

Regulation of immune response by *S*-1-propenylcysteine through autophagy-mediated protein degradation (Review)

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Abstract. Autophagy is a key event in cellular recycling processes due to its involvement in the intracellular degradation of proteins. It has been demonstrated that *S*-1-propenylcysteine (S1PC), a characteristic sulfur compound in aged garlic extract, induces the activation of autophagy. S1PC degrades the adaptor protein myeloid differentiation response protein 88 (MyD88) of downstream of Toll-like receptor (TLR) by activating autophagy *in vitro* and *in vivo*. The degradation of MyD88 inhibits the TLR signaling pathway, including the phosphorylation of interleukin 1 receptor associated kinase 4 (IRAK4) and nuclear factor (NF)- κ B p65 *in vitro*, and eventually leads to the inhibition of interleukin (IL)-6 production *in vitro* and C-C motif chemokine ligand 2 (*Ccl2*) mRNA expression *in vivo*. S1PC also increases the level of intestinal immunoglobulin A (IgA) and the number of IgA-producing cells in Peyer's patches *in vivo*. In addition, S1PC triggers the mRNA expression of X-box binding protein 1 (*Xbp1*), an inducer of IgA-producing cell differentiation via the phosphorylation of extracellular signal-regulated kinase (ERK)1/2 and the degradation of paired box protein 5 (Pax5), a suppressor of *Xbp1* mRNA expression. The present review summarizes the mechanisms through which the activation of autophagy by S1PC modulates the immune response.

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1. Introduction

Aged garlic extract (AGE) is produced by extracting and aging garlic slices in an aqueous ethanol solution for >10 months and has been shown to modulate several immune functions, such as decreasing inflammatory cytokines and chemokines in animal models (1-4). In addition, the supplementation of AGE was previously shown to increase the numbers of $\gamma\delta$ -T and natural killer (NK) cells, and to reduce the levels of inflammatory cytokines, such as interleukin (IL)-6 and tumor necrosis factor (TNF)- α , in a clinical study (5). AGE has been shown to exert immuno-enhancing and anti-inflammatory effects (1-5). *S*-1-propenylcysteine (S1PC), a major characteristic sulfur compound in AGE, which exhibits good oral bioavailability in rats and canines (6), has been shown to exert several beneficial effects, such as immunoregulatory, anti-hypertensive and blood flow-promoting effects (7-10). Moreover, in our previous recent studies, it was indicated that S1PC promoted intestinal immunoglobulin A (IgA) production and inhibited lipopolysaccharide (LPS)-induced IL-6 production (7,10).

Autophagy is a major degradation system of cellular components, including abnormal proteins, protein aggregates and damaged organelles (11-13). In addition, autophagy maintains cellular homeostasis and regulates various cellular events, such as signal transduction, cell growth, apoptosis and differentiation. Autophagy has been shown to regulate the immune response and immune cell differentiation (14-18). The inhibition of autophagy prevents monocyte-to-macrophage differentiation as it contributes to the transition from apoptosis to differentiation (19). In addition, the regulatory T (Treg) cell-specific deletion of autophagy-related gene (*Atg*) 7 triggers the loss of Treg cells by inducing apoptosis and promotes the development of inflammatory disorders (20). Thus, autophagy plays an important role in the regulation of immune developments and functions. The aim of the present review is to provide a summary and discussion of the mechanisms responsible for the immunoregulatory effects of S1PC which are mediated via the activation of autophagy.

