Advances in Experimental Medicine and Biology 1209

Jun Cui *Editor*

Autophagy Regulation of Innate Immunity

Springer

Advances in Experimental Medicine and Biology

Volume 1209

Advances in Experimental Medicine and Biology presents multidisciplinary and dynamic findings in the broad fields of experimental medicine and biology. The wide variety in topics it presents offers readers multiple perspectives on a variety of disciplines including neuroscience, microbiology, immunology, biochemistry, biomedical engineering and cancer research.

Advances in Experimental Medicine and Biology has been publishing exceptional works in the field for over 30 years and is indexed in Medline, Scopus, EMBASE, BIOSIS, Biological Abstracts, CSA, Biological Sciences and Living Resources (ASFA-1), and Biological Sciences. The series also provides scientists with up to date information on emerging topics and techniques. 2018 Impact Factor: 2.126.

More information about this series at http://www.springer.com/series/5584

Jun Cui
Editor

Autophagy Regulation of Innate Immunity

Springer

Editor
Jun Cui
School of Life Sciences
Sun Yat-sen University
Guangzhou, Guangdong, China

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-981-15-0605-5 ISBN 978-981-15-0606-2 (eBook)
https://doi.org/10.1007/978-981-15-0606-2

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

Contributors

He-Nan Bao State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, People's Republic of China

Jun Cui MOE Key Laboratory of Gene Function and Regulation, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China

Jian Deng School of Biomedical Sciences, The University of Hong Kong, Hong Kong, China

Lian He Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX, USA

Wenzhuo He State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

Shouheng Jin MOE Key Laboratory of Gene Function and Regulation, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China

Yuqing Lei Department of Neurosurgery, State Key Laboratory of Biotherapy, Collaborative Innovation Center for Biotherapy, West China Hospital, Sichuan University, Chengdu, China

Huihui Li State Key Laboratory of Biotherapy, West China Second University Hospital, Sichuan University, Chengdu, China

Jiang-Yuan Li Key Laboratory for Cell and Gene Engineering of Zhejiang Province, College of Life Sciences, Zhejiang University, Hangzhou, People's Republic of China

Eena Lin Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX, USA

Bo Liu State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu, China

Tao Liu Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

Kefeng Lu Department of Neurosurgery, State Key Laboratory of Biotherapy, Collaborative Innovation Center for Biotherapy, West China Hospital, Sichuan University, Chengdu, China

Jingrong Mao Xiangya Hospital, Central South University, Changsha, People's Republic of China

Mei-Ling Nan Advanced Medical Research Institute, Shandong University, Jinan, Shandong, China

Akinkunmi Okekunle The Postgraduate College, University of Ibadan, Ibadan, Nigeria;
Department of Epidemiology and Medical Statistics, College of Medicine, University of Ibadan, Ibadan, Nigeria

Sunil Kumar Sahu State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, People's Republic of China

Peng Tan Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX, USA

Ling-Yan Wang State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, People's Republic of China

Pei-Hui Wang Advanced Medical Research Institute, Shandong University, Jinan, Shandong, China;
School of Biomedical Sciences, The University of Hong Kong, Hong Kong, China

Yaoxing Wu MOE Key Laboratory of Gene Function and Regulation, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China

Xiaojun Xia State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

Wenjing Xiong State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

Nan Yao State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, People's Republic of China

Youqiong Ye Department of Biochemistry and Molecular Biology, University of Texas Health Science Center at Houston McGovern Medical School, Houston, TX, USA

Jiaming Yu Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, People's Republic of China

Xiao-Qiang Yu School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO, USA

Hong-Yun Zeng State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, People's Republic of China

Jing Zhang Advanced Medical Research Institute, Shandong University, Jinan, Shandong, China

Lan Zhang School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, China

Ping Zheng State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou, People's Republic of China

Yubin Zhou Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX, USA

Yun Zhu Department of Pediatric Surgery, Guangzhou Women and Children's Medical Center, Guangzhou Institute of Pediatrics, Guangzhou Medical University, Guangzhou, Guangdong, China;
School of Biomedical Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China

Chapter 1
Introduction

Jun Cui

Abstract Innate immunity plays an important role in the host defense of a variety of different pathogens, and its responses must be tightly regulated to effectively eliminate microbial invasion and avoid immune-related diseases. Autophagy is a homeostatic process that is critical for the bulk degradation of cellular constituents. Recently, accumulating evidence demonstrates the emerging role of the autophagy in the regulation of innate immune responses. In this chapter, we will have a broad overview of the function of autophagy in innate immunity, and briefly introduce the composition of this book and the content of each chapter.

Keywords Autophagy · Innate immunity · Autophagy-related proteins · Immune regulation · Cargo receptors

1.1 Overview of Innate Immunity and Autophagy

The immune system is primarily responsible for host defense, which can be further classified as the innate immune system and the adaptive immune system (only in vertebrates) [3]. The innate immune cells, such as macrophages, dendritic cells (DCs), nature killer (NK) cells, and mast cells trigger the innate immune responses, while the antigen-specific T cells and B cells initiate adaptive immunity responses [32]. A typical characteristic of the immune system is to distinguish the "self" and "non-self" components and generate effective responses to eliminate the "non-self" constitutes [24, 31]. The immune cells can be activated and undergo a set of cellular processes including proliferation and differentiation to generate proper immune responses, once they encounter their relevant ligands [4, 34]. However, when faced with "self" component, immune cells display low response or even no response. The

J. Cui (✉)
MOE Key Laboratory of Gene Function and Regulation, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou 510006, Guangdong, China
e-mail: cuij5@mail.sysu.edu.cn

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_1

state of unresponsiveness of the immune system under certain condition is termed as immune tolerance. As a particular form of an immune response, immune tolerance generally shows no reaction to a specific allogeneic antigen, while maintains a normal response to other antigens [27, 29].

The innate immunity is an ancient and conservative defense strategy, and it plays a dominant role in plants, fungi, insects, and primitive multicellular organisms, which do not have an adaptive immune system. In mammals, the innate immune system serves as the first line of host defense, and deploys the germline-encoded receptors named as pattern-recognition receptors (PRRs) to sense and respond to invading microbial pathogens [3, 16]. PRRs contain Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), NOD-like receptors (NLRs), and several nucleic acid sensors that detect viral DNA or RNA [18]. PRRs recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), thus activating a series of intracellular signaling proteins to turn on the transcription factor nuclear factor-κB (NF-κB) and IRF3/IRF7, or inflammasome pathways [36]. The PRR signaling pathways mediate the production of proinflammatory cytokines and type I interferons, which subsequently eliminates the invading pathogens and provokes the comprehensive antimicrobial states [5]. The PRR signaling must be tightly controlled to facilitate the microbial clearance timely and maintain an appropriate immune response to avoid harmful effects [3]. The abnormal PRR signaling is widely associated with the development of a variety of diseases, such as tissue pathology and dysfunction in infectious diseases, autoimmune diseases, neurological diseases, and cardiovascular diseases [6].

Autophagy, orchestrated by a number of autophagy-related (ATG) proteins, plays an essential role in the capture and delivery of cellular substrates to the double-membraned vesicles named as autophagosomes [23]. The autophagosomes further fuse with lysosomes, thus exerting the degradation and recycling of intracellular macromolecules and organelles [22]. Autophagy is a critical process to maintain cellular homeostasis, development, and tumorigenesis in mammalian cells [28]. Besides its function in the "bulk degradation" of cellular components, autophagy is also indispensable for the regulation of the immune system [21]. During viral or bacterial infection, the innate immune system could induce autophagy to provide a series of barriers against invading microbial pathogens [8]. Additionally, autophagy is highly integrated with the innate and adaptive immune systems. In humans, a failure in any part of the autophagy flux can lead to inflammatory, autoimmune, or general immune disorders [11].

Innate immune cells, such as mast cells, macrophages, neutrophils, DCs, eosinophils, basophils, NK cells, and γδ T cells, are highly heterogeneous and are produced by the differentiation of pluripotent hematopoietic stem cells residing in bone marrow, but not proliferated for their own. Autophagy regulates pluripotent hematopoietic stem cells from the bone marrow to develop into a variety of innate immune cells through complicated mechanisms [10, 26, 33, 35]. Moreover, autophagy regulates the metabolism and longevity of immune cells to maintain T/B

lymphocytes homeostasis and the distribution of lymphocyte subsets [9, 12]. During the activation of lymphocytes, autophagy controls the anergic state of lymphocytes and affects the proliferation of effector lymphocytes [9, 14]. Autophagy also plays a vital role in the formation of memory lymphocytes. In addition, autophagy affects the differentiation and function of immunomodulatory cells like regulatory T cells and regulatory DCs [1, 7]. Collectively, autophagy is essential for the formation and function of immune cells.

Accumulating evidence has indicated that ATG proteins function not only in autophagy but also in other processes, to perform widespread physiological functions in innate immune signaling pathways. PRR-mediated type I interferon, pro-inflammatory signaling, and inflammasome pathway, have deeply interplayed with autophagy [11]. TLR signaling triggers MyD88 or TRIF to interact with Beclin-1, leading to its dissociation from the B cell lymphoma-2 (BCL-2) inhibitory complex and the activation of autophagy [8]. The rapid BCL-2 phosphorylation mediated by JUN N-terminal kinase (JNK) induces autophagy through disrupting BCL2–Beclin-1 association [37]. The ATG5–ATG12 conjugation inhibits RIG-I–MAVS signaling, while ATG9A and ULK1 suppress STING-mediated type I interferon production [19, 20, 30]. Moreover, Beclin-1 suppresses not only the cGAS-mediated IFN signaling but also MAVS-mediated antiviral responses [17]. Autophagy balances and regulates immune activation to avoid excessive inflammatory responses. Autophagy can eliminate damaged or irreversible depolarized mitochondria, the process of which is often termed as mitophagy, thus reducing the release of inflammasome agonist, such as reactive oxygen species (ROS) and mitochondrial DNA, which ultimately restricts the activation of inflammasomes [11, 34].

Autophagy can be highly selective as it could specifically target intracellular substrates by cargo receptors [2]. p62/SQSTM1, NDP52, OPTN, NBR1, and Tollip are most well-defined cargo receptors, which contain both identifiable LC3-interacting region (LIR) motif to directly bind LC3/GABARAPs and ubiquitin-binding domain (UBD) to recognize the substrate labeled with poly-ubiquitin chains [15, 18]. Therefore, cargo receptors are capable to capture and deliver specific substrates to autophagosomes for selective degradation. Accumulating evidence indicate that ubiquitin and ubiquitin-like modifications, such as ISG15 [13], play essential roles in the recognition of cargo proteins in selective autophagy, however, the mechanism underlying a limited number of cargo receptors to mediate the degradation of such numerous cargoes still remains largely unclear. Secondary receptors or co-receptors, like tripartite motif (TRIM) and leucine-rich repeat containing (LRRC) family protein members bridge the key inflammatory and immune signaling molecules, such as absent in melanoma 2 (AIM2), p65/RelA, and RIG-I for p62-mediated selective autophagy degradation [25, 38]. Uncover the unidentified cargo receptors, secondary receptors/co-receptors, cargoes, and recognition signals in innate immunity remain hot topics that warrant further dissection, which can provide potential targets for selective autophagy-based immune therapy.

1.2 Parts of the Book

Part I of the book introduces the regulatory functions of autophagy on the immune system in mammals and plants. Chapters 2 and 3 discuss the crucial roles of autophagy in mammalian and plant immunity, respectively. Part II of the book introduces the detailed roles of autophagy in the microbial invasion. Chapters 4 and 5 provide an overview of autophagy in bacterial and viral pathogen infection, and the strategies that autophagy combat pathogens and pathogens utilize autophagy for their survival and proliferation. Part III of the book introduces the roles of autophagy in the regulation of innate immune signaling pathways. Chapter 6 focuses on the interplay between proinflammatory signaling and autophagy. Chapter 7 discusses the critical roles of autophagy in inflammasome activation and regulation. Chapter 8 provides a comprehensive view of the cross-regulation between autophagy and type I interferon responses. Part IV of the book introduces selective autophagy and cargo receptor network. Chapter 9 discusses the roles of cargo receptor networks built up by cargo receptor, secondary receptor/co-receptor, and recognition signal in innate immune responses. Part V of the book introduces autophagy in immune-related diseases (such as autoimmune diseases) and drug design targeting them. Chapter 10 focuses on the emerging roles of autophagy in immune-related diseases and Chapter 11 provides the application of small-molecule modulators in immune-related diseases via targeting autophagy.

In summary, this book highlights the frontier advances of autophagy in host defense, the mechanisms underpinning elimination of infected microbial pathogens by autophagy, the crosstalk between autophagy and PRR signaling pathways to shape host defense, and reveals the functions of autophagy in autoimmune diseases and offers novel thinking to design small-molecule drugs based on autophagy to treat immune-related diseases. It is essential to reveal and understand the mechanisms of autophagy in regulating innate immunity, so as to manipulate the immune responses by orchestrating autophagy. Since autophagy and innate immunity interrelate with each other, the "one size fits all" approach to intervene in autophagy flux may have some risks for immune therapy. A better strategy might be used to manipulate the selective autophagic degradation of specific substrates to modulate the innate immune responses. Therefore, immunotherapies that rely on selective autophagy may have broad application prospects in the treatment of a variety of diseases in the future.

Acknowledgements This work was supported by National Natural Science Foundation of China (31870862, 31700760 and 31800751), and Science and Technology Planning Project of Guangzhou, China (201804010385).

References

1. Alissafi T, Banos A, Boon L, Sparwasser T, Ghigo A, Wing K, Vassilopoulos D, Boumpas D, Chavakis T, Cadwell K et al (2017) Tregs restrain dendritic cell autophagy to ameliorate autoimmunity. J Clin Invest 127:2789–2804
2. Anding AL, Baehrecke EH (2017) Cleaning house: selective autophagy of organelles. Dev Cell 41:10–22
3. Cao XT (2016) Self-regulation and cross-regulation of pattern-recognition receptor signalling in health and disease. Nat Rev Immunol 16:35–50
4. Chan YK, Gack MU (2016) Viral evasion of intracellular DNA and RNA sensing. Nat Rev Microbiol 14:360–373
5. Chen K, Liu J, Cao XT (2017) Regulation of type I interferon signaling in immunity and inflammation: a comprehensive review. J Autoimmun 83:1–11
6. Chiang C, Gack MU (2017) Post-translational control of intracellular pathogen sensing pathways. Trends Immunol 38:39–52
7. Choi AMK, Ryter SW, Levine B (2013) Autophagy in human health and disease. N Engl J Med 368:651–662
8. Choi Y, Bowman JW, Jung JU (2018) Autophagy during viral infection—a double-edged sword. Nat Rev Microbiol 16:340–353
9. Clarke AJ, Simon AK (2018) Autophagy in the renewal, differentiation and homeostasis of immune cells. Nat Rev Immunol
10. Deretic V, Kimura T, Timmins G, Moseley P, Chauhan S, Mandell M (2015) Immunologic manifestations of autophagy. J Clin Invest 125:75–84
11. Deretic V, Levine B (2018) Autophagy balances inflammation in innate immunity. Autophagy 14:243–251
12. Deretic V, Saitoh T, Akira S (2013) Autophagy in infection, inflammation and immunity. Nat Rev Immunol 13:722–737
13. Du Y, Duan TH, Feng YC, Liu QX, Lin M, Cui J, Wang RF (2018) LRRC25 inhibits type I IFN signaling by targeting ISG15-associated RIG-I for autophagic degradation. EMBO J 37:351–366
14. Gapin L (2016) Development of invariant natural killer T cells. Curr Opin Immunol 39:68–74
15. Gatica D, Lahiri V, Klionsky DJ (2018) Cargo recognition and degradation by selective autophagy. Nat Cell Biol 20:233–242
16. Ivashkiv LB, Donlin LT (2014) Regulation of type I interferon responses. Nat Rev Immunol 14:36–49
17. Jin SH, Tian S, Chen YM, Zhang CX, Xie WH, Xia XJ, Cui J, Wang RF (2016) USP19 modulates autophagy and antiviral immune responses by deubiquitinating Beclin-1. EMBO J 35:866–880
18. Jin SH, Tian S, Luo M, Xie WH, Liu T, Duan TH, Wu YX, Cui J (2017) Tetherin suppresses type I interferon signaling by targeting MAVS for NDP52-mediated selective autophagic degradation in human cells. Mol Cell 68:308–322
19. Jounai N, Takeshita F, Kobiyama K, Sawano A, Miyawaki A, Xin KQ, Ishii KJ, Kawaii T, Akira S, Suzuki K et al (2007) The Atg5–Atg12 conjugate associates with innate antiviral immune responses. Proc Natl Acad Sci USA 104:14050–14055
20. Konno H, Konno K, Barber GN (2013) Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. Cell 155:688–698
21. Kumar H, Kawai T, Akira S (2011) Pathogen recognition by the innate immune system. Int Rev Immunol 30:16–34
22. Levine B, Kroemer G (2019) Biological functions of autophagy genes: a disease perspective. Cell 176:11–42
23. Levy JMM, Towers CG, Thorburn A (2017) Targeting autophagy in cancer. Nat Rev Cancer 17:528–542
24. Liu J, Qian C, Cao XT (2016) Post-translational modification control of innate immunity. Immunity 45:15–30

25. Liu T, Tang Q, Liu KP, Xie WH, Liu X, Wang HS, Wang RF, Cui J (2016) TRIM11 suppresses AIM2 inflammasome by degrading AIM2 via p62-dependent selective autophagy. Cell Rep 16:1988–2002
26. Lopez-Soto A, Bravo-San Pedro JM, Kroemer G, Galluzzi L, Gonzalez S (2017) Involvement of autophagy in NK cell development and function. Autophagy 13:633–636
27. Maurer K, Reyes-Robles T, Alonzo F, Durbin J, Torres VJ, Cadwell K (2015) Autophagy mediates tolerance to Staphylococcus aureus α-toxin. Cell Host Microbe 17:429–440
28. Riffelmacher T, Richter FC, Simon AK (2018) Autophagy dictates metabolism and differentiation of inflammatory immune cells. Autophagy 14:199–206
29. Rongvaux A (2018) Innate immunity and tolerance toward mitochondria. Mitochondrion 41:14–20
30. Saitoh T, Fujita N, Hayashi T, Takahara K, Satoh T, Lee H, Matsunaga K, Kageyama S, Omori H, Noda T et al (2009) Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. Proc Natl Acad Sci USA 106:20842–20846
31. Schlee M, Hartmann G (2016) Discriminating self from non-self in nucleic acid sensing. Nat Rev Immunol 16:566–580
32. Shibutani ST, Saitoh T, Nowag H, Munz C, Yoshimori T (2015) Autophagy and autophagy-related proteins in the immune system. Nat Immunol 16:1014–1024
33. Stranks AJ, Hansen AL, Panse I, Mortensen M, Ferguson DJP, Puleston DJ, Shenderov K, Watson AS, Veldhoen M, Phadwal K et al (2015) Autophagy controls acquisition of aging features in macrophages. J Innate Immun 7:375–391
34. Takahama M, Akira S, Saitoh T (2018) Autophagy limits activation of the inflammasomes. Immunol Rev 281:62–73
35. Ushio H, Ueno T, Kojima Y, Komatsu M, Tanaka S, Yamamoto A, Ichimura Y, Ezaki J, Nishida K, Komazawa-Sakon S et al (2011) Crucial role for autophagy in degranulation of mast cells. J Allergy Clin Immunol 127:1267–1282
36. Wang WD, Jiang MH, Liu S, Zhang SK, Liu W, Ma YW, Zhang LF, Zhang JY, Cao XT (2016) RNF122 suppresses antiviral type I interferon production by targeting RIG-I CARDs to mediate RIG-I degradation. Proc Natl Acad Sci USA 113:9581–9586
37. Wei YJ, Sinha S, Levine B (2008) Dual role of JNK1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation. Autophagy 4:949–951
38. Xian HF, Yang S, Jin SH, Zhang YX, Cui J (2019) LRRC59 modulates type I interferon signaling by restraining the SQSTM1/p 62-mediated autophagic degradation of pattern recognition receptor DDX58/RIG-I. Autophagy 1–11

Chapter 2
Autophagy Regulation of Mammalian Immune Cells

Wenzhuo He, Wenjing Xiong and Xiaojun Xia

Abstract Autophagy is a fully competent cellular machinery able to carry out the clearance of macromolecules via fusion with the lysosome. Many studies conducted in recent years have revealed that autophagy not only plays a critical role in maintaining cell homeostasis, but can also promote bacterial elimination. Additionally, autophagy exists in most eukaryotic cells including immune cells, such as lymphocytes, neutrophils, eosinophils, mast cells, and natural killer cells. Presently, there are numerous studies focusing on the roles of autophagy in regulating immune response. Autophagy regulates the innate and adaptive immunity by modulating cell differentiation, survival, phagocytosis, antigen presentation, degranulation, and cytokine production. In this chapter, we will summarize how autophagy participates explicitly in the survival and function of the mammalian adaptive and innate immune cells.

Keywords Autophagy · Immune cells · Lymphocyte · Neutrophil · Macrophage · Dendritic cell · Natural killer cell

Autophagy is a highly conserved catalytic process in almost all eukaryotic species and can transport cytoplasmic substrates, ranging from soluble macromolecules to whole organelles, for lysosomal degradation [34]. According to the mechanisms by which macromolecules are degraded, autophagy can be further classified into three types, namely, macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) [43].

Macroautophagy is the most well-studied type of autophagy. During the activation of macroautophagy, autophagy-related proteins (ATGs) generate a double-membrane vesicle which can load intracellular material or abandoned molecules, termed autophagosomes, which can then fuse with lysosomes [22, 23]. Depending

W. Z. He and W. J. Xiong—Contributed equally to this work.

W. He · W. Xiong · X. Xia (✉)
State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China
e-mail: xiaxj@sysucc.org.cn

W. He
VIP Region, Sun Yat-sen University Cancer Center, Guangzhou, China

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_2

on the specific mechanisms for recognizing different cargos, macroautophagy can be defined into specific forms, namely, mitophagy, involves the degradation of mitochondria [20], lipophagy, defined as the autophagic degradation of lipid droplets [66], and xenophagy, characterized by delivered pathogens into autophagosomes [3]. Microautophagy occurs on the lysosomal membrane of yeast and mammalian cells, such as dendritic cells (DCs), and through forming invaginations, involving cytosolic cargo, into the lysosome. Microautophagy takes place in the late endosome and is termed endosomal microautophagy [50]. CMA is a selective form of autophagy. In this situation, cytosolic proteins are transported by chaperone into lysosome for degradation [59] and CMA coordination with endoplasmic reticulum (ER) to regulate the network of physiological and pathological stress response [27].

