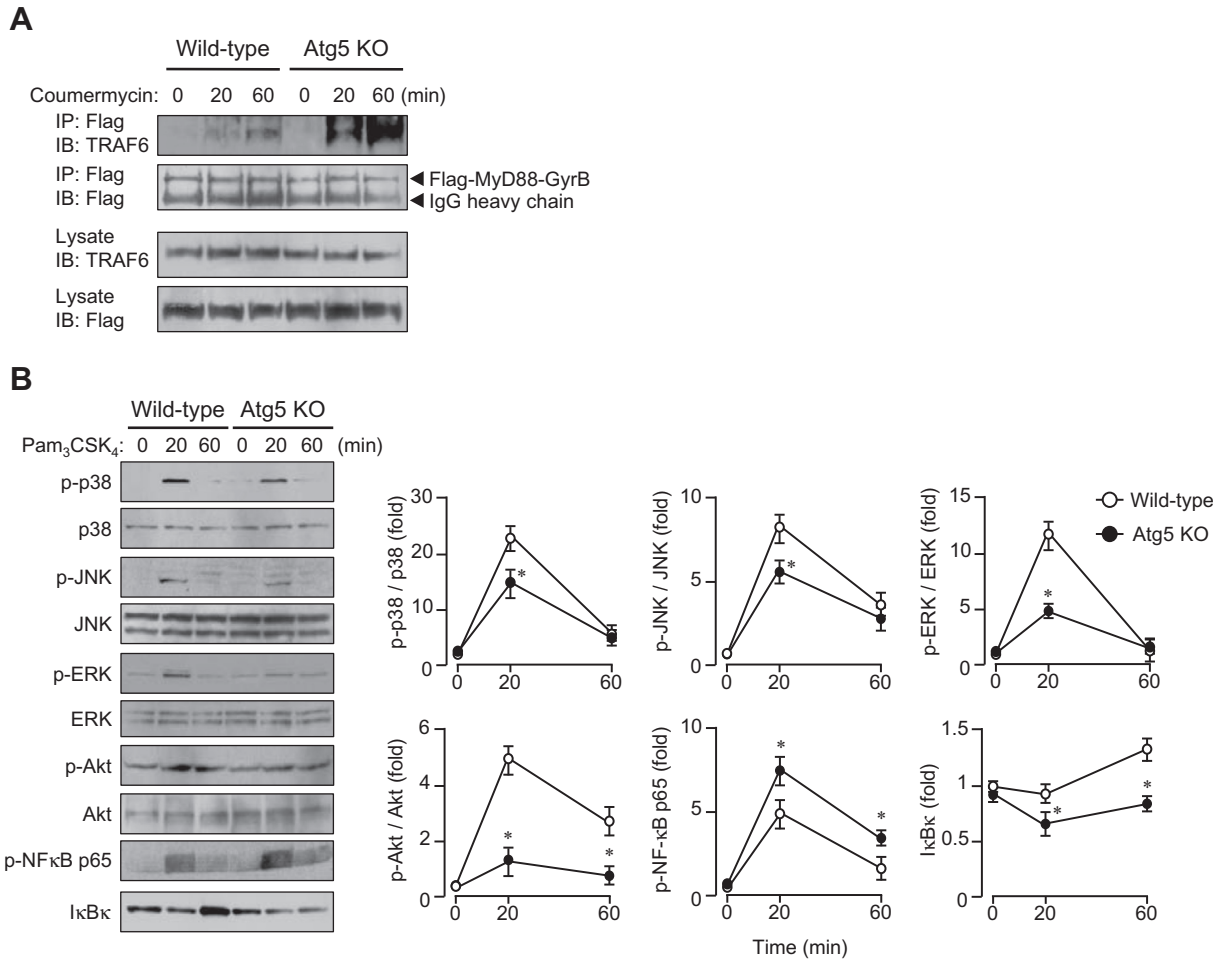

Fig. 4. Effect of Atg5 on signal transduction induced by TLR stimulation. (A) Wild-type and Atg5 KO MEFs stably expressing Flag-MyD88-GyrB were stimulated with coumermycin (1 μM) for the indicated periods. Cell lysates were immunoprecipitated with anti-Flag antibody and then subjected to immunoblotting analysis using anti-TRAF6 or anti-Flag antibodies. (B) Wild-type and Atg5 KO MEFs were stimulated with Pam_3CSK_4 (1 μg/ml) for the indicated periods. Cell lysates were subjected to immunoblotting analysis using the indicated antibodies. Densitometric quantification was performed on all of the immunoblot bands. Each value of p-p38, p-JNK, p-ERK and p-Akt was normalized to each level of total p38, total JNK, total ERK and total Akt, respectively. Results are expressed as means ± SD of three independent experiments and as fold increases by taking the control values (wild-type, 0 min) as 1. (*$P < 0.01$, for comparison with the wild-type group).

autophagic processes [27]. The present study demonstrated that the loss of Atg5 enhanced MyD88 condensed structure formation and several MyD88-mediated signaling events. Atg5 could interact with MyD88, but it did not induce MyD88 degradation. Moreover, activation of MyD88-dependent signaling by LPS stimulation did not induce LC3 punctate formation and LC3 conversion. These observations suggest that the regulatory effect of Atg5 on MyD88-dependent signaling may be exerted through association with MyD88, but not through autophagic processes.