2. S1PC induces the activation of autophagy

Autophagy is activated by several stress conditions, such as nutrient starvation, unfolded proteins and infection (11-13). Autophagy-mediated proteolysis occurs through different steps, which include the elongation of the phagophore and

delivery to lysosomes. These processes are regulated by several signaling molecules (21,22). S1PC has been shown to promote the phosphorylation of AMP-activated protein kinase (AMPK), which is a cellular energy sensor and regulates the initial steps of autophagy activation (10). AMPK triggers the phosphorylation of unc-51-like kinase 1/2 (Ulk1/2) and inhibits the phosphorylation of mammalian target of rapamycin (mTOR), a repressor of autophagy. These steps initiate the elongation of the phagophore by phosphorylating the complex of Beclin1/vesicular sorting protein 34 (VPS34) (21-23). Following the formation of the autophagosome membrane, microtubule-associated protein 1 light chain 3 (LC3-I) conjugates with phosphatidylethanolamine by ubiquitin-like enzymes, such as Atg7, Atg3 and the Atg16L:Atg5-Atg12 complex, and is converted to lipidated LC3 (LC3-II). LC3-II interacts with target proteins via adaptor protein p62 on the autophagosome membrane. The LC3-II/LC3-I ratio usually increases upon the activation of autophagy (24,25), whereas S1PC has been shown to increase the levels of both LC3-I and LC3-II. Accordingly, S1PC can not only promote the conversion of LC3-I to LC3-II, but can also increase the production of LC3-I. Subsequently, the autophagosome fuses with the lysosome and then target proteins are degraded with LC3-II and p62 (10). S1PC has been shown to induce the degradation of target proteins and p62 (10). In addition, both 3-methyladenine (3-MA), an autophagy inhibitor and compound C, an AMPK inhibitor, have been shown to block the S1PC-induced activation of autophagy (10). A schematic diagram of the mechanisms through which S1PC induces autophagy is presented in Fig. 1. It is thus suggested that S1PC triggers the activation of autophagy by inducing AMPK phosphorylation.

3. Anti-inflammatory effects of S1PC

Chronic inflammation is associated with the onset of several human conditions and diseases, including aging, allergies, autoimmune diseases, atherosclerosis, cancer, chronic wounds, cystic fibrosis, metabolic syndrome and obesity (26,27). The pattern recognition receptors (PRRs) play an important role in innate immunity and host defense by recognizing pathogen-associated molecular patterns (PAMPs). However, PRRs trigger chronic inflammation by consecutively interacting with danger-associated molecular patterns (DAMPs) released from dying cells (28-30). Toll-like receptors (TLRs), which are important members of the PRR family, recognize microbial components and cellular debris (28-30). Therefore, PRRs recognize not only pathogens, but also cellular components. The activation of TLRs recruits myeloid differentiation response protein 88 (MyD88), a common adaptor protein of TLRs, apart from TLR3, and IL-1 receptor-associated kinase 4 (IRAK4) to the plasma membrane (30-32). TLR signaling induces the production of the inflammatory cytokines, IL-6 and TNF- α , and the chemokines, C-C motif chemokine ligand 2 (CCL2) and C-X-C motif chemokine ligand 8 (CXCL8) via the activation of nuclear factor (NF)- κ B (34,35). S1PC has been shown to inhibit IL-6 production by suppressing the TLR signaling pathway via the degradation of MyD88 (10). In addition, S1PC blocks the mRNA expression of *Ccl2* in the livers of spontaneously hypertensive rats (SHRs) (10). A schematic diagram of the mechanisms through which S1PC induces the