Maintaining cell homeostasis is just one of the many functions regulated by autophagy. Autophagy also acts as a crucial regulator in the regulation of immune response by modulating the survival, differentiation, and functions of immune cells, including innate immune cells, T cells, and B cells, in mammals [11, 12]. In this chapter, we briefly introduce the current knowledge about the role of autophagy in regulating mammalian immune cells.

2.1 Autophagy and Innate Immune Cells

Innate immunity provides early defense against invading microbes. The innate immune cells recognize microbes via innate immune receptors identifying structures common to certain microbes, the so-called pathogen-associated molecular patterns (PAMPs). We will discuss the role of autophagy in various types of innate immune cells, including neutrophils, eosinophils, monocytes, macrophages, natural killer cells, dendritic cells, and mast cells.

2.1.1 Autophagy and Neutrophils

Neutrophils serve as the first line of defense in our body. The roles of autophagy in neutrophil differentiation, phagocytosis, degranulation, cell death, and neutrophil extracellular trap formation have been well studied. It is reported that autophagic activity is inversely associated with the neutrophil differentiation rate [49]. Upon mammalian target of rapamycin complex 1 (mTORC1) inhibition by rapamycin treatment in neutrophilic precursor cells, autophagy would be induced in these precursor cells to further impede their differentiation [49]. Inhibiting autophagy-mediated lipid degradation is sufficient to disturb neutrophil differentiation [46]. Reduced autophagy was found in patients with primary acute myeloid leukemia, while treatment with all-trans retinoic acid was found to induce autophagy and neutrophil differentiation [19].

Neutrophils are able to activate autophagy for eliminating pathogenic adherent-invasive *Escherichia coli* (AIEC) infections. AIEC infection of neutrophil-like PLB-985 cells can block autophagy, thereby allowing the intracellular survival of bacteria, and stimulation of autophagy by nutrient starvation or rapamycin reduced intracellular AIEC survival [7]. Autophagy also contributes to the bacteria-killing in neutrophils. It has been found that *Streptococcus pneumoniae* induced autophagy in neutrophils in a type III phosphatidylinositol-3 kinase-dependent fashion, which requires the autophagy gene *Atg5*. Phagocytosis is enhanced by autophagy, while killing is inhibited by autophagy [62]. For this, ATG5 has been found to play a unique role in the protection against *M. tuberculosis*. The loss of *Atg5* in polymorphonuclear cells has been shown to sensitize mice to *M. tuberculosis* infection [21]. Autophagy-mediated secretary pathway also contributes to IL-1β secretion in human neutrophils [16]. These results suggest that autophagy in neutrophils plays a critical role in fighting against pathogens.

In a myeloid-specific autophagy-deficient mice model, researchers observed that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-mediated reactive oxygen species (ROS) generation was decreased in autophagy-deficient neutrophils, while inhibition of NADPH oxidase reduced neutrophil degranulation [4]. These results indicate that NADPH oxidase is a key player at the intersection of autophagy and degranulation. On the other hand, these mice with autophagy deficiency showed reduced severity in neutrophil-mediated autoimmune disease.

A novel form of programmed necrotic cell death, associated with cytoplasmic organelle fusion events, was found in neutrophils exposed to Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). This form of cell death was associated with autophagy induction [65]. However, no abnormalities in morphology, granule protein content, apoptosis regulation, or migration were observed in *Atg5*-knockout neutrophils [49].

It has been recognized that neutrophil can kill microbial not only by phagocytosis but also through neutrophil extracellular traps (NETs) in the extracellular space [5]. Neutrophils isolated from patients who have survived sepsis was found to have increased autophagy induction and were primed for NET formation in response to subsequent phorbol 12-myristate 13-acetate (PMA) stimulation. In contrast, neutrophils isolated from patients who died from sepsis exhibited dysregulated autophagy and a reduced response to PMA [42]. In a sepsis-stimulated mouse model, the enhancement of autophagy was found to prolong their survival via a NET-dependent mechanism. Mincle functions as an activating receptor for pathogen-derived molecular patterns. Impaired formation of NET in *Mincle*$^{-/-}$ neutrophils correlates with an attenuated autophagy activation in vitro and in vivo. Exogenous treatment with autophagy inducer tamoxifen could rescue the NET formation in *Mincle*$^{-/-}$ neutrophils, indicating that autophagy is required in Mincle-mediated NET formation [55]. These findings suggest that autophagy in neutrophil is required for innate immune receptor-activated NET formation.

2.1.2 Autophagy and Eosinophils

Eosinophils are bone marrow-derived granulocytes and lack a well-defined function. Limited information is available on the role of autophagy in eosinophils. It has been observed that eosinophils can regulate the severity of inflammation. In a mouse model of allergic asthma, eosinophils demonstrated the prominent formation of autophagosomes and increased microtubule-associated proteins light chain 3 (LC3) expression as compared with other inflammatory cells in lung tissue. When autophagy was inhibited by 3-methyl adenine (3-MA) or *Atg5* shRNA treatment, the airway hyperresponsiveness and eosinophilia was much improved [28]. Currently, there is still no information available regarding the role of autophagy in eosinophil differentiation.

2.1.3 Autophagy and Macrophages

Pharmacologic and genetic evidence indicates that autophagy plays a plethora of functions in the differentiation of monocytes into macrophages. By using pharmacologic inhibitors, siRNA approaches, and $Atg7^{-/-}$ mice, researchers found that autophagy was required for proper colony-stimulating factor-1-driven differentiation of human and murine monocytes [17]. Another group also found that GM-CSF blocked the cleavage of the autophagy protein ATG5, and induced JNK activation to mediate the disassociation of Beclin-1 and Bcl-2, followed by the induction of autophagy [73]. Preventing autophagy induction hampered monocyte differentiation and cytokine production. Therefore, autophagy probably mediates an important transition from monocyte apoptosis to differentiation.

Autophagy can target bacteria into autophagosomes, and further fuse autophagosomes with lysosomes. For instance, group *A Streptococcus* (GAS) can escape from endosomes into the cytoplasm to evade immune clearance. However, GAS can be enveloped by autophagosome-like compartments and be killed upon fusion with lysosomes. In autophagy-deficient $Atg5^{-/-}$ cells, GAS survived, multiplied, and were released from cells, indicating that autophagy could act as an innate defense system against invading pathogens [37]. *M. tuberculosis* can be used as a second example. *M. tuberculosis* is an intracellular pathogen surviving through the interference with phagolysosome biogenesis. Induction of autophagy suppresses the intracellular survival of mycobacteria because autophagic pathways can overcome the trafficking block imposed by *M. tuberculosis* [13]. Toll-like receptors (TLRs) might also play a crucial role in this situation. TLR4 activation can recruit myeloid differentiation primary response 88 (MyD88), which can further bind to Beclin-1 to initiate autophagy [56]. On the other side, pathogens can activate cytosolic receptors and induce autophagy. During infection, nucleotide-binding oligomerization domain (Nod) proteins Nod1 and Nod 2 were found to recruit the autophagy protein Atg16l1 to the plasma membrane at the bacterial entry site. Mutant Nod2 failed to recruit Atg16l1 to the plasma membrane and consequently wrapping of invading bacteria by autophagosomes was impaired [61].

Using mice with macrophage-specific deletion of *Atg5*, researchers have shown that myeloid *Atg5* contributed to in vivo resistance to intracellular pathogens *Listeria monocytogenes* and *Toxoplasma gondii*. *Atg5* was essential for interferon-gamma (IFN-gamma)/Lipopolysaccharide (LPS)-induced attack on the *T. gondii* parasitophorous vacuole membrane and clearance of parasite, suggesting that *Atg5* function in phagocytic cells is indispensable for cellular immunity against intracellular pathogens [74].

Autophagy in macrophages also affects cytokine production. Inhibiting autophagy, by the blockade of ATG5, ATG7, LC3, and Beclin-1, can enhance IL-1β and IL-18, indicating that autophagy limits the activation of inflammasome [51]. The link between autophagy and cytokine production has been found to impact inflammatory disease outcomes. Gaucher disease is an inherited deficiency with increased secretion of cytokines. Induction of autophagy with small molecules could lower IL-1β secretion and relieve the symptoms of Gaucher disease [1]. On the contrary, the deletion of certain autophagy genes in macrophages can cause inflammation-mediated diseases such as uveitis and hepatic inflammation [29, 52].

2.1.4 Autophagy and Nature Killer Cells

Natural killer (NK) cells were initially defined as large lymphocytes with natural cytotoxic ability against tumor, and were later recognized as a separate lymphocyte lineage. Robust autophagy was found in immature NK cells and contributed to the development of NK cells [31]. By removing damaged mitochondria and reducing intracellular ROS, autophagy could protect NK cells viability [64]. Dysfunctional mitochondria accumulate in NK cells during viral infection, and autophagy is crucially needed in order to remove the depolarized mitochondria. Deletion of *Atg5* significantly reduce the absolute numbers of mature NK cells, while NK cell-specific deletion of *Atg5* caused mice succumbed to *mouse cytomegalovirus* (MCMV) infection quickly [40].

It was observed that targeting autophagy-related gene *BECN1* improved the recruitment of functional NK cells into the tumor bed. *BECN1*-defective tumors transcriptionally upregulated the expression level of chemokine gene CC chemokine ligand 5 (CCL5), which facilitates the infiltration of NK cells. Such infiltration and tumor regression were abrogated by silencing CCL5 in *BECN1*-defective tumors. These results highlight the role of targeting tumor autophagy to recruit NK cells and breaking the immunosuppressive tumor microenvironment barrier [33].

2.1.5 Autophagy and Dendritic Cells

Autophagy can direct pathogens into autophagosomes which subsequently fuse with lysosomes. The pathogens are then presented to $CD4^+$ T cells via major histocompatibility complex (MHC) class II molecules. Thus, epitopes of pathogens and intracellular antigens can be delivered to MHC class II molecules in an autophagy-dependent

manner. There are several ways by which autophagy connects dendritic cells (DCs) with adaptive immunity. The autophagy pathway facilitates the transfer of cytosolic antigens to lysosomal compartments. Autophagy, induced by starvation, promotes MHC-II presentation of peptides, and as such the inhibition of autophagy impairs MHC class II presentation. Autophagosomes can fuse with MHC class II loading compartments and targeting this pathway can promote antigen presentation to $CD4^+$ T cells by MHC class II [39]. Deletion of *Atg5* in mouse DCs could impair $CD4^+$ T cell priming, thereby causing rapid death of mice after infection [26]. Another subset of phagosomes coated with LC3 and maintained phagocytized antigens, prolonged the presentation of MHC-II molecules. A deficiency in this pathway could also impair $CD4^+$ T cell responses, suggesting that autophagy proteins could stabilize pathogen-containing phagosomes for prolonged antigen processing [47]. Autophagy in antigen-presenting cells is a key cellular event for proper presentation of peptides to $CD4^+$ T cells.

Unlike the supportive role of autophagy for $CD4^+$ T cell, autophagy impairs antigen presentation of MHC-I molecules. *Atg5* and *Atg7* knockout DCs exhibit increased MHC-I levels due to decreased degradation of MHC-I molecules [30]. Inhibition of autophagy can enhance $CD8^+$ T cell response during influenza infection.

The MHC class I pathway, which is dependent on the transporter associated with antigen processing (TAP) complex, is responsible for the endogenous presentation of most viral epitopes. Yet, there also exists a TAP-independent pathway in DCs which presents a small number of epitopes to $CD8^+$ T cells. It was found that autophagy could mediate TAP-independent antigen presentation. Through macroautophagy, the antigen is processed via a proteasome-independent pathway, and the peptide epitopes are loaded within the autophagolysosomal compartment. Despite bypassing much of the conventional MHC class I pathway, this kind of antigen presentation mediated by autophagy pathway generates similar epitope as the conventional pathway [60].

2.1.6 Autophagy and Mast Cells

Mast cells (MCs) are cells that reside in all vascularized tissues and secrete various biologically active products. It is well known that MCs are involved in various chronic inflammatory disorders, especially in IgE-mediated allergic reactions. Yet, little is known about the role of autophagy in MCs functions.

The conversion of type I to type II LC3 is a hallmark of autophagy flux. It was observed that this process was constitutively ongoing in mast cells under full nutrient conditions [63]. Although the development of MCs was not affected after the deletion of Atg7, $Atg7^{-/-}$MCs showed severe impairment of degranulation, but not cytokine production [63]. These results suggest that autophagy is essential for degranulation, rather than the development of mast cells. Such findings were with therapeutic significance suggesting that autophagy might serve as a potential target for treating allergic diseases.

2.2 Autophagy and Adaptive Immune Cells

2.2.1 Autophagy and T Cells

2.2.1.1 The Role of Autophagy in T Cell Development

T cells are the cornerstone of adaptive immune responses and autophagy is critical in shaping adaptive T cell immunity. Some reports have shown that the thymus has considerably high basal levels of constitutive autophagy when comparing with other tissues [25, 38], and T cells differentiation from early thymic emigrants to mature peripheral T cells was also controlled by autophagy. Autophagy specifically promotes the subcellular origin of peptide synthesis. Common lymphoid precursors (CLPs) migrate into the thymus and then go through the positive and negative selection to develop into mature T lymphocytes. During positive and negative selection, $CD4^+CD8^+$ double-positive T cells bind with thymic epithelial cells (TECs) or thymic DC, which express the MHC presented peptides, and then develop into $CD4^+$ or $CD8^+$ signal positive cells with the ability of MHC molecules recognition. It has been reported that autophagy promotes TECs and professional antigen-presenting cells (APC) to present intracellular MHC-II peptides [38]. During the process of T cells in early thymic emigrants to mature peripheral T cells, the mitochondrial content reduction occurs and such process is controlled by autophagy. Furthermore, inhibiting autophagy in mouse T cells induced defective mitochondria turnover, resulting in increased ROS generation and altered apoptotic proteins levels [67].

For T cell differentiation, *Atg5* or *Atg7* deficiency in mice caused reduced T cell numbers in both the thymus and peripheral lymphoid tissues but did not affect the differentiation of $CD4^+$ and $CD8^+$ T cells per se [44]. Furthermore, several studies have shown that autophagy significantly contributes to the presentation of nuclear, lysosomal, and mitochondrial peptides but not the presentation of membrane-associated peptides [2]. Moreover, one study investigated the presentation of citrullinated proteins found that autophagy selectively presented citrullinated peptides to $CD4^+$ T cells [15].

Altered calcium mobilization and defects in ER and mitochondria homeostasis were observed in autophagy-deficient T cells in mice. Moreover, mice bearing *Vps34*-deficient T cells develop an inflammatory syndrome due to the defective regulatory T cell (Treg) function, indicating that autophagy is important for regulating T cell population to maintain immune homeostasis [68] (Fig. 2.1).

2.2.1.2 Autophagy in T Cell Metabolism

As an essential catabolic process in T cell biology, autophagy functions to regulate the differentiation and function of T cell through modulating cell metabolism. Autophagy degrades proteins, lipids, and glycogen to provide energy substrates supporting

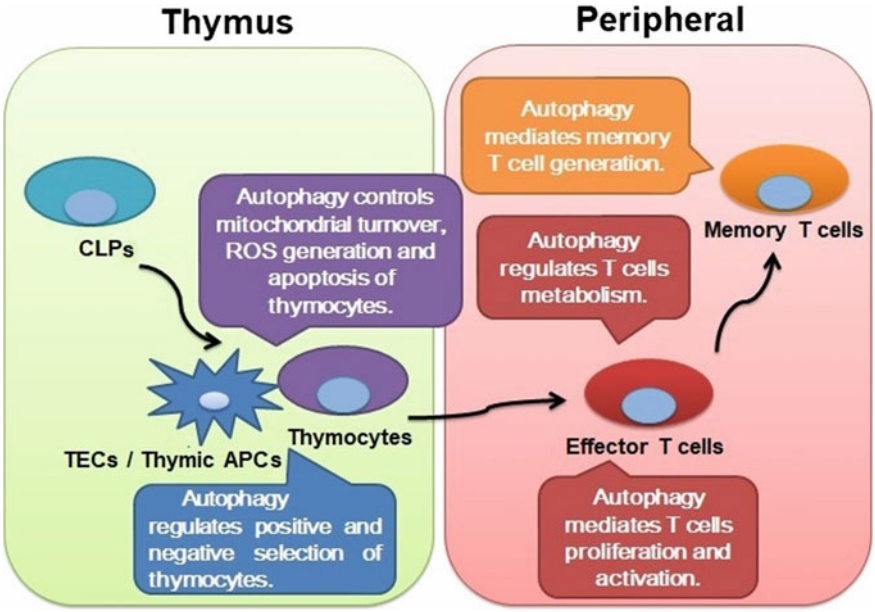


Fig. 2.1 The function of autophagy in T cell development, differentiation, and function. Common Lymphoid precursors (CLPs) migrate into the thymus and then differentiate into mature T lymphocytes and enter the periphery to become effector T cells which are stimulated with APCs. After the primary immune response, most effector T cells die and some survive as memory T cells. The role of autophagy has been indicated in different T cell statuses

T cell activation, while inhibition of this catabolism would prevent efficient T cell activation. Indeed, autophagy-deficient effector T cells exhibited a reduction of cytokine secretion and cell proliferation upon activation [14]. It has been recently proposed that autophagy regulates T cell metabolism via the mTORC1 signaling pathway. Autophagy-mediated protein degradation produces L-cysteine, which then activates mTORC1 to support metabolic changes for T cell proliferation [69].

To fully activate T cells, T cell receptor (TCR) requires engagement with co-stimulatory molecules and cytokine signaling, and during this engagement, autophagosome formation and degradation levels are increased [14]. However, *Atg5*- and *Atg7*-deficient T cells displayed impaired proliferation upon stimulation. Reduction of proliferation and cytokine production following TCR activation has been observed in *Atg7*-deficient naive $CD4^+$ and effector Th1 cells while in the presence of either PI3KC3 inhibitors or lysosomal hydrolases inhibitors, the activation and proliferation levels of T cells were found to increase [14]. In memory $CD8^+$ T cells and Treg cells, autophagy deficiency also markedly changes their metabolic profiles. Mice bearing autophagy-deficient $CD8^+$ T cells are incompetent in generating $CD8^+$ T cell memory, and deletion of *Atg7* or *Atg5* in $Foxp3^+$ T cells showed a reduction of Treg cell stability and survival. Interestingly, *Rab7*-deficient T cells, which are still able to form autophagosomes but cannot fuse with lysosomes, also showed

a reduced ability to proliferate upon TCR and CD28 engagement. Therefore, not only the sequestering of cargo to form autophagosomes is important, but also the degradation by lysosomes for proper T cell activation [48].

2.2.1.3 Autophagy and T Cell Survival

Autophagy also regulates T cell survival. It has been reported that both in naïve and activated T cells with TCR stimulation, numerous autophagy genes (*Atg5*, *Atg7*, *Atg8*, and *BECN1*) were found to be upregulated. Moreover, mice with T cell-specific gene knockout of various autophagy genes (i.e., *Atg3, Atg5*, *Atg7*, *Atg16l1*, *BECN1*, *Vps34*) showed a reduction in the frequencies of thymocytes as well as peripheral CD8 + and $CD4^+$ T cells [24, 32].

Interestingly, autophagy-deficient T cells exhibited increased levels of some proapoptotic molecules, such as procaspase-3, caspase-8, and -9 and Bim [24], the accumulation of dysregulated organelles, and increased the generation of ROS in the cytoplasm [67]; inducing cell death. Therefore, cellular levels of specific proapoptotic proteins might be regulated by the rate of autophagic degradation. In the peripheral lymphoid organs, the survival of naive T cells is dependent on TCR interactions with stromal cells and IL-7 signaling, which requires Atg3-dependent autophagy [54]. Several studies have shown that the autophagy level in T cells is increased after TCR stimulation, which is associated with rapidly increased calcium levels that are shortly activated AMPK to promote autophagy via the phosphorylation of UNC-51-like kinase 1 (ULK1) complex. On the other hand, CDKN1B, the main negative cell-cycle regulator, is accumulated in naïve autophagy-deficient T cells upon T cell activation and cannot be degraded; thereby resulting in an inefficient proliferation of autophagy-deficient T cells [18]. *Vps34* or *ATG* genes deficient mice showed a reduced number of T cells, which are probably attributed to altered regulation of T cell survival and apoptosis [70].

2.2.1.4 Autophagy and T Cell Activation

Recently, autophagy has also been shown to mediate the generation of memory $CD8^+$ T cell. In the acute phase of viral infection, the deletion of ATG gene in $CD8^+$ T cell does not dramatically limit primary CD^{8+} T cell response, suggesting that naive $CD8^+$ T cells might not be dependent on autophagy for clonal expansion. However, after the pathogens have been cleared, $CD8^+$ T cells appear to increase autophagic flux to promote the generation of memory T cells. $CD8^+$ memory T cells occur on lipolysis of futile lipid via lysosome to serve their metabolic demands and this process may be regulated by autophagy. Consequently, a lack of autophagy in $CD8^+$ T cells will result in diminished T cell survival and a defective memory response [72].

MHC-restricted antigen presentation is essential for activating T cell and promotes the intersection between innate and adaptive immune pathways. Autophagy is also essential for antigen presentation. Antigens were processed by professional antigen present cells (like DCs) to form antigenic peptides, which are loaded on class I or II MHC molecules, then presented to activate T cells. Secretion of cytokines in autophagy-deficient DCs is impaired. For example, *Atg5*-knockout DCs exhibited impaired secretion of IL-2 and IFN-γ [36], but the production of IL-12, IL-6, and TNF-α was unchanged. The ability of MHC-II pathway antigen presentation is impaired in *Atg5*-deficient DCs, which might be due to delayed fusion of lysosomes with phagosomes. Therefore, during the process of DCs triggered $CD4^+$ T cells activation and protective antiviral Th1 cell responses via MHC-II antigen presentation, *Atg5*-mediated autophagy is essential for efficient antigen presentation [41].

Mounting evidence has been established on the participation of autophagy in the regulation of proinflammatory cytokines (IL-1, IL-18, IL-17, and IL-23) and chemokines (CXCL1) production. Through numerous means, autophagy can regulate the same pathways but in complicated regulatory networks. For example, autophagy promotes the activation of inflammasome and release of its products such as IL-1 family members by an indirect effect on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway [9]. Additionally, IL-18, IL-23, and IL-17, which can mediate immune responses, can also be regulated by autophagy. IL-23, cooperating with IL-1α/IL-1β, promotes the differentiation of Th17 cell and stimulates innate-like γδ T cells to produce IL-17 [58]. Beyond that, IL-1, IL-17, and CXCL1 can cause neutrophilic tissue infiltration [53].