The present study highlights a novel role of Atg5 in the regulation of formation of MyD88 condensed structures and MyD88-dependent signal transduction. Given that excess activation of MyD88-mediated responses induces inflammatory disorders [28], Atg5 may function as one of the essential fine-tuners of MyD88-dependent signaling.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.094.

References

[1] H. Wesche, W.J. Henzel, W. Shillinglaw, S. Li, Z. Cao, MyD88: an adaptor that recruits IRAK to the IL-1 receptor complex, Immunity 7 (1997) 837–847.
[2] R. Medzhitov, P. Preston-Hurlburt, E. Kopp, A. Stadlen, C. Chen, S. Ghosh, C.A. Janeway Jr., MyD88 is an adaptor protein in the hToll/IL-1 receptor family signaling pathways, Mol. Cell 2 (1998) 253–258.
[3] M. Muzio, J. Ni, P. Feng, V.M. Dixit, IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling, Science 278 (1997) 1612–1615.
[4] S.C. Lin, Y.C. Lo, H. Wu, Helical assembly in the MyD88–IRAK4–IRAK2 complex in TLR/IL-1R signalling, Nature 465 (2010) 885–890.
[5] Z.J. Chen, Ubiquitin signalling in the NF-κB pathway, Nat. Cell Biol. 7 (2005) 758–765.
[6] Z.P. Xia, L. Sun, X. Chen, G. Pineda, X. Jiang, A. Adhikari, W. Zeng, Z.J. Chen, Direct activation of protein kinases by unanchored polyubiquitin chains, Nature 461 (2009) 114–119.
[7] S. Akira, S. Uematsu, O. Takeuchi, Pathogen recognition and innate immunity, Cell 124 (2006) 783–801.
[8] L.A. O'Neill, A.G. Bowie, The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling, Nat. Rev. Immunol. 7 (2007) 353–364.
[9] T. Kawai, S. Sato, K.J. Ishii, C. Coban, H. Hemmi, M. Yamamoto, K. Terai, M. Matsuda, J. Inoue, S. Uematsu, O. Takeuchi, S. Akira, Interferon-α induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6, Nat. Immunol. 5 (2004) 1061–1068.