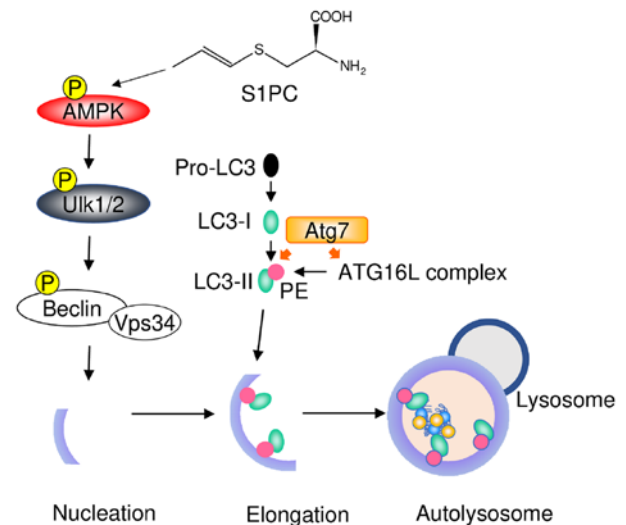


Figure 1. S1PC induces the activation of autophagy. S1PC induces the activation of the AMPK-ULK axis and triggers the elongation of phagophore. The Atg7 and Atg16L complex then catalyze the formation of PE-conjugated LC3 (LC3-II). LC3-II interacts with ubiquitinated target protein via adaptor protein p62. Finally, target protein is degraded by autophagy-lysosome degradation system. S1PC, S-1-propenylcysteine; AMPK, AMP-activated protein kinase; ULK, unc-51-like kinase; PE, phosphatidylethanolamine; LC3, light chain 3.

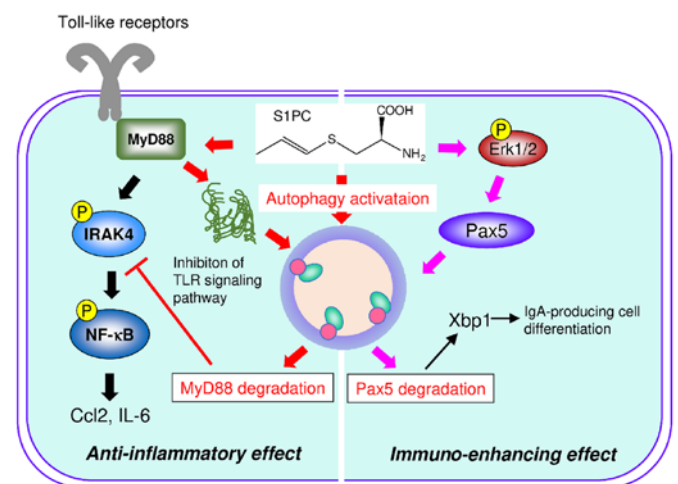


Figure 2. S1PC induces the degradation of MyD88 and Pax5 by activating autophagy. S1PC directly denatures MyD88 and then induces the formation of protein aggregates by the lysine acetylation and ubiquitination. S1PC triggers the degradation of MyD88 by inducing AMPK-mediated autophagy activation. Consequently, the degradation of MyD88 inhibits TLR signaling pathway (left part of diagram). S1PC induces the phosphorylation of ERK1/2 and triggers AMPK-induced autophagy activation. Pax5 is phosphorylated by ERK1/2 and then is degraded by autophagy. Therefore, the degradation of Pax5 induces the expression of *Xbp1* mRNA and triggered the differentiation of B cells into IgA-producing cells (right part of diagram). S1PC, S-1-propenylcysteine; MyD88, myeloid differentiation response protein 88; AMPK, AMP-activated protein kinase; TLR, Toll-like receptor; ERK1/2, extracellular-regulated kinase 1/2; Pax5, paired box protein 5; *Xbp1*, X-box binding protein 1; IRAK4, interleukin 1 receptor associated kinase 4.

degradation of MyD88 and paired box protein 5 (Pax5) by activating autophagy is presented in Fig. 2. The constituents of fresh garlic and AGE have been reported to inhibit the TLR signaling pathway. Alliin, a constituent of fresh garlic, decreases the LPS-induced phosphorylation of extracel-

lular signal-regulated kinase (ERK)1/2 in adipocytes (36). S-allylcysteine (SAC), a constituent of AGE, has been shown to reduce the production of inflammatory cytokines by inhibiting NF- κ B phosphorylation (37). The inhibitory effects of SIPC could be considered to be different from those of other garlic constituents. SIPC has been shown to degrade MyD88 by activating autophagy (see schematic diagram in Fig. 2) (10). However, the activation of autophagy alone cannot degrade MyD88 due to the inability of SAC to induce the degradation of MyD88, although SAC also activates autophagy (10). SIPC has been shown to have another distinct feature that directly denatures and aggregates MyD88, whereas SAC is unable to denature MyD88 (10). Aggregated MyD88 is modified with both acetylation and ubiquitination, and forms the histone deacetylase 6 (HDAC6)-dependent aggresome. Subsequently, ubiquitin of the aggresome interacts with p62 and is degraded by the autophagy-lysosome system (10). Thus, as discussed above, it has been suggested by both *in vitro* and *in vivo* studies that the anti-inflammatory mechanisms of SIPC involve the degradation of MyD88 by triggering the denaturation of MyD88 and the activation of autophagy.