2.2.2 Autophagy and B Cells

B cells express high basal levels of MHC-II molecules and can serve as antigen-presenting cells. They need to present antigens to primed $CD4^+$ T cells for terminal differentiation into memory or plasma cells and to further generate high-affinity antibodies. It was observed that starvation trigger autophagy in B cells and further contributed to the antigen presentation process. Disruption of autophagy in B cells impairs the citrullination of antigens. One possible explanation can be that internalized antigens co-localize with autophagosomes, which can protect the antigen from the destructive processing and facilitate cross-presentation of epitopes. Indeed, nuclear antigen Epstein–Barr Virus (EBV) nuclear antigen 1 is efficiently processed on MHC-II for presentation to T cells via autophagy [35]. Antigen presentation through autophagy can be regulated by cytokines. For example, interleukin 4 (IL-4) plays an essential role in the induction of B cell autophagy which further enhances B cells antigen presentation and contributes to B cell survival [71].

Studies have highlighted the role of autophagy in B cells survival and function restricted to certain stages and subclasses. *Atg5*-deficient mice exhibited reduced splenic B cell compartments and had higher rates of B cell apoptosis. Moreover, there was no significant defect in pro-B cells in *Atg5*-deficient mice, while the levels of pre-

and immature B cells were drastically impacted. Next, we will discuss autophagy in germinal center (GC) B cells, memory B cells, plasma cells, B1 B cells and several other types of B cell.

2.2.2.1 GC B Cells

GC B cells exhibited a high rate of autophagy after infection. During viral infection, TLR signaling in GC B cells activates lipidation of LC3. Disruption of this process leads to the expansion of memory and plasma cells. Moreover, loss lipidation of LC3 can enhance long-lived antibody responses to influenza virus. B cell activation also triggers an increased rate of autophagy and switches the form of autophagy from canonical way to non-canonical pathways. Disruption of this process is associated with alterations in the metabolic profile of B cells, which affects the GC reaction [45].

2.2.2.2 Memory B Cells

The high dependence of memory B cells and plasma cells on autophagy has also been well studied. Autophagy is indispensable for maintaining the survival of virus-specific memory B cells in mice and protective antibody responses. Mice with *Atg7*-deficient B cells failed to generate a memory B cells response upon a secondary viral challenge, while the primary immune response to influenza virus infection was normal. This means autophagy plays a role in the maintenance of memory B cells and not on the initial formation. Autophagy in memory B cells also limits mitochondrial ROS production and toxicity of peroxidized lipids. It is also possible that the mobilization of lipids through autophagy might be required for the survival of memory B cells [10]. Besides, autophagy restricts the expression of the transcriptional repressor Blimp-1 and immunoglobulins, thereby optimizing energy and viability. As a result, autophagy sustains antibody responses in vivo and is an essential intrinsic determinant of the bone marrow long-lived plasma cell niche. It was observed that a defect in autophagy of B cells also impedes the generation of plasma cells [6].

2.2.2.3 B1 B Cells

Autophagy is known to be modulated by glucose availability and involved in lipid metabolism. A similar situation exists in B cells. B1 B cells are known as innate-like and tissue-resident B cells. This kind of B cells has their own distinct properties. For example, B1 B cells can respond rapidly to certain repertoire of epitopes and are with the capacity to self-renew. B-1a B cells were reported to rely on autophagy for maintaining metabolic homeostasis and self-renewal. Several critical metabolic genes are downregulated and dysfunctional mitochondria are accumulated in the autophagy-deficient B1a B cells. Deletion of the autophagy gene *Atg7* blocks their self-renewal and further leads to a selective loss of the B-1a B cells [8].

2.2.2.4 Other B Cell Types

Autophagy also promotes marginal zone B cells development and survival. Autophagy seems to play an essential role in the maintenance of mature recirculating B cells in the bone marrow (Fraction F according to Hardy's nomenclature) [57].

2.3 Conclusion

Autophagy plays a crucial role in the maintenance of metabolic and genetic homeostasis in eukaryotic organisms. With various ATG protein complexes involved and regulated by a variety of signaling pathways, autophagy plays multiple roles in adaptive and innate immune responses. Therefore, it is essential to figure out the functions of autophagy in immune cell development, differentiation, and activation, so as to manipulate the function of immune cells by orchestrating autophagy to provide a critical theoretical basis for autophagy-related immune disease therapy. At the same time, further research is also needed for delineating the regulation of autophagy in immune response and in hope to provide valuable information for the design of therapeutic approaches.

References

1. Aflaki E, Moaven N, Borger DK, Lopez G, Westbroek W, Chae JJ, Marugan J, Patnaik S, Maniwang E, Gonzalez AN et al (2016) Lysosomal storage and impaired autophagy lead to inflammasome activation in Gaucher macrophages. Aging Cell 15:77–88
2. Aichinger M, Wu C, Nedjic J, Klein L (2013) Macroautophagy substrates are loaded onto MHC class II of medullary thymic epithelial cells for central tolerance. J Exp Med 210:287–300
3. Bah A, Vergne I (2017) Macrophage autophagy and bacterial infections. Front Immunol 8:1483
4. Bhattacharya A, Wei Q, Shin JN, Abdel Fattah E, Bonilla DL, Xiang Q, Eissa NT (2015) Autophagy is required for neutrophil-mediated inflammation. Cell Rep 12:1731–1739
5. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A (2004) Neutrophil extracellular traps kill bacteria. Science 303:1532–1535
6. Cenci S (2014) Autophagy, a new determinant of plasma cell differentiation and antibody responses. Mol Immunol 62:289–295
7. Chargui A, Cesaro A, Mimouna S, Fareh M, Brest P, Naquet P, Darfeuille-Michaud A, Hebuterne X, Mograbi B, Vouret-Craviari V et al (2012) Subversion of autophagy in adherent invasive Escherichia coli-infected neutrophils induces inflammation and cell death. PLoS One 7:e51727
8. Clarke AJ, Riffelmacher T, Braas D, Cornall RJ, Simon AK (2018) B1a B cells require autophagy for metabolic homeostasis and self-renewal. J Exp Med 215:399–413
9. Colleran A, Ryan A, O'Gorman A, Mureau C, Liptrot C, Dockery P, Fearnhead H, Egan LJ (2011) Autophagosomal IkappaB alpha degradation plays a role in the long term control of tumor necrosis factor-alpha-induced nuclear factor-kappaB (NF-kappaB) activity. J Biol Chem 286:22886–22893

10. Fribourg M, Ni J, Nina Papavasiliou F, Yue Z, Heeger PS, Leventhal JS (2018) Allospecific Memory B cell responses are dependent on autophagy. Am J Transpl 18:102–112
11. Germic N, Frangez Z, Yousefi S, Simon HU (2019) Regulation of the innate immune system by autophagy: monocytes, macrophages, dendritic cells and antigen presentation. Cell Death Differ 26:715–727
12. Germic N, Frangez Z, Yousefi S, Simon HU (2019) Regulation of the innate immune system by autophagy: neutrophils, eosinophils, mast cells, NK cells. Cell Death Differ 26:703–714
13. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V (2004) Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell 119:753–766
14. Hubbard VM, Valdor R, Patel B, Singh R, Cuervo AM, Macian F (2010) Macroautophagy regulates energy metabolism during effector T cell activation. J Immunol 185:7349–7357
15. Ireland JM, Unanue ER (2011) Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells. J Exp Med 208:2625–2632
16. Iula L, Keitelman IA, Sabbione F, Fuentes F, Guzman M, Galletti JG, Gerber PP, Ostrowski M, Geffner JR, Jancic CC et al (2018) Autophagy mediates Interleukin-1beta secretion in human neutrophils. Front Immunol 9:269
17. Jacquel A, Obba S, Boyer L, Dufies M, Robert G, Gounon P, Lemichez E, Luciano F, Solary E, Auberger P (2012) Autophagy is required for CSF-1-induced macrophagic differentiation and acquisition of phagocytic functions. Blood 119:4527–4531
18. Jia W, He MX, McLeod IX, Guo J, Ji D, He YW (2015) Autophagy regulates T lymphocyte proliferation through selective degradation of the cell-cycle inhibitor CDKN1B/p27Kip1. Autophagy 11:2335–2345
19. Jin J, Britschgi A, Schlafli AM, Humbert M, Shan-Krauer D, Batliner J, Federzoni EA, Ernst M, Torbett BE, Yousefi S et al (2018) Low Autophagy (ATG) Gene expression is associated with an immature AML blast cell phenotype and can be restored during AML differentiation therapy. Oxid Med Cell Longev 2018:1482795
20. Kim I, Rodriguez-Enriquez S, Lemasters JJ (2007) Selective degradation of mitochondria by mitophagy. Arch Biochem Biophys 462:245–253
21. Kimmey JM, Huynh JP, Weiss LA, Park S, Kambal A, Debnath J, Virgin HW, Stallings CL (2015) Unique role for ATG5 in neutrophil-mediated immunopathology during M. tuberculosis infection. Nature 528:565–569
22. Klionsky DJ, Codogno P (2013) The mechanism and physiological function of macroautophagy. J Innate Immun 5:427–433
23. Klionsky DJ, Emr SD (2000) Autophagy as a regulated pathway of cellular degradation. Science 290:1717–1721
24. Kovacs JR, Li C, Yang Q, Li G, Garcia IG, Ju S, Roodman DG, Windle JJ, Zhang X, Lu B (2012) Autophagy promotes T-cell survival through degradation of proteins of the cell death machinery. Cell Death Differ 19:144–152
25. Kuma A, Hatano M, Matsui M, Yamamoto A, Nakaya H, Yoshimori T, Ohsumi Y, Tokuhisa T, Mizushima N (2004) The role of autophagy during the early neonatal starvation period. Nature 432:1032–1036
26. Lee HK, Mattei LM, Steinberg BE, Alberts P, Lee YH, Chervonsky A, Mizushima N, Grinstein S, Iwasaki A (2010) In vivo requirement for Atg5 in antigen presentation by dendritic cells. Immunity 32:227–239
27. Li W, Zhu J, Dou J, She H, Tao K, Xu H, Yang Q, Mao Z (2017) Phosphorylation of LAMP2A by p38 MAPK couples ER stress to chaperone-mediated autophagy. Nat Commun 8:1763
28. Liu JN, Suh DH, Trinh HK, Chwae YJ, Park HS, Shin YS (2016) The role of autophagy in allergic inflammation: a new target for severe asthma. Exp Mol Med 48:e243
29. Liu K, Zhao E, Ilyas G, Lalazar G, Lin Y, Haseeb M, Tanaka KE, Czaja MJ (2015) Impaired macrophage autophagy increases the immune response in obese mice by promoting proinflammatory macrophage polarization. Autophagy 11:271–284
30. Loi M, Muller A, Steinbach K, Niven J, Barreira da Silva R, Paul P, Ligeon LA, Caruso A, Albrecht RA, Becker AC et al (2016) Macroautophagy proteins control MHC Class I levels on dendritic cells and shape anti-viral CD8(+) T Cell Responses. Cell Rep 15:1076–1087

31. Marcais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, Rabilloud J, Mayol K, Tavares A, Bienvenu J et al (2014) The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nat Immunol 15:749–757
32. Merkley SD, Chock CJ, Yang XO, Harris J, Castillo EF (2018) Modulating T cell responses via autophagy: the intrinsic influence controlling the function of both antigen-presenting cells and T cells. Front Immunol 9:2914
33. Mgrditchian T, Arakelian T, Paggetti J, Noman MZ, Viry E, Moussay E, Van Moer K, Kreis S, Guerin C, Buart S et al (2017) Targeting autophagy inhibits melanoma growth by enhancing NK cells infiltration in a CCL5-dependent manner. Proc Natl Acad Sci USA 114:E9271–E9279
34. Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147:728–741
35. Morandi E, Jagessar SA, t Hart BA, Gran B (2017) EBV infection empowers human B cells for autoimmunity: role of autophagy and relevance to multiple sclerosis. J Immunol 199:435–448
36. Munz C (2019) Autophagy proteins influence endocytosis for MHC restricted antigen presentation. Semin Cancer Biol
37. Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, Nara A, Funao J, Nakata M, Tsuda K et al (2004) Autophagy defends cells against invading group a streptococcus. Science 306:1037–1040
38. Nedjic J, Aichinger M, Emmerich J, Mizushima N, Klein L (2008) Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. Nature 455:396–400
39. Nimmerjahn F, Milosevic S, Behrends U, Jaffee EM, Pardoll DM, Bornkamm GW, Mautner J (2003) Major histocompatibility complex class II-restricted presentation of a cytosolic antigen by autophagy. Eur J Immunol 33:1250–1259
40. O'Sullivan TE, Geary CD, Weizman OE, Geiger TL, Rapp M, Dorn GW 2nd, Overholtzer M, Sun JC (2016) Atg5 is essential for the development and survival of innate lymphocytes. Cell Rep 15:1910–1919
41. Oh DS, Lee HK (2019) Autophagy protein ATG5 regulates CD36 expression and anti-tumor MHC class II antigen presentation in dendritic cells. Autophagy 1–16
42. Park SY, Shrestha S, Youn YJ, Kim JK, Kim SY, Kim HJ, Park SH, Ahn WG, Kim S, Lee MG et al (2017) Autophagy primes neutrophils for neutrophil extracellular trap formation during sepsis. Am J Respir Crit Care Med 196:577–589
43. Parzych KR, Klionsky DJ (2014) An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal 20:460–473
44. Pua HH, Dzhagalov I, Chuck M, Mizushima N, He YW (2007) A critical role for the autophagy gene Atg5 in T cell survival and proliferation. J Exp Med 204:25–31
45. Raso F, Sagadiev S, Du S, Gage E, Arkatkar T, Metzler G, Stuart LM, Orr MT, Rawlings DJ, Jackson SW et al (2018) Alphav integrins regulate germinal center B cell responses through noncanonical autophagy. J Clin Invest 128:4163–4178
46. Riffelmacher T, Clarke A, Richter FC, Stranks A, Pandey S, Danielli S, Hublitz P, Yu Z, Johnson E, Schwerd T et al (2017) Autophagy-dependent generation of free fatty acids is critical for normal neutrophil differentiation. Immunity 47(466–480):e465
47. Romao S, Gasser N, Becker AC, Guhl B, Bajagic M, Vanoaica D, Ziegler U, Roesler J, Dengjel J, Reichenbach J et al (2013) Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. J Cell Biol 203:757–766
48. Roy SG, Stevens MW, So L, Edinger AL (2013) Reciprocal effects of rab7 deletion in activated and neglected T cells. Autophagy 9:1009–1023
49. Rozman S, Yousefi S, Oberson K, Kaufmann T, Benarafa C, Simon HU (2015) The generation of neutrophils in the bone marrow is controlled by autophagy. Cell Death Differ 22:445–456
50. Sahu R, Kaushik S, Clement CC, Cannizzo ES, Scharf B, Follenzi A, Potolicchio I, Nieves E, Cuervo AM, Santambrogio L (2011) Microautophagy of cytosolic proteins by late endosomes. Dev Cell 20:131–139
51. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M et al (2008) Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature 456:264–268

52. Santeford A, Wiley LA, Park S, Bamba S, Nakamura R, Gdoura A, Ferguson TA, Rao PK, Guan JL, Saitoh T et al (2016) Impaired autophagy in macrophages promotes inflammatory eye disease. Autophagy 12:1876–1885
53. Sawant KV, Xu R, Cox R, Hawkins H, Sbrana E, Kolli D, Garofalo RP, Rajarathnam K (2015) Chemokine CXCL1-mediated neutrophil trafficking in the lung: role of CXCR53 activation. J Innate Immun 7:647–658
54. Sena LA, Li S, Jairaman A, Prakriya M, Ezponda T, Hildeman DA, Wang CR, Schumacker PT, Licht JD, Perlman H et al (2013) Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling. Immunity 38:225–236
55. Sharma A, Simonson TJ, Jondle CN, Mishra BB, Sharma J (2017) Mincle-mediated neutrophil extracellular trap formation by regulation of autophagy. J Infect Dis 215:1040–1048
56. Shi CS, Kehrl JH (2008) MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. J Biol Chem 283:33175–33182
57. Siggs OM, Stockenhuber A, Deobagkar-Lele M, Bull KR, Crockford TL, Kingston BL, Crawford G, Anzilotti C, Steeples V, Ghaffari S et al (2016) Mutation of Fnip1 is associated with B-cell deficiency, cardiomyopathy, and elevated AMPK activity. Proc Natl Acad Sci USA 113:E3706–E3715
58. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH (2009) Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity 31:331–341
59. Tekirdag K, Cuervo AM (2018) Chaperone-mediated autophagy and endosomal microautophagy: joint by a chaperone. J Biol Chem 293:5414–5424
60. Tey SK, Khanna R (2012) Autophagy mediates transporter associated with antigen processing-independent presentation of viral epitopes through MHC class I pathway. Blood 120:994–1004
61. Travassos LH, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhaes JG, Yuan L, Soares F, Chea E, Le Bourhis L et al (2010) Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat Immunol 11:55–62
62. Ullah I, Ritchie ND, Evans TJ (2017) The interrelationship between phagocytosis, autophagy and formation of neutrophil extracellular traps following infection of human neutrophils by Streptococcus pneumoniae. Innate Immun 23:413–423
63. Ushio H, Ueno T, Kojima Y, Komatsu M, Tanaka S, Yamamoto A, Ichimura Y, Ezaki J, Nishida K, Komazawa-Sakon S et al (2011) Crucial role for autophagy in degranulation of mast cells. J Allergy Clin Immunol 127(1267–1276):e1266
64. Wang S, Xia P, Huang G, Zhu P, Liu J, Ye B, Du Y, Fan Z (2016) FoxO1-mediated autophagy is required for NK cell development and innate immunity. Nat Commun 7:11023
65. Wang X, He Z, Liu H, Yousefi S, Simon HU (2016) Neutrophil necroptosis is triggered by ligation of adhesion molecules following GM-CSF priming. J Immunol 197:4090–4100
66. Ward C, Martinez-Lopez N, Otten EG, Carroll B, Maetzel D, Singh R, Sarkar S, Korolchuk VI (2016) Autophagy, lipophagy and lysosomal lipid storage disorders. Biochim Biophys Acta 1861:269–284
67. Watanabe R, Fujii H, Shirai T, Saito S, Ishii T, Harigae H (2014) Autophagy plays a protective role as an anti-oxidant system in human T cells and represents a novel strategy for induction of T-cell apoptosis. Eur J Immunol 44:2508–2520
68. Wei J, Long L, Yang K, Guy C, Shrestha S, Chen Z, Wu C, Vogel P, Neale G, Green DR et al (2016) Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. Nat Immunol 17:277–285
69. Whang MI, Tavares RM, Benjamin DI, Kattah MG, Advincula R, Nomura DK, Debnath J, Malynn BA, Ma A (2017) The ubiquitin binding protein TAX1BP1 mediates autophagasome induction and the metabolic transition of activated T cells. Immunity 46:405–420
70. Willinger T, Flavell RA (2012) Canonical autophagy dependent on the class III phosphoinositide-3 kinase Vps34 is required for naive T-cell homeostasis. Proc Natl Acad Sci USA 109:8670–8675
71. Xia F, Deng C, Jiang Y, Qu Y, Deng J, Cai Z, Ding Y, Guo Z, Wang J (2018) IL4 (interleukin 4) induces autophagy in B cells leading to exacerbated asthma. Autophagy 14:450–464

72. Xu X, Araki K, Li S, Han JH, Ye L, Tan WG, Konieczny BT, Bruinsma MW, Martinez J, Pearce EL et al (2014) Autophagy is essential for effector CD8(+) T cell survival and memory formation. Nat Immunol 15:1152–1161
73. Zhang Y, Morgan MJ, Chen K, Choksi S, Liu ZG (2012) Induction of autophagy is essential for monocyte-macrophage differentiation. Blood 119:2895–2905
74. Zhao Z, Fux B, Goodwin M, Dunay IR, Strong D, Miller BC, Cadwell K, Delgado MA, Ponpuak M, Green KG et al (2008) Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens. Cell Host Microbe 4:458–469

Chapter 3
Autophagy in Plant Immunity

Hong-Yun Zeng, Ping Zheng, Ling-Yan Wang, He-Nan Bao, Sunil Kumar Sahu and Nan Yao

Abstract The highly conserved catabolic process of autophagy delivers unwanted proteins or damaged organelles to vacuoles for degradation and recycling. This is essential for the regulation of cellular homeostasis, stress adaptation, and programmed cell death in eukaryotes. In particular, emerging evidence indicates that autophagy plays a multifunctional regulatory role in plant innate immunity during plant–pathogen interactions. In this review, we highlight existing knowledge regarding the involvement of autophagy in plant immunity, mechanisms functioning in the induction of autophagy upon pathogen infection, and possible directions for future research.

Keywords Autophagy · Plant innate immunity · Plant–Pathogen interaction · Biotic stress

3.1 Introduction

In the catabolic process known as autophagy, double-membrane vesicular structures termed autophagosomes enclose dysfunctional or unnecessary cellular components and deliver them to the vacuole/lysosomes for degradation and recycling [24]. Recent studies have made remarkable progress in understanding autophagy, not only in terms of its molecular mechanisms but also in regard to its broad physiological roles [57]. This highly regulated, the conserved pathway is continuously maintained at a basal level for homeostasis, and upregulated under stress, ensuring nutrient recycling and cellular and organismal homeostasis during stress [70]. Besides its role in survival, autophagy is reported to be involved in the regulation and execution of programmed cell death (PCD) [34]. Autophagy can act as a nonspecific catabolic pathway (bulk autophagy) for nutrient remobilization and energy supply or as a selective mechanism (selective autophagy) to eliminate superfluous and harmful compounds, including

H.-Y. Zeng · P. Zheng · L.-Y. Wang · H.-N. Bao · S. K. Sahu · N. Yao (✉)
State Key Laboratory of Biocontrol, Guangdong Provincial Key Laboratory of Plant Resources, School of Life Sciences, Sun Yat-sen University, Guangzhou 510275, People's Republic of China
e-mail: yaonan@mail.sysu.edu.cn

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_3

aggregated proteins, damaged organelles, and even invasive pathogens, depending on the mechanisms that recruit cellular cargoes to the developing autophagosomes [26, 70].

Three main types of autophagy have been described in plant cells: the conserved micro-autophagy and macro-autophagy processes, as well as mega-autophagy, an extreme form of autophagy functioning in PCD during development and pathogen attack [1]. Macro-autophagy (the focus of this review, and hereafter referred to as autophagy) is the most widely studied form. Autophagy is generally divided into five stages: initiation, nucleation, elongation and completion, fusion, and degradation. In the initial stage, a cup-shaped bilayer membrane structure, called a phagophore, containing the unwanted cytoplasmic component is formed. The membrane then further extends to form a complete bilayer membrane structure called an autophagosome. Next, the autophagosome is transported to the lysosome or vacuole, and the autophagosome membrane fuses with the lysosome or tonoplast membrane. Finally, the autophagic bodies enveloping the contents are degraded by various hydrolases [60].