[10] J.C. Kagan, R. Medzhitov, Phosphoinositide-mediated adaptor recruitment controls Toll-like receptor signaling, Cell 125 (2006) 943–955.
[11] T. Nishiya, E. Kajita, T. Horinouchi, A. Nishimoto, S. Miwa, Distinct roles of TIR and non-TIR regions in the subcellular localization and signaling properties of MyD88, FEBS Lett. 581 (2007) 3223–3229.
[12] F. Jaunin, K. Burns, J. Tschopp, T.E. Martin, S. Fakan, Ultrastructural distribution of the death-domain-containing MyD88 protein in HeLa cells, Exp. Cell Res. 243 (1998) 67–75.
[13] T. Into, M. Inomata, S. Niida, Y. Murakami, K. Shibata, Regulation of MyD88 aggregation and the MyD88-dependent signaling pathway by sequestosome 1 and histone deacetylase 6, J. Biol. Chem. 285 (2010) 35759–35769.
[14] N. Mizushima, M. Komatsu, Autophagy: renovation of cells and tissues, Cell 147 (2011) 728–741.
[15] D.J. Klionsky, F.C. Abdalla, H. Abeliovich, et al., Guidelines for the use and interpretation of assays for monitoring autophagy, Autophagy 8 (2012) 445–544.
[16] M. Tsukada, Y. Ohsumi, Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*, FEBS Lett. 333 (1993) 169–174.
[17] D.J. Klionsky, J.M. Cregg, W.A. Dunn Jr., S.D. Emr, Y. Sakai, I.V. Sandoval, A. Sibirny, S. Subramani, M. Thumm, M. Veenhuis, Y. Ohsumi, A unified nomenclature for yeast autophagy-related genes, Dev. Cell 5 (2003) 539–545.
[18] N. Mizushima, Y. Ohsumi, T. Yoshimori, Autophagosome formation in mammalian cells, Cell Struct. Funct. 27 (2002) 421–429.
[19] Y. Ohsumi, Molecular dissection of autophagy: two ubiquitin-like systems, Nat. Rev. Mol. Cell Biol. 2 (2001) 211–216.
[20] T. Into, M. Inomata, M. Nakashima, K. Shibata, H. Häcker, K. Matsushita, Regulation of MyD88-dependent signaling events by S nitrosylation retards Toll-like receptor signal transduction and initiation of acute-phase immune responses, Mol. Cell. Biol. 28 (2008) 1338–1347.
[21] A. Kuma, M. Hatano, M. Matsui, A. Yamamoto, H. Nakaya, T. Yoshimori, Y. Ohsumi, T. Tokuhisa, N. Mizushima, The role of autophagy during the early neonatal starvation period, Nature 432 (2004) 1032–1036.
[22] M. Inomata, S. Niida, K. Shibata, T. Into, Regulation of Toll-like receptor signaling by NDP52-mediated selective autophagy is normally inactivated by A20, Cell. Mol. Life Sci. 69 (2012) 963–979.
[23] W.X. Ding, X.M. Yin, Sorting, recognition and activation of the misfolded protein degradation pathways through macroautophagy and the proteasome, Autophagy 4 (2008) 141–150.
[24] M.A. Farrar, J. Alberol-Ila, R.M. Perlmutter, Activation of the Raf-1 kinase cascade by coumermycin-induced dimerization, Nature 383 (1996) 178–181.
[25] H. Häcker, V. Redecke, B. Blagoev, I. Kratchmarova, L.C. Hsu, G.G. Wang, M.P. Kamps, E. Raz, H. Wagner, G. Häcker, M. Mann, M. Karin, Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6, Nature 439 (2006) 204–207.
[26] N. Jounai, F. Takeshita, K. Kobiyama, A. Sawano, A. Miyawaki, K.Q. Xin, K.J. Ishii, T. Kawai, S. Akira, K. Suzuki, K. Okuda, The Atg5–Atg12 conjugate associates with innate antiviral immune responses, Proc. Natl. Acad. Sci. USA 104 (2007) 14050–14055.
[27] T. Saitoh, N. Fujita, T. Hayashi, K. Takahara, T. Satoh, H. Lee, K. Matsunaga, S. Kageyama, H. Omori, T. Noda, N. Yamamoto, T. Kawai, K. Ishii, O. Takeuchi, T. Yoshimori, S. Akira, Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response, Proc. Natl. Acad. Sci. USA 106 (2009) 20842–20846.
[28] F.Y. Liew, D. Xu, E.K. Brint, L.A. O'Neill, Negative regulation of Toll-like receptor-mediated immune responses, Nat. Rev. Immunol. 5 (2005) 446–458.