4. Immuno-enhancing effects of SIPC

The intestine is the largest tissue of the immune system and is the first defense line of the body against foreign antigens, such as infectious pathogens, toxins and food allergens (38,39). IgA is the most abundant secreted antibody involved in protecting intestinal epithelial cells (40). Immunoglobulin class switching from IgM to IgA is induced by the action of both cell-cell contact and cytokines in Peyer's patches (PPs) and becomes rapidly plasmablasts. The oral administration of SIPC has been shown to increase the intestinal IgA level and IgA-producing cells in PPs (7). In addition, SIPC has been found to act on B cells and increase IgA production by promoting the differentiation of B cells into IgA-producing B cells *in vitro*. Therefore, SIPC is more likely to promote the expression of transcription factors related to immunoglobulin class switching. Several transcription factors, including X-box binding protein 1 (Xbp1), and B cell-induced maturation protein-1 (Blimp1) regulate immunoglobulin class switching (41). SIPC increases the expression of *Xbp1* mRNA *in vitro* and *in vivo*, whereas the mRNA expression of Blimp1 is not affected. Xbp1 requires the formation of pre-plasmablasts, the early process of plasma cell differentiation that is independent of Blimp1 function. It is possible that SIPC induces the early process of plasma cell differentiation. The mRNA expression of *Xbp1* is repressed by Pax5. It is known that ERK1/2 triggers the degradation of Pax5 by inducing its phosphorylation (41). SIPC has been found to induce the degradation of Pax5 by enhancing ERK1/2 phosphorylation (see schematic diagram in Fig. 2) (7). Therefore, on the whole, it is suggested that SIPC induces the degradation of Pax5 by activating both ERK1/2 and autophagy, and then triggers the differentiation of B cells into IgA-producing cells by increasing the mRNA expression of *Xbp1*.

5. Conclusions and future perspectives

SIPC, a major characteristic sulfur compound in AGE, induces the activation of autophagy via the phosphorylation

of AMPK. The activation of autophagy regulates the immune response through the degradation of key molecules. SIPC has been shown not only to induce the activation of autophagy, but also to trigger the post-translational modification of target proteins. Thus, it is suggested that SIPC selectively induces the degradation of proteins. In addition, it is suggested that SIPC exerts immuno-enhancing and anti-inflammatory effects, and may contribute to the maintenance of immune homeostasis by regulating autophagy.

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Authors' contributions

JIS and YK conceived this review. JIS, SM and MU analyzed the relevant literature. JIS wrote the first draft of the manuscript and produced the figures. JIS, SM, MU and YK critically revised the manuscript. All authors have reviewed and approved the final manuscript.

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Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Kyo E, Uda N, Kasuga S and Itakura Y: Immunomodulatory effects of aged garlic extract. *J Nutr* 131: 1075S-1079S, 2001.
2. Morihara N, Hino A, Miki S, Takashima M and Suzuki JI: Aged garlic extract suppresses inflammation in apolipoprotein E-knockout mice. *Mol Nutr Food Res* 61: 1700308, 2017.
3. Morihara N, Hino A, Yamaguchi T and Suzuki J: Aged Garlic Extract Suppresses the Development of Atherosclerosis in Apolipoprotein E-Knockout Mice. *J Nutr* 146: 460S-463S, 2016.
4. Miki S, Inokuma K-I, Takashima M, Nishida M, Sasaki Y, Ushijima M, Suzuki JI and Morihara N: Aged garlic extract suppresses the increase of plasma glycated albumin level and enhances the AMP-activated protein kinase in adipose tissue in TSOD mice. *Mol Nutr Food Res* 61: 61, 2017.
5. Nantz MP, Rowe CA, Muller CE, Creasy RA, Stanilka JM and Percival SS: Supplementation with aged garlic extract improves both NK and $\gamma\delta$ -T cell function and reduces the severity of cold and flu symptoms: A randomized, double-blind, placebo-controlled nutrition intervention. *Clin Nutr* 31: 337-344, 2012.