At present, more than 30 autophagy-related (*ATG*) genes have been identified in yeast, animal, and plant cells. ATG proteins are divided into several functional groups based on their roles in autophagy. For instance, the ATG1/13 protein kinase complex participates in the induction and regulation of autophagy and the PI3 K/ATG6/ATG14 complex is involved in the nucleation of autophagic vesicles. The ATG2/9/18 complex provides and transports membranes. ATG8/PE and ATG12/5 are ubiquitin-like protein systems involved in the elongation and maturation of autophagic vesicles and the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), homotypic fusion and vacuole protein sorting (HOPS), and Rab protein complexes participate in the fusion of autophagosomes with vacuoles or lysozymes [42, 47].

Accumulating evidence has implicated autophagy in development, reproduction, metabolism, senescence, and tolerance to abiotic and biotic stresses in plants [1, 37]. In this review, we focus on the contribution of autophagy to plant–pathogen interactions and discuss the intricate molecular mechanisms underlying these processes. Pathogens can be generally divided into biotrophs and necrotrophs. Biotrophs feed on living host tissue and often cause minimal damage to the plant, whereas necrotrophs kill host tissue and feed on the remains [17]. Hemibiotrophs are biotrophic in one stage of infection and necrotrophic in another stage [49].

Plants have developed multilayered strategies to fight against infections from a broad range of pathogenic organisms [31]. Upon infection, plant pathogenic fungi, oomycetes, viruses, and bacteria face the plant cell wall as a first barrier [72]. When this barrier is breached, the microbe- or damage-associated molecules can be recognized by plant surface-localized or intracellular immune receptors. Perception of pathogen-associated molecular patterns (PAMPs), such as flagellin, EF-Tu, or chitin, by membrane pattern-recognition receptors (PRRs) leads to the activation of basal defenses known as PAMP-triggered immunity (PTI) [89]. For instance, the *Arabidopsis thaliana* immune receptor kinase PRR FLAGELLIN SENSING2 (FLS2) recognizes bacterial flagellin [7]. PAMP recognition stimulates the generation

of reactive oxygen species (ROS), intracellular calcium influx, transient activation of mitogen-activated protein kinases (MAPKs), and the production of salicylic acid (SA) [68]. Pathogen-derived signaling molecules from bacteria, fungi, and viruses, as well as host-resistance-related signaling molecules such as SA or ROS, also induce autophagy (Fig. 3.1a) [43, 44, 61, 62, 81, 85].

To counteract PTI and interrupt other plant processes, adapted pathogens interfere with PTI by secreting effector proteins. Effectors, in turn, can be recognized

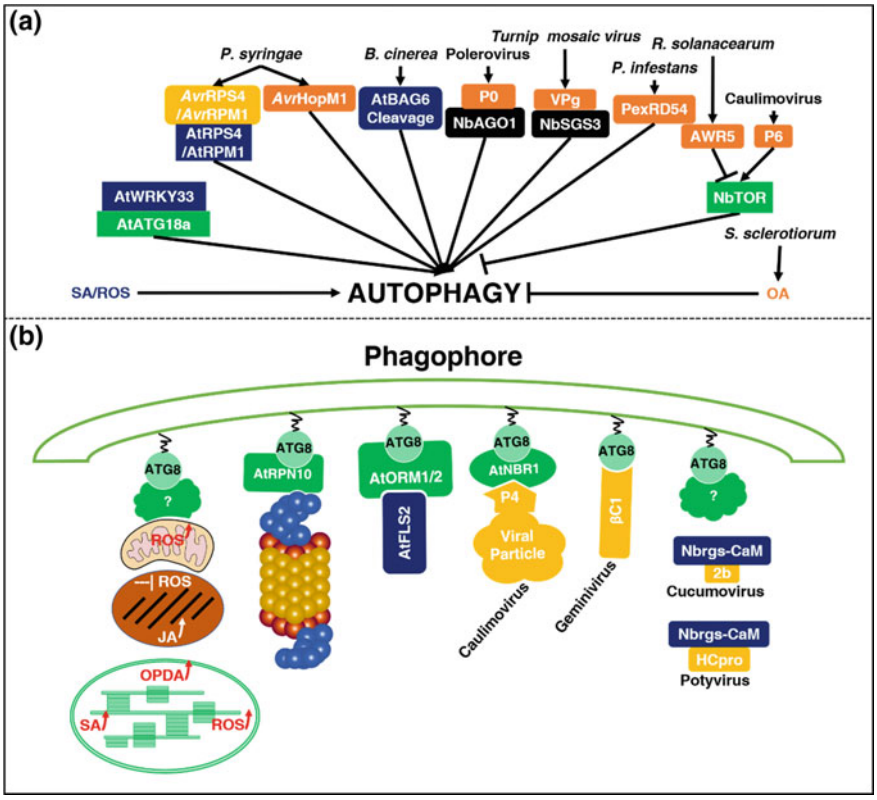


Fig. 3.1 Autophagy is an integral part of plant immunity and a pivotal target of pathogens. **a** Modulation of plant autophagy by hosts and pathogens. **b** The immunity-related cargo receptors and cargoes involved in autophagy. WRKY33: a WRKY transcription factor. *Avr*RPS4, *Avr*RPM1, *Avr*HopM1, AWR5, and PexRD54: pathogen effectors. RPS4 and RPM1: NB-LRR immune receptors. BAG6: BCL2-ASSOCIATED ATHANOGENE FAMILY PROTEIN 6. AGO1: ARGONAUTE1, a key component of the RNA-induced silencing complex. SGS3: SUPPRESSOR OF GENE SILENCING 3, an important component of the RNA silencing pathway. TOR: TARGET OF RAPAMYCIN, a negative regulator of autophagy. ATG8: AUTOPHAGY8. RPN10: REGULATORY PARTICLE NON-ATPASE10. ORMs: orosomucoid proteins. FLS2: FLAGELLIN SENSING2. NBR1: NEXT TO BRCA1 GENE1. Rgs-CaM: a calmodulin-like protein. P0, P4, P6, VPg, βC1, 2b, and HCpro: virus proteins. OA: oxalic acid. SA: salicylic acid. JA: jasmonic acid. ROS: reactive oxygen species. At: *Arabidopsis thaliana*. Nb: *Nicotiana benthamiana*

by nucleotide-binding-leucine-rich repeat (NB-LRR) immune receptors to initiate the second layer of defense, known as effector-triggered immunity (ETI) [4]. Based on their N-terminal sequences, plant NB-LRRs can be further divided into two subclasses, Toll-interleukin-1 receptor/nucleotide-binding site/leucine-rich repeat (TIR-NB-LRR) and coiled-coil/nucleotide-binding site/leucine-rich repeat (CC-NB-LRR) [9]. Unlike PTI, ETI is a strong immune response and is often accompanied by hypersensitive response (HR)-related cell death at attempted infection sites, which is believed to restrict further pathogen ingress [5, 18].

Stimulation of immunity responses occurs not only at the site of pathogen recognition, but also in distal regions of the plant, a phenomenon termed as systemic acquired resistance (SAR), which is in effect an innate immune response providing protection against a broad range of biotrophic pathogens [59]. SAR is considered a consequence of the concerted action of massive ETI- and/or PTI-triggered transcriptional changes, the increased cellular concentration of SA, the activation of multiple downstream signaling cascades mainly transduced by NONEXPRESSER OF PR GENES1 (NPR1), and the production of antimicrobial peptides such as pathogenesis-related (PR) proteins [59].

In contrast to bacteria and fungi, plant viruses can live inside plant cells. To restrain the reproduction of intracellular viruses, plants have developed a general immune mechanism that silences viral RNA expression, termed as RNA silencing or RNA interference (RNAi), which could be regarded as a viral-PAMP-triggered immunity [12, 55, 66]. However, most viruses counter the RNAi defense by expressing factors that suppress RNA silencing [8].

The roles of plant autophagy in various biotic stresses have been extensively explored over the past decade. Autophagy has been shown to regulate basal resistance as well as immunity- and disease-related cell death responses to microbial pathogens with different infection strategies. However, the molecular mechanisms that underpin defense-related autophagy in plants are poorly understood, in part because autophagy functions in numerous physiological processes [26]. Moreover, it has been difficult to dissect the defense-related roles of autophagy against pathogens with standard genetic approaches because autophagy-defective mutants commonly have pleiotropic effects on plant development and other cellular processes [37].

Nevertheless, information gained from studying the pathogen-produced proteins that manipulate the plant autophagy machinery has revealed novel autophagy-related defense components and shed light on the functions of defense-related autophagy [10, 11, 20, 23, 75]. In this review, we discuss and elaborate on the multidimensional roles of autophagy in plant immunity and delve into how autophagy is implicated in immunity.

3.2 Plant Autophagy is Involved in Multiple Immune Signaling Pathways

Several studies have examined the roles of autophagy in plant basal resistance to virulent biotrophic and hemibiotrophic pathogens. However, how autophagy controls plant basal immunity to microbial infection in either a positive or a negative way remains a mystery. For instance, during the first 2 days after bacterial infection the abundance of the virulent bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 increases significantly in *AtATG6*-silenced Arabidopsis plants compared to the wild-type plants [61]. Moreover, mutation of *ATG7* in Arabidopsis leads to increased growth of the virulent Emwa isolate, but not the virulent Noco2 isolate of the biotrophic oomycete *Hyaloperonospora arabidopsidis*. This suggests that autophagy positively regulates basal immunity against some downy-mildew-causing fungal species [28, 38]. *ATG6* is suggested to play a positive role in immunity to the powdery mildew fungus *Blumeria graminis* f. sp. *tritici* in wheat (*Triticum* sp.) as well as in the symbiotic interaction with the bacterium *Rhizobium tropici* and the arbuscular mycorrhizal fungus *Rhizophagus irregularis* in common bean (*Phaseolus vulgaris*) [15, 86].

In contrast to the above observations where autophagy positively regulates resistance, observations in autophagy-defective Arabidopsis mutants for *ATG5*, *ATG7*, *ATG10*, or *ATG18a* suggest that autophagy negatively regulates plant basal immunity to virulent bacterial pathogens [39, 84]. A similar observation has been reported in the response to infection by several obligately biotrophic powdery-mildew-causing fungal species. Mutations in several Arabidopsis *ATG* genes, including *ATG2*, *ATG5*, *ATG7*, *ATG10*, or *ATG18a*, result in enhanced resistance to the biotrophic powdery mildew pathogen *Golovinomyces cichoracearum* [79]. In addition, Ustum et al. observed significantly increased proliferation of *Pst* in *AtATG5* overexpression lines compared to the wild-type control [75]. Surprisingly, loss of function of *AtATG7* leads to two distinctly different results, either susceptible or resistant to *Pst* DC3000 [28, 84]. While the conclusions derived from different observations seem to be controversial, it is clear that autophagy is involved in the regulation of multiple immunity signaling pathways.

It has been suggested that elevated SA levels are responsible for the enhanced resistance of some autophagy-defective mutants to *Pst* DC3000 [38, 39]. As SA levels in the autophagy-defective mutants are sensitive to developmental and environmental conditions, the discrepancy in the assessments of basal immunity to the bacterial pathogen could be explained by differences in the autophagy-defective mutants used in the studies, as well as in their ages and growth conditions [88]. Moreover, high SA levels among the aging *atg* mutants gradually lead to the accumulation of ROS, ubiquitinated proteins, and endoplasmic reticulum stress, which result in early senescence and unrestricted cell death induced by pathogens [6, 51, 54]. Nevertheless, how autophagy represses the excessive increase of SA as well as ROS production during aging remains unknown.

It is likely that autophagy controls the turnover of cellular structures and organelles related to these stress signals to inhibit unnecessary immunity activation and maintain cellular homeostasis. Plants do this via proteaphagy, chlorophagy, mitophagy, and pexophagy, during development or in response to abiotic stresses (Fig. 3.1b) [30, 41, 46, 69]. Consistent with this hypothesis, proteaphagy, which was initially discovered in plants as a mechanism to selectively recycle proteasomes during nitrogen starvation, was recently reported to be involved in plant–microbe interactions [46, 75]. The known selective autophagy components, including receptors and cargo proteins, that function in development or abiotic stress responses [1, 47] may also contribute directly or indirectly to plant immunity. In particular, it is unclear whether these proteins are essential for the function of autophagy during combinatorial stress that has biotic and abiotic components.

Besides its role in the regulation of cellular homeostasis, autophagy was recently demonstrated to function in selective degradation of the immune receptor kinase FLS2 [84]. Orosomucoid (ORM) proteins of Arabidopsis, which are negative regulators of sphingolipid biosynthesis, act as selective autophagy receptors to mediate the degradation of FLS2, revealing a new mechanism for recycling nonactivated immune receptors to maintain a functional immune system (Fig. 3.1b). While overexpression of *ORM1* or *ORM2* largely diminishes FLS2 accumulation and signaling and enhances plant susceptibility to *Pst* DC3000, deficiency of *ORM1* or *ORM2* increases FLS2 accumulation and signaling and elevates resistance against *Pst* DC3000 [84]. Intriguingly, FLS2 abundance is restored to a normal level in autophagy-defective mutants (*atg7* and *atg10*) overexpressing *ORM1* or *ORM2*, suggesting that autophagy components are necessary for ORM-mediated FLS2 degradation [84]. Therefore, ORMs, the first two autophagy cargo receptors reported to directly degrade immunity-related proteins in plants, appear to be key for uncovering molecular connections between immunity and autophagy.

Arabidopsis TRAF1a and TRAF1b (TUMOR NECROSIS FACTOR RECEPTOR-ASSOCIATED FACTOR 1a and b) may integrate autophagy with plant immunity. They are proposed to act as adaptors for SEVEN IN ABSENTIA (SINAT)1 and SINAT2, which mediate proteasomal degradation of ATG6 under normal growth conditions, thereby suppressing autophagy [65]. Conversely, when SINAT1/2 levels decline, the TRAF1a/b interaction with SINAT6 leads to the stabilization of ATG6 and, thus, the activation of autophagy upon starvation [65]. In addition to the fact that TRAF1a/b were previously shown to associate with the SCF-type E3 ligase complex to form a plant-type TRAFasome that modulates the turnover of NB-LRR immune sensors, including SUPPRESSOR OF NPR1-1, CONSTITUTIVE 1, and RIBOSOMAL PROTEIN S2, TRAF1a/b may serve as an intermediary to integrate the NB-LRR signaling pathway with autophagy processes [29]. Despite the high probability that autophagy plays a role in the negative regulation of NB-LRR function, more direct and convincing evidence is required to reveal the direct connection between ETI and autophagy [63]. Recently, Zheng et al. [87] deciphered the role of Arabidopsis alkaline ceramidase (*At*ACER) in the

autophagy process and elaborated on the crucial role played by *At*ACER in the maintenance of a dynamic loop between sphingolipids and autophagy for cellular homeostasis under various environmental stresses.

In contrast, the mechanism of autophagy in promoting basal resistance against bacteria remains largely undefined. In cassava (*Manihot esculenta*), MeWRKY20 and MeATG8a/8f/8 h are reported to play an essential role in resistance against bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* by forming various transcriptional modules and interacting complexes (Fig. 3.1a) [83].

Overall, autophagy participates in plant immunity partly through interplay with multiple immunity pathways, for example, by preventing overactivation of the signaling pathways of SA, PTI, and maybe even ETI, or by boosting immunity in a different context.

3.3 Autophagy Modulates Host Life and Death During Pathogen Attack

Apart from being a survival mechanism to maintain cellular homeostasis and to respond to environmental stresses, such as nutrient depletion or pathogen attack, autophagy is also responsible for programmed cell death [56]. Despite the compelling evidence revealing the participation of autophagy in immunity, some doubts and apparent contradictions remain concerning the function(s) of autophagy as a pro-survival or pro-death pathway [2, 22, 27, 51, 74, 88].

Liu et al. [44] first discovered that autophagy can have a negative role during the HR in plants, and they found that silencing of *N. benthamiana BECLIN1/ATG6* as well as other autophagy-related genes leads to the unrestricted spread of cell death beyond the primary virus infection sites to uninfected tissues and leaves distal to the *Tobacco mosaic virus* (TMV)-inoculated leaf [44]. Consistent with this, neither the HR PCD induced by avirulent *Pst* DC3000 (*Avr*Rpm1) nor disease-associated cell death mediated by virulent *Pst* DC3000 could be contained in the *AtATG6*-silenced plants [61]. Loss of function of *ATG5* in Arabidopsis also enhances the HR PCD in plants infected by avirulent *Pst* DC3000 (*Avr*Rpm1) [85]. Moreover, the *atg* mutant phenotype of accelerated PCD requires SA signaling pathways, as the unrestricted cell death is suppressed in *atg*5 *sid2* (*salicylic acid induction deficient 2*) and *atg5 npr1* double mutants [85]. Likewise, several Arabidopsis autophagy mutants (*atg5*, *atg7*, *atg10*, and *atg18a*) fail to control the spread of cell death after being challenged with the necrotrophic fungal pathogens *Alternaria brassicicola* or *Botrytis cinerea*, as well as the fungal toxin fumonisin B1 [36, 39]. Furthermore, mutations in several *ATG* genes result in enhanced resistance and dramatic pathogen-induced cell death in response to a biotrophic powdery-mildew-causing pathogen (*G. cichoracearum*), which is consistent with the speculation that cell death contributes to resistance against biotrophic pathogens [79].

Surprisingly, although spontaneous cell death, early senescence, and disease resistance require the SA signaling pathway, powdery-mildew-induced cell death is not fully suppressed by inactivation of SA signaling [79, 85]. The fact that cell death could be uncoupled from disease resistance implied that cell death is necessary but not sufficient for resistance to powdery mildew in *atg* mutants [79]. In addition, Arabidopsis ASSOCIATED MOLECULE WITH THE SH3 DOMAIN OF STAM1 (AMSH1), a deubiquitinating enzyme, interacts with the ESCRT-III subunit VACUOLAR PROTEIN SORTING2.1 (VPS2.1) for autophagic degradation, while the impairment of both genes causes early senescence and the double mutants exhibit more resistance to the powdery mildew pathogen *Erysiphe cruciferarum* [32]. Similarly, the enhanced resistance is also associated with increased spontaneous cell death [32]. Thus, autophagy seems to repress cell death, resulting in different outcomes depending on the pathogen lifestyle and the immunity response against avirulent bacteria and some biotrophic fungi, at least in part by restraining SA-dependent cell death.

Nevertheless, a series of subsequent studies yielded conflicting results. Pathogen-induced cell death is observed in *atg7* and *atg9* mutants after inoculation with *Pst* DC3000 (*Avr*RPS4), *Pst* DC3000 (*Avr*Rpm1), or an avirulent isolate (Noco2) of the oomycete *H. arabidopsidis* (Fig. 3.1a) [28]. In addition, the initiation of HR cell death via TIR-type immune receptors required the defense regulator ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1). Moreover, PCD triggered by CC-type immune receptors via NON-RACE-SPECIFIC DISEASE RESISTANCE 1 (NDR1) either is autophagy independent or utilizes autophagic components with cathepsins and other unidentified cell death mediators. These observations suggest that pathogen-induced autophagy contributes positively to HR cell death initiated through TIR-type immune receptors [28].

In a similar way, an Arabidopsis small GTP-binding protein, RabG3b, is suggested to contribute to HR cell death in response to *Pst* DC3000 (*Avr*Rpm1) via the activation of autophagy [35]. Furthermore, loss of *NO CATALASE ACTIVITY1* (*NCA1*) function leads to a strong suppression of RPM1-triggered autophagic degradation and cell death [19]. Later reports have elaborated that autophagy acts as a positive regulator of HR cell death in young plants, when these functions are not masked by the cumulative stresses of aging [6, 54]. In *Nicotiana benthamiana*, cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) interacts with ATG3, and activating ATG3-dependent autophagy by silencing *GAPDH* significantly enhances *N*-gene-mediated HR and resistance against TMV [21]. Likewise, an autophagy deficiency caused by the deletion of *VPS35* homologs, which function in autophagy-associated vacuolar processes, impairs the pathogen-triggered HR after infection with *Pst* DC3000 expressing *Avr*Rps4, *Avr*Rps4, or *Avr*Rpt2 [21]. Based on these results, it appears that autophagy triggers and promotes cell death during pathogen infection.

Surprisingly, silencing of the gene encoding the highly conserved cell death regulator and ATG6-interacting protein Bax inhibitor-1 (BI-1) in tobacco reduces the autophagic activity and enhances *N*-gene-mediated cell death in response to TMV,

while overexpression of plant BI-1 boosts autophagic activity and causes autophagy-dependent cell death [82]. These results compellingly substantiate both pro-survival and pro-death effects of autophagy in different physiological contexts.

Overall, all of these seemingly inconsistent studies indicate that autophagy plays complex and multifaceted roles in the promotion or restriction of different cell death pathways in plants. Based on morphological criteria, pathogen-induced PCD displays features of both vacuolar and necrotic cell death [76]. It is widely known that autophagy can either promote or restrict different forms of PCD in plants, as it does in animals. At the site of bacterial infection, distinct NB-LRR immune receptors (such as RPS4 and RPM1) recognize cognate effectors (such as *Avr*RPS4 and *Avr*RPM1) and then induce the HR, which contributes to the induction of autophagy, with SA and ROS accumulation triggered by the HR [28]. Here, autophagy serves as an effector of the HR PCD upstream of the "point of no return" in an NPR1-independent manner [51]. In uninfected tissue beyond HR lesions, higher expression of *PR* genes mediated by NPR1 causes endoplasmic reticulum stress, which induces autophagy after the establishment of SAR, followed by the HR [6, 16, 54]. In older tissues, aging-related accumulation of ROS and SA contributes to NPR1-dependent ER stress and activation of autophagy [54]. Hence, in the latter two contexts, autophagy acts as a downstream mechanism for clearance of damaged proteins and structures to prevent cell death, which depends on NPR1 [51]. Accordingly, the positive effect of autophagy in HR PCD is counteracted by the cytoprotective functions of autophagy, explaining the premature senescence and runaway cell death observed outside of HR lesions in autophagy-deficient plants. In this regard, it is tempting to assume that the stress-induced runaway cell death in *atg* mutants is linked to the necrotic PCD mode, although this hypothesis needs to be addressed through an in-depth morphological characterization [74].