6. Amano H, Kazamori D and Itoh K: Pharmacokinetics and *N*-acetylation metabolism of *S*-methyl-L-cysteine and *trans*-*S*-1-propenyl-L-cysteine in rats and dogs. *Xenobiotica* 46: 1017-1025, 2016.
7. Suzuki J, Yamaguchi T, Matsutomo T, Amano H, Morihara N and Kodera Y: *S*-1-Propenylcysteine promotes the differentiation of B cells into IgA-producing cells by the induction of Erk1/2-dependent Xbp1 expression in Peyer's patches. *Nutrition* 32: 884-889, 2016.
8. Matsutomo T, Ushijima M, Kodera Y, Nakamoto M, Takashima M, Morihara N and Tamura K: Metabolomic study on the antihypertensive effect of *S*-1-propenylcysteine in spontaneously hypertensive rats using liquid chromatography coupled with quadrupole-Orbitrap mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 1046: 147-155, 2017.
9. Matsutomo T, Ushijima M, Kunimura K and Ohtani M: Metabolomic study reveals the acute hypotensive effect of *S*-1-propenylcysteine accompanied by alteration of the plasma histidine level in spontaneously hypertensive rats. *J Pharm Biomed Anal* 168: 148-154, 2019.
10. Suzuki JI, Kodera Y, Miki S, Ushijima M, Takashima M, Matsutomo T and Morihara N: Anti-inflammatory action of cysteine derivative *S*-1-propenylcysteine by inducing MyD88 degradation. *Sci Rep* 8: 14148, 2018.
11. Mizushima N, Yoshimori T and Ohsumi Y: The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 27: 107-132, 2011.
12. Eskelinen E-L and Saftig P: Autophagy: A lysosomal degradation pathway with a central role in health and disease. *Biochim Biophys Acta* 1793: 664-673, 2009.
13. Saha S, Panigrahi DP, Patil S and Bhutia SK: Autophagy in health and disease: A comprehensive review. *Biomed Pharmacother* 104: 485-495, 2018.
14. Cuervo AM and Macian F: Autophagy, nutrition and immunology. *Mol Aspects Med* 33: 2-13, 2012.
15. Arroyo DS, Gaviglio EA, Peralta Ramos JM, Bussi C, Rodriguez-Galan MC and Iribarren P: Autophagy in inflammation, infection, neurodegeneration and cancer. *Int Immunopharmacol* 18: 55-65, 2014.
16. Deretic V and Levine B: Autophagy balances inflammation in innate immunity. *Autophagy* 14: 243-251, 2018.
17. Into T, Horie T, Inomata M, Gohda J, Inoue JI, Murakami Y and Niida S: Basal autophagy prevents autoactivation or enhancement of inflammatory signals by targeting monomeric MyD88. *Sci Rep* 7: 1009, 2017.
18. Riffelmacher T, Richter FC and Simon AK: Autophagy dictates metabolism and differentiation of inflammatory immune cells. *Autophagy* 14: 199-206, 2018.
19. Zhang Y, Morgan MJ, Chen K, Choksi S and Liu ZG: Induction of autophagy is essential for monocyte-macrophage differentiation. *Blood* 119: 2895-2905, 2012.
20. Wei J, Long L, Yang K, Guy C, Shrestha S, Chen Z, Wu C, Vogel P, Neale G, Green DR, *et al*: Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. *Nat Immunol* 17: 277-285, 2016.
21. Abounit K, Scarabelli TM and McCauley RB: Autophagy in mammalian cells. *World J Biol Chem* 3: 1-6, 2012.
22. He C and Klionsky DJ: Regulation mechanisms and signaling pathways of autophagy. *Annu Rev Genet* 43: 67-93, 2009.