Notably, during an attempted or successful host invasion, pathogen-induced PCD is observed and can be part of either the immune response or the disease [53]. Undoubtedly, the HR is related to the immune response, which prevents the spread of avirulent biotrophic and hemibiotrophic pathogens beyond the initial infection sites [31]. In contrast, disease-associated cell death often develops during virulent infections and is induced by necrotrophic pathogens to allow nutrient acquisition and proliferation [50]. Supporting this view, Arabidopsis mutants lacking *ATG5*, *ATG10*, or *ATG18a* develop necrosis and show enhanced hyphal growth upon infection with the necrotrophic fungal pathogens *A. brassicicola* and *Plectosphaerella cucumerina* [39]. The *atg* mutants do not exhibit enhanced immunity to the obligate biotrophic oomycete *H. arabidopsidis* (*Ha*), which is consistent with the view that autophagy controls plant basal immunity to microbial infection in a pathogen-lifestyle-dependent manner in either a positive or a negative way [39]. These facts strongly suggest that unrestricted necrotic cell death evoked by destructive necrotrophic pathogens in autophagy-deficient genotypes facilitates increased necrotrophic pathogen growth [38]. Arabidopsis *atg* (*atg5*, *atg7*, and *atg18a*) mutants exhibit increased susceptibility to the necrotrophic pathogens *B. cinerea* and *A. brassicicola* [36]. Furthermore, the association of Arabidopsis WRKY33, a critical tran-

scription factor required for resistance to necrotrophic pathogens, with ATG18a is responsible for the increased susceptibility of *atg* mutants [36] (Fig. 3.1a). Moreover, the jasmonic-acid signaling pathway is also involved [36].

Similarly, Katsiarimpa et al. [32] demonstrated that, as compared to the wild type, a loss-of-function mutant of Arabidopsis AMSH1, which is required for autophagic degradation, displays increased susceptibility, noticeable by the complete wilting and necrosis that spread along the entire leaf, after inoculation with the necrotrophic fungus *A. brassicicola*. Recently, Minina et al. [52] reported that Arabidopsis *ATG5*- or *ATG7*-overexpressing plants exhibit increased resistance to *A. brassicicola*. In another recent report, Arabidopsis BAG6 (BCL2-ASSOCIATED ATHANOGENE FAMILY PROTEIN 6) was identified as an upstream regulator of autophagy (Fig. 3.1a). Investigation of its relevance to *B. cinerea* infection revealed that Arabidopsis *bag6* mutants are defective in autophagy induction and show increased susceptibility, whereas the elevated autophagy levels and induced cell death in *BAG6* overexpression plants enhanced resistance to *B. cinerea* [43]. Furthermore, BAG6 cleavage triggers autophagy in the host and coincides with disease resistance, while mutation of the cleavage site blocks cleavage and inhibits autophagy in plants, which compromises disease resistance [43]. This finding provided a key link between fungal recognition and the induction of cell death and resistance. Together, these studies indicate that autophagy seems to boost plant survival in response to necrotrophic pathogens.

It is a common view that autophagy suppresses disease-associated cell death triggered by necrotrophic fungi, such as *B. cinerea* and *Sclerotinia sclerotiorum*, while promoting the immunity-related HR triggered by avirulent strains of *Pst*, *H. arabidopsidis*, and TMV (Fig. 3.2) [74]. However, how autophagy exerts either a protective or a destructive role during an immune response is not well understood. The role of autophagy in cell survival is likely due to homeostatic functions required to counterbalance infection-induced systemic responses such as SA signaling, ROS production, accumulation of misfolded/aggregated proteins, and endoplasmic reticulum stress [6, 54, 85]. By contrast, our knowledge of the pro-death mechanism of autophagy is still relatively limited. It is thought that removal of negative cell death regulators, possibly by selective autophagy, is involved in this process. On the basis of these considerations, more cell-death-related targets of autophagy during pathogen infection need to be isolated.

Overall, autophagy plays both positive and negative roles in the regulation of cell death in response to pathogen attack. It is well established that plants deploy autophagy to restrain disease-induced cell death and promote an immunity-related HR in certain pathological situations to safeguard against infectious diseases (Fig. 3.2). Nonetheless, how the host tightly controls the orchestrated death of the cell remains obscure.

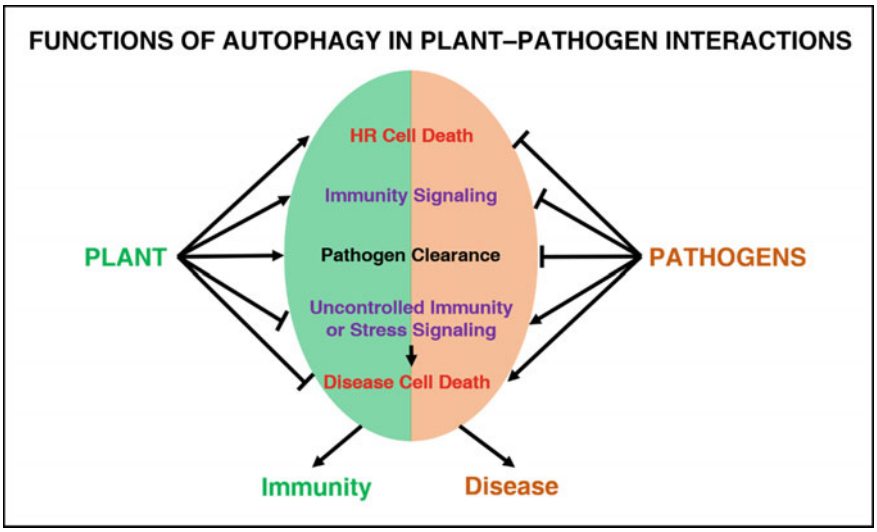


Fig. 3.2 The function of host autophagy in plant–pathogen interactions. Autophagy either prevents the overactivation of innate immune signaling pathways or boosts immunity during pathogen infection. Autophagy suppresses disease-induced (necrotic) cell death and promotes immunity-related HR. Autophagy serves as a cleaner to target, sequester, and eliminate invading pathogens and pathogenic proteins

3.4 Host Plants Degrade Pathogens or Pathogenic Proteins via Autophagy

In mammals, autophagy preferentially targets, sequesters, and eliminates invading bacteria, viruses, and parasites [33, 40, 58, 71]. In particular, in metazoans, autophagy is a key component of host defenses against viruses [14]. Similar antiviral functions of autophagy have been long suspected to exist in the plant kingdom. Previous microscopy observations of viral particles inside a vacuole have supported the existence of an active process for clearance of viruses [48, 67]. Nakahara et al. demonstrated that autophagy-like protein degradation seems to degrade viral silencing suppressors such as CMV protein 2b and potyvirus protein HCpro [55] (Fig. 3.1b). Hafrén et al. [20] also demonstrated that NBR1 associates with the non-assembled and virus-particle-forming capsid protein P4 of *Cauliflower mosaic virus* (CaMV) and orchestrates autophagic degradation of non-assembled P4 and virus particles in tobacco in a process similar to the xenophagy described in animal cells (Fig. 3.1b). This report provides evidence for the removal of a plant pathogen by xenophagy and for the pro- or antiviral roles of autophagy in compatible plant–virus interactions. Haxim et al. [23] reported that the plant autophagic machinery targets the virulence factor βC1 of *Cotton leaf curl Multan virus* (CLCuMuV) for degradation through its interaction with NbATG8 (Fig. 3.1b). The authors found that CLCuMuV infection induces autophagy and that destroying autophagy by silencing

the autophagy-related genes *ATG5* and *ATG7* reduces plant resistance to CLCuMuV, whereas activating autophagy by silencing CYTOSOLIC GLYCOLYTIC ENZYMES GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASES genes enhances plant resistance to viral infection. Moreover, abolishing the interaction between NbATG8f and βC1 abrogates the autophagic antiviral defense against CLCuMuV [23]. These findings reveal a battery of exciting new molecular mechanisms of plant autophagy involved in recognizing and eliminating invading pathogens and pathogenic proteins (Fig. 3.2) in a manner similar to that seen in animals.

3.5 Pathogens Manipulate Plant Autophagy to Counteract Host Defenses

It is becoming clear that adapted phytopathogens have evolved avenues not only to evade autophagic clearance but also to modulate and co-opt autophagy for their own benefits [37]. Considering the long-lasting coevolutionary battle between plants and pathogens, it is not surprising that some microbes have evolved sophisticated strategies to modulate autophagy for their benefits (Figs. 3.1 and 3.2).

Arabidopsis ARGONAUTE1 (AGO1), a key component of the RNA-induced silencing complex (RISC), can be degraded by the host autophagy pathway after it is hijacked by the viral suppressor of RNA silencing protein P0 (Fig. 3.1a) [11]. Intriguingly, the same authors found that the autophagy pathway degrades AtAGO1 even in a nonviral context [11]. In another analogous case, VPg, a viral suppressor of RNA silencing, of *Turnip mosaic virus* (TuMV) (a single-stranded RNA potyvirus) is reported to target Arabidopsis SUPPRESSOR OF GENE SILENCING3 (SGS3), another important component of the RNA silencing pathway, to trigger its degradation through both the autophagy and ubiquitin-proteasome pathways (Fig. 3.1a) [3].

Furthermore, the CaMV P6 protein is reported to bind and activate TARGET OF RAPAMYCIN (TOR) to diminish SA-dependent autophagy and attenuate resistance responses of host plants to bacterial pathogens, either *Pst* DC3000 or the effector-delivery-deficient *Pst* mutant *hrc* (Fig. 3.1a) [90]. In addition, the viral protein domain required for suppression of these plant defense responses is indispensable for the binding and activation of the TOR kinase but not for silencing suppression (Fig. 3.1a) [90]. These findings imply that autophagy steered by viral proteins benefits not only the virus itself but also nonviral pathogens in secondary infections, thereby supporting the view that pathogens can impede autophagy-dependent immunity (Fig. 3.2). Coincidentally, the bacterial wilt pathogen *Ralstonia solanacearum* deploys the ALANINE-TRYPTOPHANARGININE TRYAD 5 (AWR5) effector to inhibit TOR-related activity and stimulate autophagy in yeast (Fig. 3.1a) [64]. This modulation of TOR seems to be a common target for invaders.

Another instance of the manipulation of the host autophagy machinery to counteract host defenses by a plant pathogen was reported by Dagdas et al. [10]. PexRD54, an effector from the hemibiotrophic oomycete *Phytophthora infestans*, associates

with the host autophagy protein NbATG8CL to prevent the interaction of ATG8 with Joka2/NBR1, stimulating autophagosome formation (Fig. 3.1a). As plants use Joka2-mediated selective autophagy to safeguard against infectious diseases from *P. infestans*, the authors speculate that PexRD54 depletes the autophagy cargo receptor Joka2 out of ATG8CL complexes and interferes with Joka2's positive effect on pathogen defense [10]. This convincing evidence implies that pathogens can avoid clearance from the host by subversion of its particular autophagy process (Fig. 3.2).

It appears that suppression of the early onset of host cell death by autophagy-mediated survival functions provides an advantage for (hemi)biotrophic pathogens [74]. By contrast, some necrotrophic (and maybe some hemibiotrophic) pathogens take advantage of the autophagy pathway by promoting autophagy-mediated cell death functions. Besides infecting and killing host cells, adapted pathogens are in direct competition with their hosts to acquire nutrients, causing starvation after successfully colonizing the plants [13]. In this regard, it is reasonable to assume that distinct autophagy processes are subverted and employed by the pathogen to support, for example, nutrient acquisition during the establishment of infection [2]. Therefore, it is tempting to assume that plants have developed, during the coevolution with their pathogens, an elaborate autophagy system with paradoxical roles: as a promoter of cell death at the onset of pathogen-triggered HR and as an inhibitor of cell death following containment of invading pathogens (Fig. 3.2). Supporting this hypothesis, emerging evidence indicates that these two processes can be targeted by intruders. Necrotrophic plant pathogens were long thought to rely mainly on their capacity to kill the host plant and degrade dead host tissue to obtain nourishment [77]. Traditionally, the resulting disease symptoms have been attributed to the direct killing of host tissue via secretion of toxic metabolites by the pathogen [77].

Nevertheless, emerging data from several pathosystems illustrate more subtle interactions between necrotrophic fungi and their host plants [73, 80]. For instance, necrotrophic fungi such as *S. sclerotiorum* and *Botrytis* exploit two distinct pathways for PCD in host plants for their own success [73, 78]. The necrotrophic phytopathogen *S. sclerotiorum* hijacks host autophagy pathways, triggers unrestricted host cell death, and establishes successful infection via oxalic acid [73]. The authors found that the restricted cell death of Arabidopsis plants triggered by oxalic-acid-deficient, nonpathogenic *S. sclerotiorum* mutants is autophagic. Autophagy deficiency rescues the nonpathogenic mutant phenotype, suggesting that *S. sclerotiorum* secretes oxalic acid to suppress antimicrobial autophagy. This is not unique; similar autophagy-mediated mechanisms are steered by another pathogen with a completely different lifestyle, the nonhost *Ustilago maydis*. The wild-type strain of *U. maydis* triggers an HR-like cell death in barley (*Hordeum vulgare*) that shows features of necrotic cell death. In contrast, *U. maydis* mutants lacking the Pep1 effector induce host responses that involved hallmarks of autophagy, indicating that Pep1 serves as an autophagy inhibitor [25]. These findings lead us to speculate that the autophagy-dependent regulation of cell death might be a common virulence scheme shared by various pathogens.

Intriguingly, besides the compelling data for antiviral functions of autophagy mentioned above, it has also been reported that antiviral xenophagy is counteracted by

protective functions of autophagy-resistant CaMV inclusion bodies [20]. Although Arabidopsis *atg*5 and *atg*7 loss-of-function mutants are susceptible to CaMV, the authors hypothesize that a second, nonselective NBR1-independent autophagy pathway promotes plant viability during infection and serves as a proviral mechanism to extend the time span for virus production and potential CaMV transmission [20]. Incongruent functions of autophagy, which are manipulated by pathogens, have also been observed in plant–bacteria interactions. *Pst* uses the effector *Avr*HopM1 as a principal mediator to activate autophagy and stimulate the autophagic removal of proteasomes (proteaphagy) to support bacterial proliferation in Arabidopsis (Fig. 3.1a) [75]. Intriguingly, in contrast to the probacterial effects of *Pst*-induced proteaphagy, NBR1-dependent selective autophagy counteracts disease progression and limits the formation of HopM1-mediated water-soaked lesions [75]. The distinct contributions of autophagy to hosts and pathogens imply that autophagy is an effective weapon used by both plants and their pathogens.

To summarize, we highlight and integrate recent findings illustrating that pathogens subvert and steer plant autophagy to undermine resistance, restrict immunity-related cell death, boost disease-induced cell death, and/or avoid being recognized and cleared from the host cells, and ultimately overcome host immunity and cause disease (Fig. 3.2). Hence, it is fair to envisage that plants employ, and their foes exploit, autophagy to benefit defense and disease, respectively. However, the mechanistic details of autophagy during the interaction between plants and pathogens are not yet fully understood.

3.6 Conclusions and Future Directions

Tremendous progress has been made during the last decade in unraveling the roles of autophagy in the field of plant–microbe interactions. From these studies, it is clear that autophagy influences the outcomes of plant–pathogen attack through interfacing with many important facets of plant immunity, including modulation of multiple innate immunity signaling pathways, execution of HR/disease-induced cell death, and pathogen clearance (Figs. 3.1 and 3.2). In addition, during the long-lasting evolutionary battle with host organisms, pathogens have acquired sophisticated weaponry to trick plants (Figs. 3.1 and 3.2). However, the evidence illustrating the regulation of plant immunity by autophagy is still limited both in number of examples and in mechanistic details.

Because of the extensive involvement of autophagy in a variety of developmental processes, it is difficult to unravel more precise and unknown functions and the associated molecular mechanisms of autophagy in plant–microbe interactions. Use of appropriate methods, materials, timing, and improved technology will facilitate future research. However, it is necessary to be cautious when selecting autophagic mutants and inhibitors/activators for specific studies. To dissect these mechanisms in greater detail, researchers should focus on inducible expression or silencing of core autophagy-related genes in the plant during infection. In addition, a useful

prospective approach would be to further explore the available collection of *atg* mutants and analyze the roles of autophagy in different immune responses of various host–pathogen systems. Moreover, the specific components and mechanisms of autophagy, as well as its functions in different contexts, for example in microbes with diverse lifestyles, need to be addressed. Particular selective autophagy pathways are thought to be involved in the plant–microbe interactions [26]. However, very few autophagy cargo receptors and substrates have been identified in plants. A recent study describes a new class of ATG8 interactors that exploit ubiquitin-interacting motif-like sequences for high-affinity binding to an alternative ATG8 interaction site [45]. By using the new binding sites, the authors ultimately identified a new class of adaptors and receptors, extending the reach of selective autophagy.

The very complex outcomes of disease in autophagy-deficient plants, in general, imply that selective processes with distinct functions may operate in parallel during the full autophagic response [26]. It is therefore tempting to assume that applications of reverse genetics using key autophagy proteins, especially ATG8, and the cargo receptors as lure proteins, in the presence or absence of pathogens, might shed light on the study to further reveal the highly sophisticated and multifaceted integration of autophagy into the plant immune system. In general, the identification of interacting proteins of effectors, pathogenic proteins, or even pathogens themselves might represent a potential strategy to further dissect the molecular mechanisms underlying autophagy-mediated immunity in plants.

Acknowledgements This work was supported by the National Natural Science Foundation of China (31771357), the Natural Science Foundation of Guangdong Province (2017A030311005), and the Fundamental Research Funds for the Central Universities (18lgpy51).

References

1. Avin-Wittenberg T (2019) Autophagy and its role in plant abiotic stress management. Plant Cell Environ 42:1045–1053
2. Batoko H, Dagdas Y, Baluska F, Sirko A (2017) Understanding and exploiting autophagy signaling in plants. Essays Biochem 61:675–685
3. Cheng X, Wang A (2017) The Potyvirus silencing suppressor protein VPg mediates degradation of SGS3 via ubiquitination and autophagy pathways. J Virol 91:e01478-16
4. Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814
5. Coll NS, Epple P, Dangl JL (2011) Programmed cell death in the plant immune system. Cell Death Differ 18:1247–1256
6. Coll NS, Smidler A, Puigvert M, Popa C, Valls M, Dangl JL (2014) The plant metacaspase AtMC1 in pathogen-triggered programmed cell death and aging: functional linkage with autophagy. Cell Death Differ 21:1399–1408
7. Couto D, Zipfel C (2016) Regulation of pattern recognition receptor signalling in plants. Nat Rev Immunol 16:537–552
8. Csorba T, Pantaleo V, Burgyan J (2009) RNA silencing: an antiviral mechanism. Adv Virus Res 75. https://doi.org/10.1016/S0065-3527(09)07502-2

9. Cui H, Tsuda K, Parker JE (2015) Effector-triggered immunity: from pathogen perception to robust defense. Annu Rev Plant Biol 66:487–511
10. Dagdas YF, Belhaj K, Maqbool A, Chaparro-Garcia A, Pandey P, Petre B, Tabassum N, Cruz-Mireles N, Hughes RK, Sklenar J (2016). An effector of the Irish potato famine pathogen antagonizes a host autophagy cargo receptor. eLife 5:e10856
11. Derrien B, Baumberger N, Schepetilnikov M, Viotti C, De Cillia J, Ziegler-Graff V, Isono E, Schumacher K, Genschik P (2012) Degradation of the antiviral component ARGONAUTE1 by the autophagy pathway. Proc Natl Acad Sci USA 109:15942–15946
12. Ding SW, Voinnet O (2007) Antiviral immunity directed by small RNAs. Cell 130:413–426
13. Dodds PN, Lagudah ES (2016) Starving the enemy. Science 354:1377–1378
14. Dong X, Levine B (2013) Autophagy and viruses: adversaries or allies? J Innate Immun 5:480–493
15. Estrada-Navarrete G, Cruz-Mireles N, Lascano R, Alvarado-Affantranger X, Hernandez-Barrera A, Barraza A, Olivares JE, Arthikala MK, Cardenas L, Quinto C et al (2016) An autophagy-related kinase is essential for the symbiotic relationship between *phaseolus vulgaris* and both Rhizobia and Arbuscular mycorrhizal fungi. Plant Cell 28:2326–2341
16. Fu ZQ, Dong X (2013) Systemic acquired resistance: turning local infection into global defense. Annu Rev Plant Biol 64:839–863
17. Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227
18. Greenberg JT, Yao N (2004) The role and regulation of programmed cell death in plant-pathogen interactions. Cell Microbiol 6:201–211
19. Hackenberg T, Juul T, Auzina A, Gwizdz S, Malolepszy A, Van Der Kelen K, Dam S, Bressendorff S, Lorentzen A, Roepstorff P et al (2013) Catalase and NO CATALASE ACTIVITY1 promote autophagy-dependent cell death in Arabidopsis. Plant Cell 25:4616–4626
20. Hafrén A, Macia J-L, Love AJ, Milner JJ, Drucker M, Hofius D (2017) Selective autophagy limits cauliflower mosaic virus infection by NBR1-mediated targeting of viral capsid protein and particles. Proc Natl Acad Sci USA 114:E2026–E2035
21. Han S, Wang Y, Zheng X, Jia Q, Zhao J, Bai F, Hong Y, Liu Y (2015) Cytoplastic Glyceraldehyde-3-Phosphate Dehydrogenases interact with ATG3 to negatively regulate autophagy and immunity in *Nicotiana benthamiana*. Plant Cell 27:1316–1331
22. Han S, Yu B, Wang Y, Liu Y (2011) Role of plant autophagy in stress response. Protein Cell 2:784–791
23. Haxim Y, Ismayil A, Jia Q, Wang Y, Zheng X, Chen T, Qian L, Liu N, Wang Y, Han S et al (2017) Autophagy functions as an antiviral mechanism against geminiviruses in plants. eLife 6:e23897
24. He C, Klionsky DJ (2009) Regulation mechanisms and signaling pathways of autophagy. Annu Rev Genet 43:67–93
25. Hof A, Zechmann B, Schwammbach D, Hückelhoven R, Doehlemann G (2014) Alternative cell death mechanisms determine epidermal resistance in incompatible Barley-Ustilago interactions. Mol Plant Microbe Interact 27:403–414
26. Hofius D, Li L, Hafren A, Coll NS (2017) Autophagy as an emerging arena for plant-pathogen interactions. Curr Opin Plant Biol 38:117–123
27. Hofius D, Munch D, Bressendorff S, Mundy J, Petersen M (2011) Role of autophagy in disease resistance and hypersensitive response-associated cell death. Cell Death Differ 18:1257–1262
28. Hofius D, Schultz-Larsen T, Joensen J, Tsitsigiannis DI, Petersen NH, Mattsson O, Jorgensen LB, Jones JD, Mundy J, Petersen M (2009) Autophagic components contribute to hypersensitive cell death in Arabidopsis. Cell 137:773–783
29. Huang S, Chen X, Zhong X, Li M, Ao K, Huang J, Li X (2016) Plant TRAF proteins regulate NLR immune receptor turnover. Cell Host Microbe 19:204–215
30. Izumi M, Ishida H, Nakamura S, Hidema J (2017) Entire photodamaged chloroplasts are transported to the central vacuole by autophagy. Plant Cell 29:377–394
31. Jones JD, Dangl JL (2006) The plant immune system. Nature 444:323–329

32. Katsiarimpa A, Kalinowska K, Anzenberger F, Weis C, Ostertag M, Tsutsumi C, Schwechheimer C, Brunner F, Hückelhoven R, Isono E (2013) The deubiquitinating enzyme AMSH1 and the ESCRT-III subunit VPS2.1 are required for autophagic degradation in Arabidopsis. Plant Cell 25:2236–2252
33. Knodler LA, Celli J (2011) Eating the strangers within: host control of intracellular bacteria via xenophagy. Cell Microbiol 13:1319–1327
34. Kourtis N, Tavernarakis N (2008) Autophagy and cell death in model organisms. Cell Death Differ 16:21–30
35. Kwon SI, Cho HJ, Kim SR, Park OK (2013) The Rab GTPase RabG3b positively regulates autophagy and immunity-associated hypersensitive cell death in Arabidopsis. Plant Physiol 161:1722–1736
36. Lai Z, Wang F, Zheng Z, Fan B, Chen Z (2011) A critical role of autophagy in plant resistance to necrotrophic fungal pathogens. Plant Journal 66:953–968
37. Leary AY, Sanguankiattichai N, Duggan C, Tumtas Y, Pandey P, Segretin ME, Salguero Linares J, Savage ZD, Yow RJ, Bozkurt TO (2018) Modulation of plant autophagy during pathogen attack. J Exp Bot 69:1325–1333
38. Lenz HD, Haller E, Melzer E, Gust AA, Nürnberger T (2011) Autophagy controls plant basal immunity in a pathogenic lifestyle-dependent manner. Autophagy 7:773–774
39. Lenz HD, Haller E, Melzer E, Kober K, Wurster K, Stahl M, Bassham DC, Vierstra RD, Parker JE, Bautor J et al (2011) Autophagy differentially controls plant basal immunity to biotrophic and necrotrophic pathogens. Plant J 66:818–830
40. Levine B, Deretic V (2007) Unveiling the roles of autophagy in innate and adaptive immunity. Nat Rev Immunol 7:767–777
41. Li F, Chung T, Vierstra RD (2014) AUTOPHAGY-RELATED11 plays a critical role in general autophagy-and senescence-induced mitophagy in Arabidopsis. Plant Cell 26:788–807
42. Li F, Vierstra RD (2012) Autophagy: a multifaceted intracellular system for bulk and selective recycling. Trends Plant Sci 17:526–537
43. Li Y, Kabbage M, Liu W, Dickman MB (2016) Aspartyl protease mediated cleavage of AtBAG6 is necessary for autophagy and fungal resistance in plants. Plant Cell 28:233–247
44. Liu Y, Schiff M, Czymmek K, Talloczy Z, Levine B, Dinesh-Kumar SP (2005) Autophagy regulates programmed cell death during the plant innate immune response. Cell 121:567–577
45. Marshall RS, Hua Z, Mali S, McLoughlin F, Vierstra RD (2019) ATG8-binding UIM proteins define a new class of autophagy adaptors and receptors. Cell 177(766–781):e724
46. Marshall RS, Li F, Gemperline DC, Book AJ, Vierstra RD (2015) Autophagic degradation of the 26S proteasome is mediated by the dual ATG8/Ubiquitin receptor RPN10 in Arabidopsis. Mol Cell 58:1053–1066
47. Marshall RS, Vierstra RD (2018) Autophagy: the master of bulk and selective recycling. Annu Rev Plant Biol 69:173–208
48. Martelli GP, Russo M (1973) Electron microscopy of artichoke mottled crinkle virus in leaves of Chenopodium quinoa Willd. J Ultrastruct Res 42:93–107
49. Mendgen K, Hahn M (2002) Plant infection and the establishment of fungal biotrophy. Trends Plant Sci 7:352–356
50. Mengiste T (2012) Plant immunity to necrotrophs. Annu Rev Phytopathol 50:267–294
51. Minina EA, Bozhkov PV, Hofius D (2014) Autophagy as initiator or executioner of cell death. Trends Plant Sci 19:692–697
52. Minina EA, Moschou PN, Vetukuri RR, Sanchez-Vera V, Cardoso C, Liu Q, Elander PH, Dalman K, Beganovic M, Lindberg Yilmaz J et al (2018) Transcriptional stimulation of rate-limiting components of the autophagic pathway improves plant fitness. J Exp Bot 69:1415–1432
53. Mukhtar MS, McCormack ME, Argueso CT, Pajerowska-Mukhtar KM (2016) Pathogen tactics to manipulate plant cell death. Curr Biol 26:R608–R619
54. Munch D, Rodriguez E, Bressendorff S, Park OK, Hofius D, Petersen M (2014) Autophagy deficiency leads to accumulation of ubiquitinated proteins, ER stress, and cell death in Arabidopsis. Autophagy 10:1579–1587

55. Nakahara KS, Masuta C, Yamada S, Shimura H, Kashihara Y, Wada TS, Meguro A, Goto K, Tadamura K, Sueda K et al (2012) Tobacco calmodulin-like protein provides secondary defense by binding to and directing degradation of virus RNA silencing suppressors. Proc Natl Acad Sci USA 109:10113–10118
56. Nelson C, Baehrecke EH (2014) Eaten to death. FEBS J 281:5411–5417
57. Ohsumi Y (2013) Historical landmarks of autophagy research. Cell Res 24:9–23
58. Orvedahl A, Levine B (2008) Eating the enemy within: autophagy in infectious diseases. Cell Death Differ 16:57–69
59. Pajerowska-Mukhtar KM, Emerine DK, Mukhtar MS (2013) Tell me more: roles of NPRs in plant immunity. Trends Plant Sci 18:402–411
60. Parzych KR, Klionsky DJ (2014) An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal 20:460–473
61. Patel S, Dinesh-Kumar SP (2008) Arabidopsis ATG6 is required to limit the pathogen-associated cell death response. Autophagy 4:20–27
62. Patel S, Dinesh-Kumar SP (2014) Arabidopsis ATG6 is required to limit the pathogen-associated cell death response. Autophagy 4:20–27
63. Pecenkova T, Sabol P, Kulich I, Ortmannova J, Zarsky V (2016) Constitutive negative regulation of R proteins in Arabidopsis also via autophagy related pathway? Front Plant Sci 7:260
64. Popa C, Li L, Gil S, Tatjer L, Hashii K, Tabuchi M, Coll NS, Arino J, Valls M (2016) The effector AWR5 from the plant pathogen Ralstonia solanacearum is an inhibitor of the TOR signalling pathway. Sci Rep 6:27058
65. Qi H, Xia FN, Xie LJ, Yu LJ, Chen QF, Zhuang XH, Wang Q, Li F, Jiang L, Xie Q et al (2017) TRAF family proteins regulate autophagy dynamics by modulating AUTOPHAGY PROTEIN6 stability in Arabidopsis. Plant Cell 29:890–911
66. Ruiz-Ferrer V, Voinnet O (2009) Roles of plant small RNAs in biotic stress responses. Annu Rev Plant Biol 60:485–510
67. ŠArić A, Wrischer M (1975) Fine structure changes in different host plants induced by Grapevine Fanleaf Virus. J Phytopathol 84:97–104
68. Seyfferth C, Tsuda K (2014) Salicylic acid signal transduction: the initiation of biosynthesis, perception and transcriptional reprogramming. Front Plant Sci 5:697
69. Shibata M, Oikawa K, Yoshimoto K, Kondo M, Mano S, Yamada K, Hayashi M, Sakamoto W, Ohsumi Y, Nishimura M (2013) Highly oxidized peroxisomes are selectively degraded via autophagy in Arabidopsis. Plant Cell 25:4967–4983
70. Shibutani ST, Saitoh T, Nowag H, Münz C, Yoshimori T (2015) Autophagy and autophagy-related proteins in the immune system. Nat Immunol 16:1014–1024
71. Sridhar S, Botbol Y, Macian F, Cuervo AM (2012) Autophagy and disease: always two sides to a problem. J Pathology 226:255–273
72. Stael S, Kmiecik P, Willems P, Van Der Kelen K, Coll NS, Teige M, Van Breusegem F (2015) Plant innate immunity–sunny side up? Trends Plant Sci 20:3–11
73. Tyler B, Kabbage M, Williams B, Dickman MB (2013) Cell death control: the interplay of apoptosis and autophagy in the pathogenicity of *Sclerotinia sclerotiorum*. PLoS Pathog 9:e1003287
74. Ustun S, Hafren A, Hofius D (2017) Autophagy as a mediator of life and death in plants. Curr Opin Plant Biol 40:122–130
75. Ustun S, Hafren A, Liu Q, Marshall RS, Minina EA, Bozhkov PV, Vierstra RD, Hofius D (2018) Bacteria exploit autophagy for proteasome degradation and enhanced virulence in plants. Plant Cell 30:668–685
76. van Doorn WG, Beers EP, Dangl JL, Franklin-Tong VE, Gallois P, Hara-Nishimura I, Jones AM, Kawai-Yamada M, Lam E, Mundy J et al (2011) Morphological classification of plant cell deaths. Cell Death Differ 18:1241–1246
77. van Kan JA (2006) Licensed to kill: the lifestyle of a necrotrophic plant pathogen. Trends Plant Sci 11:247–253
78. Veloso J, van Kan JAL (2018) Many shades of grey in Botrytis-host plant interactions. Trends Plant Sci 23:613–622

79. Wang Y, Nishimura MT, Zhao T, Tang D (2011) ATG2, an autophagy-related protein, negatively affects powdery mildew resistance and mildew-induced cell death in Arabidopsis. Plant Journal 68:74–87
80. Williams B, Kabbage M, Kim HJ, Britt R, Dickman MB (2011) Tipping the balance: Sclerotinia sclerotiorum secreted oxalic acid suppresses host defenses by manipulating the host redox environment. PLoS Pathog 7:e1002107
81. Xiong Y, Contento AL, Nguyen PQ, Bassham DC (2007) Degradation of oxidized proteins by autophagy during oxidative stress in Arabidopsis. Plant Physiol 143:291–299
82. Xu G, Wang S, Han S, Xie K, Wang Y, Li J, Liu Y (2017) Plant Bax Inhibitor-1 interacts with ATG6 to regulate autophagy and programmed cell death. Autophagy 13:1161–1175
83. Yan Y, Wang P, He C, Shi H (2017) MeWRKY20 and its interacting and activating autophagy-related protein 8 (MeATG8) regulate plant disease resistance in cassava. Biochem Biophys Res Commun 494:20–26
84. Yang F, Kimberlin AN, Elowsky CG, Liu Y, Gonzalez-Solis A, Cahoon EB, Alfano JR (2019) A plant immune receptor degraded by selective autophagy. Mol Plant 12:113–123
85. Yoshimoto K, Jikumaru Y, Kamiya Y, Kusano M, Consonni C, Panstruga R, Ohsumi Y, Shirasu K (2009) Autophagy negatively regulates cell death by controlling NPR1-dependent salicylic acid signaling during senescence and the innate immune response in Arabidopsis. Plant Cell 21:2914–2927
86. Yue J, Sun H, Zhang W, Pei D, He Y, Wang H (2015) Wheat homologs of yeast ATG6 function in autophagy and are implicated in powdery mildew immunity. BMC Plant Biology 15
87. Zheng P, Wu JX, Sahu SK, Zeng HY, Huang LQ, Liu Z, Xiao S, Yao N (2018) Loss of alkaline ceramidase inhibits autophagy in Arabidopsis and plays an important role during environmental stress response. Plant Cell Env 41:837–849
88. Zhou J, Yu J-Q, Chen Z (2014) The perplexing role of autophagy in plant innate immune responses. Molecular Plant Pathology 15:637–645
89. Zipfel C (2014) Plant pattern-recognition receptors. Trends Immunol 35:345–351
90. Zvereva AS, Golyaev V, Turco S, Gubaeva EG, Rajeswaran R, Schepetilnikov MV, Srour O, Ryabova LA, Boller T, Pooggin MM (2016) Viral protein suppresses oxidative burst and salicylic acid-dependent autophagy and facilitates bacterial growth on virus-infected plants. New Phytol 211:1020–1034

Chapter 4
Autophagy Regulation of Bacterial Pathogen Invasion

Yuqing Lei, Huihui Li and Kefeng Lu

Abstract Autophagy pathway is highly conserved in all eukaryotic species and responsible for targeting of cytosol components, such as protein aggregates, damaged or unnecessary organelles, and intracellular bacterial pathogens for lysosome-dependent degradation. Besides severing as a catabolic process, autophagy pathway furthermore has been discovered to function pivotally in both innate and adaptive immune responses. At present, it has been well demonstrated that certain types of bacteria could be targeted by autophagy upon their invasion. However, several bacterial pathogens have developed strategies to evade this degradation and clearance. Here, we review the role and mechanism of autophagy in the regulation of bacteria invasion, which may facilitate the designing of clinical drugs for efficient and safe cure of infection diseases caused by toxic bacteria.

Keywords Autophagy · Bacteria · Invasion · Degradation · Exnophagy

4.1 Introduction

The autophagy process is highly conserved among eukaryotes from yeast to humans [40] and plays fundamental roles in a variety of both physiological and pathological conditions [13, 31]. In the cell, autophagy pathway selectively targets intracellular pathogens, removes damaged or excessive organelles, and eliminates potentially toxic protein aggregates. Autophagy is functional for the clearance of proteins and other macromolecules for nutrients under starvation conditions. Moreover, autophagy plays fundamental roles in a variety of both physiological and pathological conditions, such as survival during starvation, aging, metabolic diseases, cancer, and neurodegeneration [31]. Furthermore, numerous studies have linked autophagy with

Y. Lei · K. Lu (✉)
Department of Neurosurgery, State Key Laboratory of Biotherapy, Collaborative Innovation Center for Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, China
e-mail: lukf@scu.edu.cn

H. Li
State Key Laboratory of Biotherapy, West China Second University Hospital, Sichuan University, Chengdu 610041, China

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_4

the innate immune responses including function in regulation of invasion, clearance, tolerance, and inflammation [8]. Autophagy could be effectively activated by immuno-receptors such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) that respond to bacterial pathogens and toxic damage-associated molecular pattern molecules (DAMPs) [6, 66]. Autophagy also functions in adaptive immune reaction by production of antigen peptides that could be presented to T cells through MHC I and MHC II molecules [16]. Furthermore, autophagy is functional for polarization of Th1/Th2 cells and activation of macrophages [67].

Autophagy is initiated at a special region associated with the endoplasmic reticulum (ER), Golgi apparatus, and ER-Golgi intermediate compartment (ERGIC) [21]. The autophagosomes fuse with the lysosomes (in mammalian cells) or the vacuoles (in yeast and plants) for final degradation [37, 64]. The autophagy process involves a group of factors called ATG (autophagy related) proteins [37]. Growth and expansion of the phagophore require class III phosphatidylinositol 3-kinase (PI3 K) complex I that comprises lipid kinase Vps34, regulatory kinase Vps15, Beclin-1/Atg6, and Atg14 [5, 30]. The PI3 K complex generates phosphatidylinositol-3-phosphate (PI3P) from phosphatidylinositol to change the lipid composition of the phagophore [44, 62]. As the only transmembrane protein essential in autophagy, Atg9 traffics and transports membrane components for the growing of phagophore [28]. Upon closing, completed formation of the phagophore, the mature autophagosome moves to, docks on and subsequently fuses with lysosome/vacuole, in a process that is mediated by the SNARE proteins syntaxin-17, SNAP29 and VAMP8 as well as the HOPS complex [68].

Upon first observation, autophagy was thought to be a nonselective degradation process. However, it has been revealed clearly that this process can selectively target protein aggregates (aggrephagy); cellular organelles such as mitochondria (mitophagy), peroxisomes (pexophagy), endoplasmic reticulum (ER-phagy), ribosomes (ribophagy), lipid droplets (lipophagy), and bacterial and virus pathogens (xenophagy) [48, 65]. In host cells, autophagy is an efficient pathway for selective engulfment and degradation of bacterial pathogens. Here, we will review generally and briefly the function of autophagy in bacterial invasion.

4.2 Autophagosome Scaffold LC3 as a Platform for Receptor-Bacteria Recruitment

Autophagy is a very evolutionarily conserved degradation pathway for lysosomal degradation of long-lived proteins, big aggregates, and whole organelles. Autophagy in yeast is usually kept at a very low level in rich medium culture condition and is dramatically induced under starvation or rapamycin treatment. Whereas in mammalian cells, autophagy level is different depending on cell background, although in most cases it is constitutively activated [7, 11, 19].

Autophagy process begins from the formation of a double-membrane vesicle called autophagosome, where LC3 is conjugated to engulf cytosolic cargoes [40].

Autophagosome grows from a single spot called phagophore assembly site (PAS), where the following steps of initiation, nucleation, elongation, and finally the closure of the cup-shaped double-layer membrane, phagophore, happen successively [50]. Genetic screens led to the identification of over 40 ATG genes function in different steps in autophagosome formation [58]. Initiation of autophagy is regulated by the Atg1/Atg13/Atg17 kinase complex, which is inhibited by target of rapamycin (TOR) kinase at rich medium culture condition. The next step, nucleation of phagophore at PAS is controlled by a lipid kinase complex containing Vps34, regulatory subunits Atg14, Atg6/Vps30, and Vps15. The following elongation step is controlled by Atg9, the only transmembrane protein in autophagy pathway that provides lipid membrane for the expanding phagophore through shuttling between various vesicle compartments and PAS depending on Atg1 and Vps34. Besides Atg9, two highly conserved ubiquitin-like protein (Atg12 and Atg8) conjugation systems, Atg12–Atg5 and Atg8-phosphatidylethanolamine (PE), also contribute to this process [46]. Ubiquitin is a small protein containing 76 amino acids and is highly conserved from yeast to human. It is tightly folded to form a globular structure composed of five-stranded beta-sheet wrapped surrounding a central helix. Ubiquitin is synthesized first as a precursor protein. Subsequent proteolytic cleavage exposes its active C-terminal glycine amino acid, which allows ubiquitin to be conjugated to a lysine (or N-terminal methionine) in the substrate protein or in the first ubiquitin moiety. A cascade of catalytic enzymes involving activating (E1), conjugating (E2) and ligating (E3) enzymes, generate ubiquitin conjugates containing either multiple mono-ubiquitin or poly-ubiquitin chains (mostly Lys48, Lys63, or linear). Diverse types of ubiquitin modification confer diverse functions, such as regulation of cytoplasm membrane receptor endocytosis, targeting proteins for degradation or functioning in signalling complex assembly [20]. Proteins containing ubiquitin-binding domains (UBDs) can act as ubiquitin receptors through interaction with ubiquitin non-covalently. The ubiquitin conjugates can be reversed by a large class of de-ubiquitinating proteases (DUBs) that cleave the ubiquitin moieties from their substrates. Atg12 was the first ubiquitin-like protein (UBL) to be identified in autophagy pathway [54]. Different from ubiquitin, it is synthesized as a C-terminal glycine-exposed form. Autophagy core protein Atg7 (E1) directly transfers Atg12 onto Atg10 (E2) in a cascade manner. Atg12 is finally conjugated to Atg5. The Atg12–Atg5 conjugate then recruits factors important for phagophore elongation and closure. The Atg12–Atg5 conjugate works together with dimerized coiled-coil protein Atg16, which promotes the association of this conjugate with PAS and phagophore elongation. The main role of Atg12–Atg5–Atg16 complex functions as E3 for the LC3-PE conjugation. LC3, another UBL functions in autophagy, similar to ubiquitin, is synthesized as a precursor and cleaved by cysteine protease Atg4. Matured form of LC3 with exposed C-terminal glycine is activated by and conjugated to Atg7 (E1), transferred to Atg3 (E2) and then finally conjugated to PE lipid incorporated in phagophore with the help of Atg12–Atg5 conjugate. Conjugation of LC3 to phagophore is essential for expansion and also important to function as a platform for recruitment of cargoes, which is mediated by autophagy receptors [2].

4.3 Autophagy Receptors for Degradation of Bacterial Pathogens

Autophagy was first considered to be a bulk degradation pathway, but it is now accepted that many autophagy receptors exist for recognition of distinct cargo substrates. Based on the category of cargoes, several types of selective autophagy have been found: aggrephagy (clearance of protein aggregates, mitophagy (clearance of damaged mitochondria), ribophagy (clearance of excessive ribosomes), xenophagy (clearance of invading pathogens), pexophagy (clearance of peroxisomes), ER-phagy (clearance of endoplasmic reticulum), nucleophagy (clearance of nuclear envelope), lipophagy (clearance of liposomes) [1]. Selective autophagy mediated by different receptors plays an important role in maintaining intracellular homeostasis.

How is it achieved for selectively targeting different cargos (such as pathogenic bacteria) by autophagy? One way is to tag the invading bacteria by ubiquitin chains through different ubiquitin ligases. After the cargo bacteria get ubiquitinated, they are recognized by several autophagy receptors. These receptor proteins serve as a bridge between the cargo bacteria and LC3 on the membranes of the nascent autophagosomes. Thus, these receptor proteins share three common feature domains: LC3 interacting region (LIR) domain, oligomerization domain and ubiquitin moiety binding domain [4, 41]. At present, there are at least four key autophagy receptors including p62, NDP52, OPTN, and TAX1BP1. p62, also called SQSTM1, is the first molecule identified to function as a mammalian autophagy receptor. Initially, p62 is implied for the function in selective autophagy degradation of ubiquitinated protein aggregates. p62 interacts with several ubiquitin ligases such as TRIM50, TRAF6, and MURF2 that ubiquitinate substrates of p62 [52]. Notably, p62 also functions in pexophagy and mitophagy. The role of p62 in antibacterial autophagy was first explored in eliminating the invading *Salmonella Typhimurium* [69]. Soon later, several other types of bacterial pathogens including *Shigella flexneri* and *Mycobacterium tuberculosis* were also found to be subject to selective autophagy mediated by p62 [18]. It has been found that p62 co-localizes with *M. tuberculosis* in host cells after its invasion and controls its survival and replication in macrophages. Consistently, knockdown of p62 upregulates the invasion and survival of infected *M. tuberculosis* in macrophages. Besides, p62 also exerts a role in anti-inflammation through suppressing inflammatory responses induced by globular adiponectin [60]. In addition to p62 itself, its modulating proteins are also found to be involved in xenophagy. For insistence, TBK1 kinase can stimulate p62 function in bacterial autophagy through phosphorylation of Serine 403 at the UBA domain of p62. Such phosphorylation effectively increases the function of p62 for clearance of *M. tuberculosis*. p62 is also shown to be activated through TAK1-mediated phosphorylation, which promotes the binding of p62 with Keap-1 and finally inhibits inflammatory reactions induced by TLR, NLR, or IL-1 cytokines [24].

NDP52 is an important autophagy receptor protein initially found in mitophagy to maintain cell health by clearance of damaged mitochondria [26]. It was also found to function in the regulation of bacterial invasion. NDP52 could transfer different

types of bacterial pathogens such as *Streptococcus pyogenes*, *Salmonella enterica*, and *S. flexneri* by autophagy for their selective degradation [36].