23. Kim J and Guan KL: AMPK connects energy stress to PIK3C3/VPS34 regulation. *Autophagy* 9: 1110-1111, 2013.
24. Tanida I, Ueno T and Kominami E: LC3 and Autophagy. *Methods Mol Biol* 445: 77-88, 2008.
25. Cherra SJ III, Kulich SM, Uechi G, Balasubramani M, Mountzouris J, Day BW and Chu CT: Regulation of the autophagy protein LC3 by phosphorylation. *J Cell Biol* 190: 533-539, 2010.
26. Chung HY, Kim DH, Lee EK, Chung KW, Chung S, Lee B, Seo AY, Chung JH, Jung YS, Im E, *et al*: Redefining Chronic Inflammation in Aging and Age-Related Diseases: Proposal of the Senoinflammation Concept. *Aging Dis* 10: 367-382, 2019.
27. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X and Zhao L: Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 9: 7204-7218, 2017.
28. Falck-Hansen M, Kassiteridi C and Monaco C: Toll-like receptors in atherosclerosis. *Int J Mol Sci* 14: 14008-14023, 2013.
29. Piccinini AM and Midwood KS: DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm* 2010: pii: 672395, 2010.
30. Drexler SK and Foxwell BM: The role of toll-like receptors in chronic inflammation. *Int J Biochem Cell Biol* 42: 506-518, 2010.
31. Troutman TD, Bazan JF and Pasare C: Toll-like receptors, signaling adapters and regulation of the pro-inflammatory response by PI3K. *Cell Cycle* 11: 3559-3567, 2012.
32. Deguine J and Barton GM: MyD88: A central player in innate immune signaling. *F1000Prime Rep* 6: 97, 2014.
33. Takeda K and Akira S: TLR signaling pathways. *Semin Immunol* 16: 3-9, 2004.
34. Boyd JH, Divangahi M, Yahiaoui L, Gvozdic D, Qureshi S and Petrof BJ: Toll-like receptors differentially regulate CC and CXCL chemokines in skeletal muscle via NF-kappaB and calcineurin. *Infect Immun* 74: 6829-6838, 2006.
35. Bassett SA and Barnett MP: The role of dietary histone deacetylases (HDACs) inhibitors in health and disease. *Nutrients* 6: 4273-4301, 2014.
36. Quintero-Fabián S, Ortuño-Sahagún D, Vázquez-Carrera M and López-Roa RI: Alliin, a garlic (*Allium sativum*) compound, prevents LPS-induced inflammation in 3T3-L1 adipocytes. *Mediators Inflamm* 2013: 381815, 2013.
37. Arreola R, Quintero-Fabián S, López-Roa RI, Flores-Gutiérrez EO, Reyes-Grajeda JP, Carrera-Quintanar L and Ortuño-Sahagún D: Immunomodulation and anti-inflammatory effects of garlic compounds. *J Immunol Res* 2015: 401630, 2015.
38. Ley RE, Peterson DA and Gordon JI: Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 124: 837-848, 2006.
39. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, *et al*: MetaHIT Consortium: A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464: 59-65, 2010.
40. Fagarasan S and Honjo T: Intestinal IgA synthesis: Regulation of front-line body defences. *Nat Rev Immunol* 3: 63-72, 2003.
41. Yasuda T, Hayakawa F, Kurahashi S, Sugimoto K, Minami Y, Tomita A and Naoe T: B cell receptor-ERK1/2 signal cancels PAX5-dependent repression of BLIMP1 through PAX5 phosphorylation: A mechanism of antigen-triggering plasma cell differentiation. *J Immunol* 188: 6127-6134, 2012.



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