Because of the redundancy of receptors, more than one receptor can target the same bacteria to autophagosomes. It has been found that p62 and NDP52 together target *Shigella* to autophagosomes, while p62 and NDP52 are recruited separately to *Listeria* [24]. NDP52 could interact with all the human LC3 orthologs with a preference for LC3C by its noncanonical LIR (CLIR) domain in antibacterial autophagy function. Ubiquitin ligase Parkin and TBK1 modify the function of NDP52 in bacterial autophagy [26]. GTPase protein Rab35 has been found to control Group A Streptococcus (GAS) degradation by autophagy through binding with NDP52 [36]. Besides, NDP52 also functions in the downregulation of inflammation by inhibiting the NF-κB signalling pathway. Optineurin (OPTN) is a 67 kDa size protein functioning in various tissues and has several domains including C-terminal zinc-finger, leucine zipper domain, an LIR domain, ubiquitin-binding UBAN domain, and coiled-coil motifs that mediate its oligomerization [57]. OPTN is found to function as autophagy receptor in mitophagy, aggrephagy, and xenophagy. Studies have found that OPTN can restrict the growth of *S. enterica* upon invasion [32]. Similarly, TBK1 phosphorylates OPTN within its LIR domain at Ser-177 and regulates its activity in selective autophagy. Besides, OPTN can inhibit inflammation by negatively regulating NF-κB signalling pathway [57]. In addition, OPTN also reduces ER-Stress in intestinal cell by targeting IRE1-α for degradation, which inhibits the ER based inflammation response [36]. On the other hand, OPTN mediates IRF3 activation, which results in type I IFN production for bacterial clearance [56]. TAX1BP1, also called CALCOCO3, is a closely related paralog of NDP52. Its function in xenophagy was first demonstrated by its involvement in the autophagic clearance of *S. typhimurium* [61]. The removal of the bacteria relied on the binding of TAX1BP1 to myosin motor VI that functions in the fusion of autophagosomes with lysosomes [47]. TAX1BP1 overexpression in the heart alleviates inflammatory reaction, oxidative stress, and cell apoptosis in Streptozotocin (STZ)-infection mouse models. It has been shown that TAX1BP1 can interact with MAVS virus, which induces the recruitment of ubiquitin ligase Itch to MAVS for its ubiquitination and degradation leading to restricted cell apoptosis [9].

4.4 Bacterial Pathogens that are Regulated by Autophagy upon Invasion

Although the exact mechanism of bacterial recognition by autophagy has not been discovered clearly, it is known that these recognition processes depend on the ubiquitination of the substrates [45]. Autophagy receptors including p62, NBR1, NDP52, OPTN, and TAX1BP1 mentioned above are a subset of pattern recognition receptors (PRRs). These receptors target ubiquitinated substrates to autophagosomes through binding LC3 [12]. At present, it has become clear that autophagy has a crucial role in the elimination of many types of pathogens [23].

The capacity to degrade bacteria by autophagy was first demonstrated in GAS. In 2004, it was found that in GAS-infected HeLa cells, almost all of the bacteria were recruited into autophagosomes. When autophagy was blocked in ATG5$^{-/-}$ cells, the bacteria survived within the cells [38]. Interestingly, upon invasion, the CD46 receptor could induce autophagy and GAS elimination by activating BECN1 and PI3 K complex [55]. Upon invasion, bacteria are first targeted by endosomes, several Rab GTPase family members that are found both in endocytosis and autophagy are found to be involved in bacterial autophagy, such as Rab7, Rab23, and Rab9A [42]. Several studies have shown that *S. typhimurium* is also a substrate of the autophagy pathway. *S. typhimurium* is an intracellular bacterium that usually resides in a *Salmonella*-containing vacuole (SCV) after invasion. In the cytosol, *S. typhimurium* is coated with ubiquitinated proteins detected and bound by p62, which co-localizes with LC3 and LAMP1 [59]. OPTN also plays an important role in the elimination of *S. typhimurium*. The kinase TBK1, which functions in activation of the transcription of type I interferons, could phosphorylate Serine177 of OPTN, thus enhances the affinity of LC3 binding with OPTN. *M. tuberculosis* usually infects and survives in human alveolar macrophages. Upon invasion, *M. tuberculosis* could arrest phagosome maturation and phagolysosomal fusion, which inhibits the processing and presentation of bacterial antigens. In 2004, studies showed that autophagy could efficiently inhibit the replication of *M. tuberculosis* in macrophages through elimination [23]. Interestingly, it has been found that vitamin D, or 1, 25-dihydroxy vitamin D (1,25D3), induces autophagy in human monocytes via the transcription of *BECN1*. Recently, it was found that upon invasion, *M. tuberculosis* containing phagosomes were highly labeled by LC3 and ATG12 [63]. Other than regulation of bacteria by autophagy, certain bacteria could also inhibit autophagy. For example, *Legionella pneumophila* could enhance the secretion of autophagosomes. Upon invasion, *Legionella* is internalized into a phagosome, then autophagy proteins such as ATG7 and LC3 are recruited and eventually facilitate the degradation of bacteria in lysosomes. *L. pneumophila* could evade autophagy by the secreted effector protein RavZ. RavZ is an ATG4-like cysteine protease that could hydrolyze the amide bond at the C-terminal of LC3 that is conjugated to phosphatidylethanolamine (PE) [10]. Beside of *L. pneumophila*, *S. flexneri* is also found to interfere with autophagy pathway. As a gram-negative pathogen, *S. flexneri* can escape from endosome upon invasion and transfer into cellular cytosol. *S. flexneri* then secrets toxin factors such as IcsB and IcsA (VirG) that can reduce binding of autophagy components like ATG5, which eventually inhibits the recognition of bacteria by the autophagy [43]. *Listeria monocytogenes* is an example of bacterial pathogen that can evade recognition by autophagy upon invasion. At the first phase of infection by *Listeria*, autophagy functions as host immune defense. In ATG5-deficient host cells, comparing to wild-type cells, *L. monocytogenes* rapidly replicates in mouse embryonic fibroblasts, which suggests a pivotal role for autophagy [49]. *Listeria* uses its surface-expressed ActA and InIK proteins to prevent its ubiquitination and recruitment of autophagy receptors such as p62 and NDP52 [15]. There are also certain bacteria that could exploit

autophagic vacuole for multiplication, such as *Coxiella burnetiid* and *Porphyromonas gingivalis*, two types of bacterial pathogens that are associated with cardiovascular diseases [14, 51].

4.5 LC3-Associated Phagocytosis

Recently, a pathway named as LC3-associated phagocytosis (LAP) has been found for the function of autophagy induced by bacterial invasion (Fig. 4.1). The detailed mechanism of the LAP has not been completely understood yet. What is known at present is that LAP uses several common components of the autophagy machinery especially the LC3 conjugation system. For instance, the ubiquitin-like reaction systems for conjugation of LC3 to the membrane lipid PE, ATG12 conjugation system consists of ATG7 (E1-like) and ATG10 (E2-like), the LC3 conjugation system of ATG7 (E1-like), ATG3 (E2-like) and a complex of ATG16L1, ATG5, and ATG12 (E3-like as a complex), have been found to be similarly needed for LAP and canonical autophagy [34]. However, there are also some differences in the molecular machinery between LAP and canonical autophagy. For example, canonical autophagy is induced by the upstream kinases mTORC1 and AMPK by activating the initiation complex composed of ULKs, FIP200, ATG13, and ATG101 [40]. By contrast, LAP is induced with no necessity of this initiation complex [34]. Instead, LAP is induced by cellular surface receptors such as TLRs, Dectin-1, Dectin-2, and Mac-1/CR3/integrin αmβ2

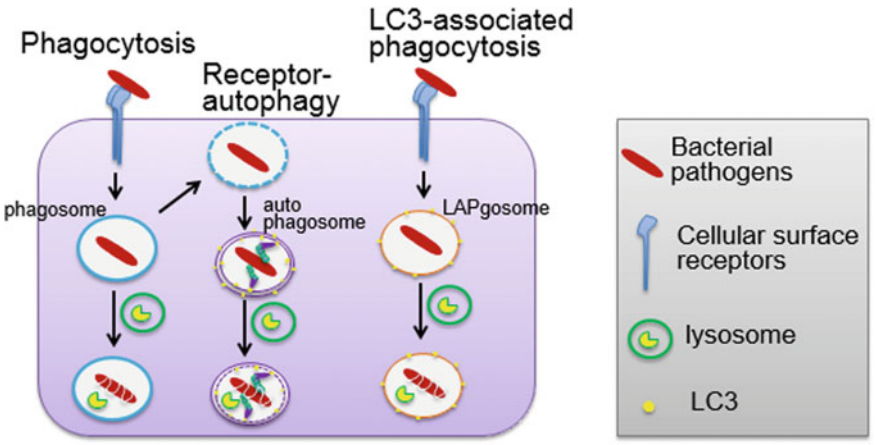


Fig. 4.1 Schematic overview of phagocytosis, canonical autophagy and LC3-associated phagocytosis upon the invasion of bacterial pathogens. Left, upon invasion, extracellular bacteria are recognized by specific surface receptors for phagocytosis. Middle, when pathogens evade from phagocytosis, they are modified by ubiquitination and recognized by autophagy receptors for degradation. Right, a noncanonical autophagy pathway called LC3-associated phagocytosis referred as LAPosomes, during which LC3 can be recruited to phagosomes directly

[25]. Both initiation complex and surface receptor activation (during LAP) can induce generation of the membrane phosphor-lipid PI3P at the target sites, which is mediated by class III PI(3)K complexes (PI3 KC3) [3]. PI3 KC3 contains the core components VPS34, VPS15, Beclin-1, and different Beclin-1 binding proteins. The LAP associated PI3KC3 contains UVRAG and Rubicon which is essential for PI3P formation during LAP but negatively regulates canonical autophagy [29]. In the process of canonical autophagy, a complex containing WIPI and ATG2 bind to the PI3P generated in the target site of membranes. The WIPI-ATG2 complex then binds to PI3P and ATG16L1, which helps recruit the LC3 conjugation systems, resulting in catalytic linkage LC3 to the target membrane. For LAP pathway, ATG16L1 recruitment to the PI3P-containing target membrane sites is also essential. The second big difference in LAP and autophagy induction is that phagocyte oxidase Nox2 generated reactive oxygen species (ROS) is specifically needed for LAP but not for autophagy [17]. To activate the production of ROS, Nox2 forms a catalytic complex with the cytosolic subunits p67, p47, p40, and Rac1/2. Rubicon upregulates Nox2 activities [34]. At the moment, how Nox2-derived ROS stimulates LAP is not clear at all.

The main function of the LAP pathway is to directly promote the fusion of phagosomes with lysosomes for quick degradation of the cargo. For example, fusion of phagosomes containing microbes like *Aspergillus fumigatus*, *Legionella dumoffii*, and *L. monocytogenes* were efficiently targeted to lysosomes by LAP pathway [22]. Additionally, LAP also enhances the fusion with lysosomes of dead cells containing phagosomal vesicles [33]. Moreover, LAP can also delay phagosome maturation, which then results in enhanced antigen presentation by MHC II [53]. The molecular mechanisms how LAP enhances its fusion with lysosomes are not well understood. The fusion of vesicles with lysosomes relies on a number of factors such as membrane lipid composition factors, combining machinery components Rab7, RILP, PLEKHM1, and the HOPS-SNARE complex [39]. At present, the specific composition of membrane lipid of LAPosome is totally unknown. For fusion of LAPosomes with lysosomes, LC3 proteins like the GABARAP family have been found to directly bind and recruit PLEKHM1 which is an adapter protein needed for Rab7 and HOPS complex recruitment to autophagosomes [35]. Another mechanism is that LAP can promote phagosome fusion with lysosomes through enhancement of specific SNARE complex. The SNARE complex mediating fusion of phagosomes with lysosomes is composed of STX17, SNAP29, VTI1b, and lysosomal membrane anchored VAMP8 [27]. The detail and clear verification of function LAP need further molecular and genetic studies.

4.6 Conclusion

Upon invasion of bacterial pathogens, autophagy, both canonical and noncanonical autophagy pathways are stimulated react for defense. The pathogens are eventually eliminated in lysosomes transferred by autophagy process. Sensing the bacterial pathogens upon invasion induces autophagy, especially noncanonical autophagy

LAP, which is a quick and direct way from invasion to lysosomal degradation. The escaped pathogens are again selectively targeted by autophagy through different bridging receptors. Autophagy is also activated by pathogen invasion, to sequester, degrade, and present antigens to host cells, leading to activated immune response against invading bacteria. However, there are also harmful effects conferred by autophagy on host cells. Through specific virulence factors, invading pathogens can evade or inhibit autophagy, or even take advantage of autophagosome for proliferation. Another thing is that in certain situations, over-enhanced autophagy activity causes excess immune response and inflammation. Thus, autophagy plays an important role in invaded host cells for the appropriate immune response to bacterial pathogens, and clinical drugs are then expected based on autophagy for treatments of various infectious diseases caused by bacterial pathogens.

Acknowledgments The authors thank members from K.F. Lu laboratory for advice and help in preparing the manuscript. The K.F. Lu laboratory was supported by the National Key R&D Program of China under grant 2017YFA0506300 (to K.L.) and the National Natural Science Foundation under grants 31770820 (to K.L.).

References

1. Abert C, Martens S (2019) Studies of receptor-Atg8 interactions during selective autophagy. Methods Mol Biol 1880:189–196
2. Abreu S, Kriegenburg F, Gomez-Sanchez R, Mari M, Sanchez-Wandelmer J, Skytte RM, Soares GR, Zens B, Schuschnig M, Hardenberg R et al (2017) Conserved Atg8 recognition sites mediate Atg4 association with autophagosomal membranes and Atg8 deconjugation. EMBO Rep 18:765–780
3. Backer JM (2016) The intricate regulation and complex functions of the class III phosphoinositide 3-kinase Vps34. Biochem J 473:2251–2271
4. Behrends C, Fulda S (2012) Receptor proteins in selective autophagy. Int J Cell Biol 2012:673290
5. Bilanges B, Posor Y, Vanhaesebroeck B (2019) PI3K isoforms in cell signalling and vesicle trafficking. Nat Rev Mol Cell Biol 20:515–534
6. Biswas D, Qureshi OS, Lee WY, Croudace JE, Mura M, Lammas DA (2008) ATP-induced autophagy is associated with rapid killing of intracellular mycobacteria within human monocytes/macrophages. BMC Immunol 9:35
7. Cappelletti C, Galbardi B, Kapetis D, Vattemi G, Guglielmi V, Tonin P, Salerno F, Morandi L, Tomelleri G, Mantegazza R et al (2014) Autophagy, inflammation and innate immunity in inflammatory myopathies. PLoS One 9:e111490
8. Chaumorcel M, Souquere S, Pierron G, Codogno P, Esclatine A (2008) Human cytomegalovirus controls a new autophagy-dependent cellular antiviral defense mechanism. Autophagy 4:46–53
9. Choi YB, Shembade N, Parvatiyar K, Balachandran S, Harhaj EW (2017) TAX1BP1 restrains virus-induced apoptosis by facilitating itch-mediated degradation of the mitochondrial adaptor MAVS. Mol Cell Biol 37
10. Choy A, Dancourt J, Mugo B, O'Connor TJ, Isberg RR, Melia TJ, Roy CR (2012) The legionella effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation. Science 338:1072–1076
11. De Paepe B, Creus KK, De Bleecker JL (2009) Role of cytokines and chemokines in idiopathic inflammatory myopathies. Curr Opin Rheumatol 21:610–616

12. Deretic V (2012) Autophagy: an emerging immunological paradigm. J Immunol 189:15–20
13. Dikic I, Elazar Z (2018) Mechanism and medical implications of mammalian autophagy. Nat Rev Mol Cell Biol 19:349–364
14. Dorn BR, Dunn WA, Progulske-Fox A (2001) Porphyromonas gingivalis traffics to autophagosomes in human coronary artery endothelial cells. Infect Immun 69:5698–5708
15. Dortet L, Mostowy S, Louaka AS, Gouin E, Nahori M, Wiemer EAC, Dussurget O, Cossart P (2011) Recruitment of the major vault protein by InlK: a listeria monocytogenes strategy to avoid autophagy. Plos Pathog 7
16. English L, Chemali M, Duron J, Rondeau C, Laplante A, Gingras D, Alexander D, Leib D, Norbury C, Lippe R et al (2009) Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. Nat Immunol 10:480–487
17. Filomeni G, De Zio D, Cecconi F (2015) Oxidative stress and autophagy: the clash between damage and metabolic needs. Cell Death Differ 22:377–388
18. Franco LH, Nair VR, Scharn CR, Xavier RJ, Torrealba JR, Shiloh MU, Levine B (2017) The ubiquitin ligase Smurf1 functions in selective autophagy of mycobacterium tuberculosis and anti-tuberculous host defense. Cell Host Microbe 22:421–423
19. Fujioka Y, Suzuki SW, Yamamoto H, Kondo-Kakuta C, Kimura Y, Hirano H, Akada R, Inagaki F, Ohsumi Y, Noda NN (2014) Structural basis of starvation-induced assembly of the autophagy initiation complex. Nat Struct Mol Biol 21:513–521
20. Gadhave K, Bolshette N, Ahire A, Pardeshi R, Thakur K, Trandafir C, Istrate A, Ahmed S, Lahkar M, Muresanu DF et al (2016) The ubiquitin proteasomal system: a potential target for the management of Alzheimer's disease. J Cell Mol Med 20:1392–1407
21. Ge L, Melville D, Zhang M, Schekman R (2013) The ER-Golgi intermediate compartment is a key membrane source for the LC3 lipidation step of autophagosome biogenesis. Elife 2:e947
22. Gluschko A, Herb M, Wiegmann K, Krut O, Neiss WF, Utermohlen O, Kronke M, Schramm M (2018) The beta2 Integrin Mac-1 Induces Protective LC3-associated phagocytosis of listeria monocytogenes. Cell Host Microbe 23:324–337
23. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V (2004) Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell 119:753–766
24. Hashimoto K, Simmons AN, Kajino-Sakamoto R, Tsuji Y, Ninomiya-Tsuji J (2016) TAK1 regulates the Nrf2 antioxidant system through modulating p62/SQSTM1. Antioxid Redox Sig 25:953–964
25. Hayashi K, Taura M, Iwasaki A (2018) The interaction between IKKalpha and LC3 promotes type I interferon production through the TLR9-containing LAPosome. Sci Sig 11
26. Heo JM, Ordureau A, Paulo JA, Rinehart J, Harper JW (2015) The PINK1-PARKIN Mitochondrial ubiquitination pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. Mol Cell 60:7–20
27. Itakura E, Kishi-Itakura C, Mizushima N (2012) The hairpin-type tail-anchored SNARE syntaxin 17 targets to autophagosomes for fusion with endosomes/lysosomes. Cell 151:1256–1269
28. Jin M, Klionsky DJ (2014) Regulation of autophagy: modulation of the size and number of autophagosomes. FEBS Lett 588:2457–2463
29. Kim YM, Jung CH, Seo M, Kim EK, Park JM, Bae SS, Kim DH (2015) mTORC1 phosphorylates UVRAG to negatively regulate autophagosome and endosome maturation. Mol Cell 57:207–218
30. Levine B, Liu R, Dong X, Zhong Q (2015) Beclin orthologs: integrative hubs of cell signaling, membrane trafficking, and physiology. Trends Cell Biol 25:533–544
31. Levine B, Kroemer G (2019) Biological functions of autophagy genes: a disease perspective. Cell 176:11–42
32. Liu W, Jiang Y, Sun J, Geng S, Pan Z, Prinz RA, Wang C, Sun J, Jiao X, Xu X (2018) Activation of TGF-beta-activated kinase 1 (TAK1) restricts Salmonella Typhimurium growth by inducing AMPK activation and autophagy. Cell Death Dis 9:570
33. Martinez J, Almendinger J, Oberst A, Ness R, Dillon CP, Fitzgerald P, Hengartner MO, Green DR (2011) Microtubule-associated protein 1 light chain 3 alpha (LC3)-associated phagocytosis is required for the efficient clearance of dead cells. Proc Natl Acad Sci USA 108:17396–17401

34. Martinez J, Malireddi RKS, Lu Q, Cunha LD, Pelletier S, Gingras S, Orchard R, Guan J, Tan H, Peng J et al (2015) Molecular characterization of LC3-associated phagocytosis reveals distinct roles for Rubicon, NOX2 and autophagy proteins. Nat Cell Biol 17:893–906
35. McEwan DG, Popovic D, Gubas A, Terawaki S, Suzuki H, Stadel D, Coxon FP, Miranda DSD, Bhogaraju S, Maddi K et al (2015) PLEKHM1 regulates autophagosome-lysosome fusion through HOPS complex and LC3/GABARAP proteins. Mol Cell 57:39–54
36. Minowa-Nozawa A, Nozawa T, Okamoto-Furuta K, Kohda H, Nakagawa I (2017) Rab35 GTPase recruits NDP52 to autophagy targets. EMBO J 36:3405
37. Mizushima N, Yoshimori T, Ohsumi Y (2011) The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol 27:107–132
38. Nakagawa I, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, Nara A, Funao J, Nakata M, Tsuda K et al (2004) Autophagy defends cells against invading group A Streptococcus. Science 306:1037–1040
39. Nakamura S, Yoshimori T (2017) New insights into autophagosome-lysosome fusion. J Cell Sci 130:1209–1216
40. Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 10:458–467
41. Nguyen TD, Shaid S, Vakhrusheva O, Koschade SE, Klann K, Tholken M, Baker F, Zhang J, Oellerich T, Surun D et al (2019) Loss of the selective autophagy receptor p62 impairs murine myeloid leukemia progression and mitophagy. Blood 133:168–179
42. Nozawa T, Aikawa C, Goda A, Maruyama F, Hamada S, Nakagawa I (2012) The small GTPases Rab9A and Rab23 function at distinct steps in autophagy during group A Streptococcus infection. Cell Microbiol 14:1149–1165
43. Ogawa M, Yoshikawa Y, Kobayashi T, Mimuro H, Fukumatsu M, Kiga K, Piao Z, Ashida H, Yoshida M, Kakuta S et al (2011) A Tecpr1-dependent selective autophagy pathway targets bacterial pathogens. Cell Host Microbe 9:376–389
44. Ohsumi Y (2001) Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol Cell Biol 2:211–216
45. Peng H, Yang J, Li G, You Q, Han W, Li T, Gao D, Xie X, Lee BH, Du J et al (2017) Ubiquitination of p62/sequestosome1 activates its autophagy receptor function and controls selective autophagy upon ubiquitin stress. Cell Res 27:657–674
46. Periyasamy-Thandavan S, Jiang M, Schoenlein P, Dong Z (2009) Autophagy: molecular machinery, regulation, and implications for renal pathophysiology. Am J Physiol Renal Physiol 297:F244–F256
47. Petkova DS, Verlhac P, Rozieres A, Baguet J, Claviere M, Kretz-Remy C, Mahieux R, Viret C, Faure M (2017) Distinct contributions of autophagy receptors in measles virus replication. Viruses 9
48. Piano ME, Folgiero V, Marcellini V, Romania P, Bellacchio E, D'Alicandro V, Bocci C, Carrozzo R, Martinelli D, Petrini S et al (2018) The Vici syndrome protein EPG5 regulates intracellular nucleic acid trafficking linking autophagy to innate and adaptive immunity. Autophagy 14:22–37
49. Py BF, Lipinski MM, Yuan J (2007) Autophagy limits Listeria monocytogenes intracellular growth in the early phase of primary infection. Autophagy 3:117–125
50. Reggiori F, Klionsky DJ (2013) Autophagic processes in yeast: mechanism, machinery and regulation. Genetics 194:341–361
51. Rodrigues PH, Belanger M Jr, Dunn W, Progulske-Fox A (2008) Porphyromonas gingivalis and the autophagic pathway: an innate immune interaction? Front Biosci-Landmrk 13:178–187
52. Rogov V, Dotsch V, Johansen T, Kirkin V (2014) Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. Mol Cell 53:167–178
53. Romao S, Gasser N, Becker AC, Guhl B, Bajagic M, Vanoaica D, Ziegler U, Roesler J, Dengjel J, Reichenbach J et al (2013) Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. J Cell Biol 203:757–766
54. Ryabovol VV, Minibayeva FV (2016) Molecular mechanisms of autophagy in plants: role of ATG8 proteins in formation and functioning of autophagosomes. Biochem (Mosc) 81:348–363

55. Sakurai A, Maruyama F, Funao J, Nozawa T, Aikawa C, Okahashi N, Shintani S, Hamada S, Ooshima T, Nakagawa I (2010) Specific behavior of intracellular Streptococcus pyogenes that has undergone autophagic degradation is associated with bacterial streptolysin O and host small G proteins Rab5 and Rab7. J Biol Chem 285:22666–22675
56. Slowicka K, Vereecke L, Mc GC, Sze M, Maelfait J, Kolpe A, Saelens X, Beyaert R, van Loo G (2016) Optineurin deficiency in mice is associated with increased sensitivity to Salmonella but does not affect proinflammatory NF-kappaB signaling. Eur J Immunol 46:971–980
57. Slowicka K, van Loo G (2018) Optineurin functions for optimal immunity. Front Immunol 9:769
58. Suzuki H, Osawa T, Fujioka Y, Noda NN (2017) Structural biology of the core autophagy machinery. Curr Opin Struct Biol 43:10–17
59. Thurston TL, Wandel MP, von Muhlinen N, Foeglein A, Randow F (2012) Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature 482:414–418
60. Tilija PN, Park PH (2017) Role of p62 in the suppression of inflammatory cytokine production by adiponectin in macrophages: involvement of autophagy and p21/Nrf2 axis. Sci Rep 7:393
61. Tumbarello DA, Manna PT, Allen M, Bycroft M, Arden SD, Kendrick-Jones J, Buss F (2015) The autophagy receptor TAX1BP1 and the molecular motor myosin VI are required for clearance of Salmonella Typhimurium by autophagy. PLoS Pathog 11:e1005174
62. Wan W, You Z, Zhou L, Xu Y, Peng C, Zhou T, Yi C, Shi Y, Liu W (2018) mTORC1-regulated and HUWE1-mediated WIPI2 degradation controls autophagy flux. Mol Cell 72:303–315
63. Watson RO, Manzanillo PS, Cox JS (2012) Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell 150:803–815
64. Wen X, Klionsky DJ (2016) An overview of macroautophagy in yeast. J Mol Biol 428:1681–1699
65. Xu Y, Eissa NT (2010) Autophagy in innate and adaptive immunity. Proc Am Thorac Soc 7:22–28
66. Yano T, Mita S, Ohmori H, Oshima Y, Fujimoto Y, Ueda R, Takada H, Goldman WE, Fukase K, Silverman N et al (2008) Autophagic control of listeria through intracellular innate immune recognition in drosophila. Nat Immunol 9:908–916
67. Yue C, Li J, Jin H, Hua K, Zhou W, Wang Y, Cheng G, Liu D, Xu L, Chen Y et al (2019) Autophagy is a defense mechanism inhibiting invasion and inflammation during high-virulent haemophilus parasuis infection in PK-15 cells. Front Cell Infect Microbiol 9:93
68. Zhao YG, Zhang H (2019) Autophagosome maturation: an epic journey from the ER to lysosomes. J Cell Biol 218:757–770
69. Zheng YT, Shahnazari S, Brech A, Lamark T, Johansen T, Brumell JH (2009) The adaptor protein p62/SQSTM1 targets invading bacteria to the autophagy pathway. J Immunol 183:5909–5916

Chapter 5
Autophagy and Viral Infection

Jingrong Mao, Eena Lin, Lian He, Jiaming Yu, Peng Tan and Yubin Zhou

Abstract Autophagy is an intracellular recycling process that maintains cellular homeostasis by orchestrating immunity upon viral infection. Following viral infection, autophagy is often initiated to curtail infection by delivering viral particles for lysosomal degradation and further integrating with innate pattern recognition receptor signaling to induce interferon (IFN)-mediated viral clearance. However, some viruses have evolved anti-autophagy strategies to escape host immunity and to promote viral replication. In this chapter, we illustrate how autophagy prevents viral infection to generate an optimal anti-viral milieu, and then concentrate on how viruses subvert and hijack the autophagic process to evade immunosurveillance, thereby facilitating viral replication and pathogenesis. Understanding the interplays between autophagy and viral infection is anticipated to guide the development of effective anti-viral therapeutics to fight against infectious diseases.

Keywords Autophagy · Viral infection · Immune response · Viral replication

5.1 Introduction

Autophagy, an evolutionarily conserved degradative process, is required to maintain organismal homeostasis and promote the clearance of intracellular waste and invading pathogens by the host immune system [76, 77]. Triggered by various physiological processes, autophagy is a frequent by-product of infection due to the cellular

E. Lin · L. He · P. Tan (✉) · Y. Zhou (✉)
Institute of Biosciences and Technology, College of Medicine,
Texas A&M University, Houston, TX 77030, USA
e-mail: tpengsysu@gmail.com

Y. Zhou
e-mail: yubinzhou@tamu.edu

J. Mao
Xiangya Hospital, Central South University, Changsha 410008, People's Republic of China

J. Yu
Zhongshan School of Medicine, Sun Yat-sen University,
Guangzhou 510080, People's Republic of China

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_5

stress caused by viral infection and replication [22, 93]. Autophagy is also selective for the recognition and degradation of specific cargoes tagged by ubiquitination by a group of E3 ligase family proteins such as the tripartite motif (TRIM) proteins [142]. Depending on the cargos being sorted for destruction, selective autophagy can be classified into mitophagy (damaged mitochondria), pexophagy (peroxisome), ribophagy (ribosomes), ER-phagy (ER), glycophagy (glycogen), xenophagy (pathogens), and lipophagy (lipid droplets) [119]. Specifically, the xenophagy, a type of selective autophagy specifically senses intracellular microorganisms, including viruses, and physically targets them to autophagosomes for further degradation [75]. Autophagy, programmed to dispose of cytoplasmic components, is first activated by the innate immune system to degrade and clear invading viruses [22, 34], and then facilitates antigen processing followed by the induction of adaptive immune responses at later stages of infection [22, 103, 114].

Although autophagy aims at clearances, some viruses, those persisting and adaptable ones, have evolved a variety of strategies to inhibit, escape or manipulate multiple steps during autophagy to the elemental goal of survival and propagation. Physically, these viruses settle down in the membrane-bound, the protected environment offered by the autophagosome; and metabolically, they utilize autophagy-generated energy and metabolites. In short, these viruses suppress the autophagic process to avoid being degraded or use the autophagosome as the site for replication. Lipophagy, another form of autophagy that degrades intracellular lipid droplets, can also be manipulated by viruses [53]. Lipid droplets serve as a desirable platform for virion assembly, and directly inducing lipophagy allows viruses to sustain the high ATP levels needed for viral replication. In brief, current evidence supports the notion that viruses have evolved strategies to either combat or exploit autophagy to benefit their own life cycle and survival.

The viral proteins [19] (Fig. 5.1, Step 1) or any single step in the viral life cycle, including virus attachment and entry, membrane fusion, exposure of viral components and replication, may trigger autophagy [105]. We provide herein several representative examples to illustrate how viruses induce autophagy at multiple phases during infection (Fig. 5.1, Steps 2–5).

The very first chance for viruses to induce autophagy is through virion binding [20]. CD46 serves as the binding and entry receptor for the measles virus (MeV) to induce autophagy by interacting with the phosphatidylinositol 3-kinase (VPS34)/Beclin-1 complex through a scaffold protein Golgi associated PDZ and coiled coil motif-containing (GOPC) (Fig. 5.1, Step 2). This pathway is only sensitive to vaccinal/attenuated strains that utilize CD46 to infect cells [59, 91, 96, 106, 112, 118]. In fact, MeV also activates autophagy by targeting an autophagy associated protein, named as immunity-associated GTPase family M (IRGM) [45, 46, 107].

Autophagy can also be activated by viral membrane fusion. Human immunodeficiency virus type 1 (HIV-1) envelope glycoproteins (gp120 and gp41, called Env) up-regulate autophagy with their fusogenic activity between HIV-infected cells and uninfected CD4 T cells, which could be prevented by HIV fusion inhibitors T20 and C34, leading to the cell death of uninfected T cells (Fig. 5.1, Step 3). There is not much known about the specific pathways and mechanisms that make up this process,

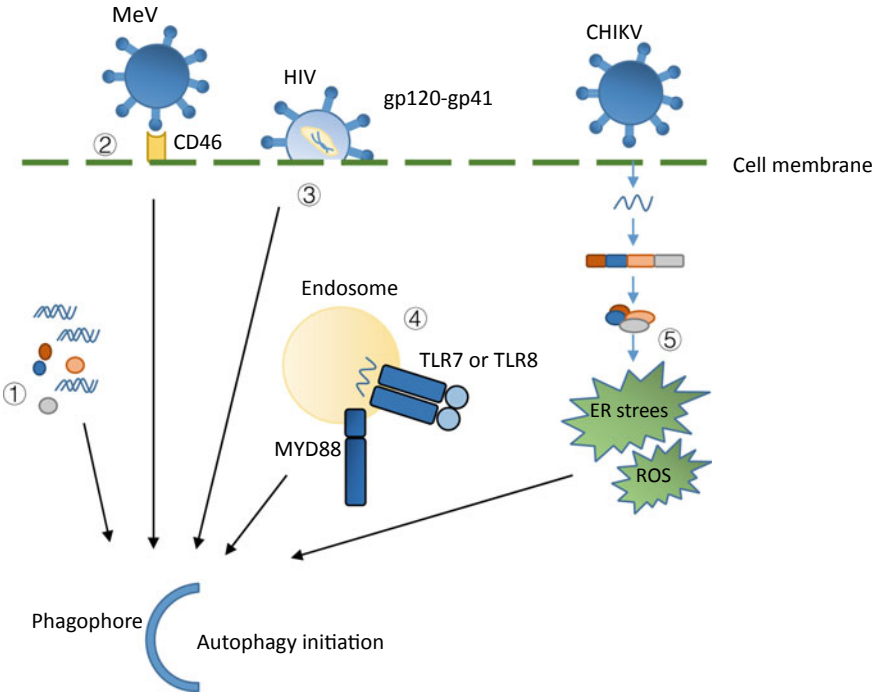


Fig. 5.1 Viral infection induces autophagy initiation. (1) Viral protein itself is able to trigger autophagy. (2) The engagement of CD46, a ubiquitous human surface pathogen receptor for measles virus (MeV) is sufficient to induce autophagy through a CD46-Cyt1/GOPC pathway. (3) Fusion activity of the HIV-1 envelope glycoproteins gp120 and gp41 induce autophagy in uninfected CD4 T cells through binding to CD4 and CXCR4, leading to HIV entry, T cell apoptosis, and immunodeficiency. (4) The recognition process of certain viruses via TLR7 requires transport of cytosolic viral replication intermediates into the lysosome by autophagy. (5) Chikungunya virus (CHIKV)-triggered autophagy is mediated by the ER and oxidative stress pathways

although it hints that bioactive lipids involved in this fusion process and increased reactive oxygen species (ROS) production may mediate the activation of autophagy [33]. It is also important to note that the signaling activity of CD4 and CXCR4 are not associated with autophagy activated by Env [33].

Events following the fusion initiate autophagy via distinct mechanisms. For instance, the delivery of viral components into the cytosol can lead to cytosolic pattern recognition receptor (PRR)-induced autophagy. This is best illustrated by viral particles containing single-stranded RNA (ssRNA) such as vesicular stomatitis virus (VSV): the cytosolic viral replication intermediates in VSV-infected plasmacytoid dendritic cells (pDCs) can be introduced and transported by autophagy to lysosome compartment for the recognition by Toll-like receptor (TLR) 7, which leads to the activation type I interferon (IFN) signaling and the production of IFN-α. Atg5-deficient pDCs were not allowed to recognize VSV infection through TLR7,

further demonstrating the critical step of VSV-induced autophagy in the host defense of viral infection (Fig. 5.1, Step 4) [71].

Viral replication offers a distinct model of deterioration in homeostasis leading to up-regulated autophagy [57]. One such example is the chikungunya virus (CHIKV) whose replication competent form has been reported to promote autophagy through endoplasmic reticulum (ER) stress and the generation of ROS [60]. ER stress is thought to be activated via the accumulation of viral polyproteins [51], which trigger the unfolded protein response (UPR) to restore homeostasis [51, 57]. In the case of CHIKV, its replication promotes the activation of inositol-requiring Ser/Thr protein kinase/endonuclease a (IRE-1a) pathway for provoking the UPR-elicited autophagy through the splicing of X-box-binding protein 1. CHIKV replication also induces increased ROS and reactive nitrogen species, which stimulates autophagy via AMPK-mediated inhibition of the mechanistic target of rapamycin complex 1 (mTORC1) (Fig. 5.1, Step 5) [60].

In summary, multiple steps during virus infection can activate autophagy by sensing of viral genomes or proteins, acting indirectly through cellular stress pathways to modulate homeostasis, and/or directly interacting with autophagy regulatory proteins.

5.2 Virus-Mediated Inhibition of Autophagy

Autophagy is a compilated but well-coordinated cellular event, which can be further divided into the processes of induction, nucleation of the phagophore, elongation, fusion, and degradation artificially [79]. Smart enough, viruses have evolved a variety of strategies to escape or manipulate these autophagic processes to benefit their own replication and propagation.

5.2.1 Inhibition of Autophagy Prior to the Initiation Phase

Mechanistic target of rapamycin (mTOR) works as a central homeostatic regulator of cell growth by promoting anabolic–metabolic processes like nucleotide synthesis and suppressing catabolic processes such as autophagy [64]. In light of the central role of mTORC1 in the prevention of autophagy, it is by no means out of the ordinary that some viruses have evolved tactics to boost mTORC1 activity, leading to the indirect suppression of the Beclin-1/PI3KIII complex and subsequent autophagy. HIV-1, whose envelope activates the mTOR pathway in dendritic cells (DCs), causes autophagy exhaustion [12]. Fusion-defective HIV-1 and CD4 agonist antibodies recapitulate these discoveries, underlining that HIV-1 might well suppress autophagy preceding viral entry (Fig. 5.2). Furthermore, the v-G protein-coupled receptor (v-GPCR), a Kaposi's sarcoma-associated herpesvirus (KSHV) protein, activates the mTOR pathway to negatively regulate autophagy (Fig. 5.2) [8].

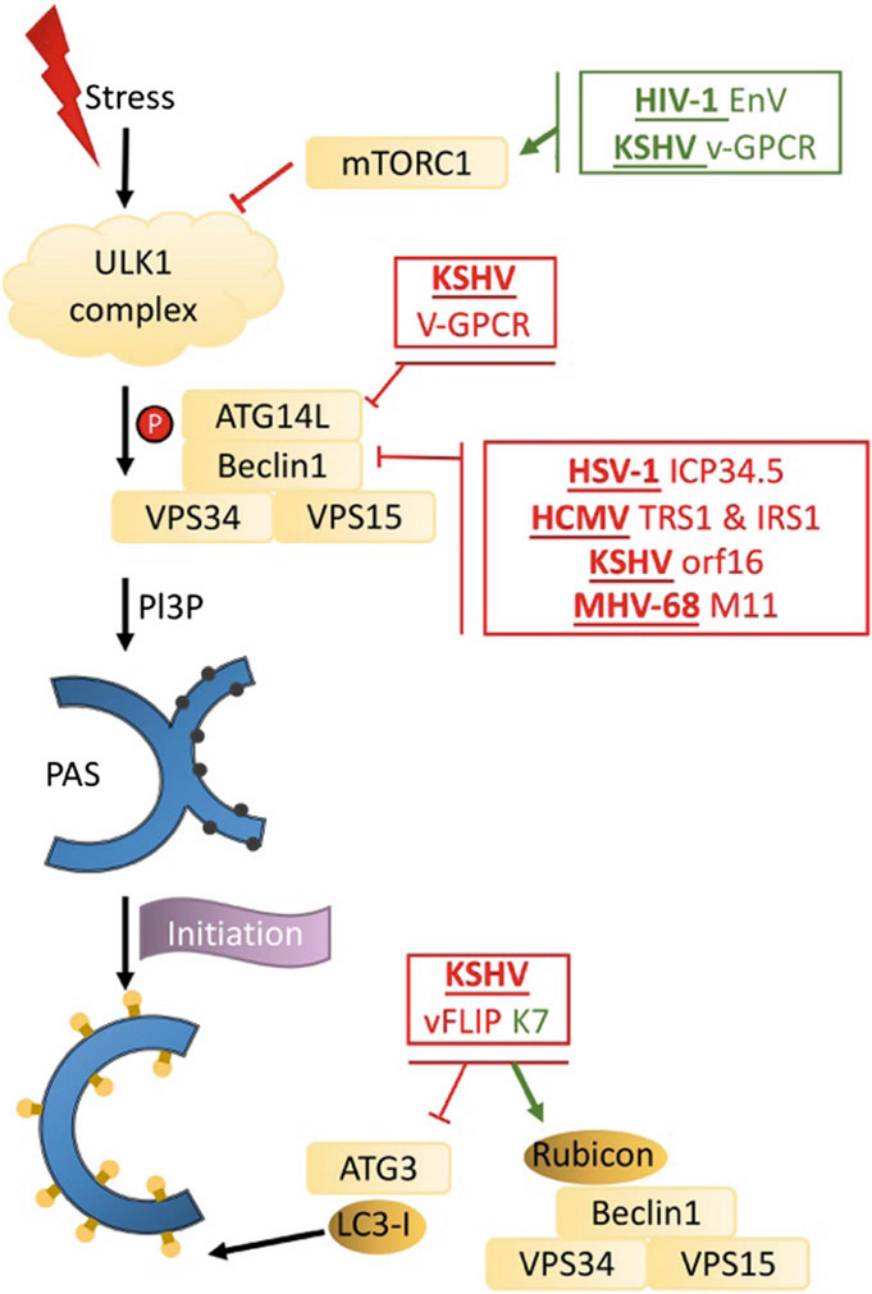
Aside from activating mTOR, v-GPCR is able to mimic the cellular homolog GPCR and down-regulates autophagy via suppressing ATG14L expression (Fig. 5.2) [152]. Interestingly, Beclin-2 may affect the v-GPCR protein level, enhancing its endolysosomal degradation [37].

5.2.2 *Inhibition of Vesicle Nucleation*

Nucleation of the phagophore is impelled by the induction of the Beclin-1/PI3KIII complex. Herpes simplex virus type 1 (HSV-1) encodes the neurovirulence factor ICP34.5 that binds to Beclin-1 and suppresses autophagy (Fig. 5.2) [100]. Human cytomegalovirus (HCMV) encodes a functional homolog of ICP34.5 called TRS1 that works against autophagy as well (Fig. 5.2) [17]. Unlike ICP34.5, the PKR binding domain of TRS1 is irrelevant to autophagy inhibition. Instead, TRS1 interacts with Beclin-1 through its N-terminal region, and this binding is indispensable to suppress autophagy. Besides TRS1, IRS1, another protein encoded by HCMV, has also been proven to inhibit autophagy by interacting with Beclin-1(Fig. 5.2) [95]. A myriad of viruses encode viral BCL-2 (vBCL-2), a protein mimicking its cellular counterpart (cBCL-2), and inhibit autophagy by directly interacting with Beclin-1 [104]. Biochemical and structural analyses showed that, compared with cBCL-2, vBCL-2 lacks the regulatory loop of cBCL-2, which is required for its phosphorylation by JUN N-terminal kinase (JNK). As a result, the association of cBCL-2 with Beclin-1 segregates Beclin-1 from the autophagy initiation complex, thereby attenuating the autophagosome formation [67, 149]. Human gamma-herpesvirus 4 (Epstein–Barr virus, EBV) encodes BHRF1 and BALF-1, two ortholog proteins of cellular Bcl-2, but their inhibitory effects on autophagy remain unclear [3]. Most members of the gamma-herpesvirus family encode and express vBcl-2 during their productive lytic infection process. For example, KSHV and murine γ-herpesvirus 68 (MHV68) use ORF16 and M11 to antagonize autophagy (Fig. 5.2) [30]. These studies have collectively illustrated that vBCL-2 has evolved to become a highly mighty autophagy suppressor.

5.2.3 *Inhibition of Vesicle Elongation and Autophagosome Formation*

KSHV encodes a homolog of the cellular FLICE-like inhibitor protein (FLIP; also known as ORF71), called vFLIP, that prevents ATG3 from binding to and processing LC3 in the autophagosome elongation process (Fig. 5.2) [72]. HSHV also expresses K7 that boosts the Rubicon–Beclin-1 interplay to attenuate the enzymatic activity of VPS34, thus hampering the fusion of autophagosomes with lysosomes (Fig. 5.2) [80].


Stress
HIV-1 EnV
KSHV v-GPCR
mTORC1
ULK1
complex
KSHV
V-GPCR
P
ATG14L
Beclin1
VPS34
VPS15
HSV-1 ICP34.5
HCMV TRS1 & IRS1
KSHV orf16
MHV-68 M11
PI3P
PAS
Initiation
KSHV
vFLIP K7
ATG3
LC3-I
Rubicon
Beclin1
VPS34
VPS15

◀**Fig. 5.2 Viral infection suppresses autophagy**. Viruses have evolved a variety of strategies to escape or manipulate autophagic steps to benefit their own survival. HIV-1 envelope up-regulates the mTOR pathway in DCs, resulting in autophagy exhaustion which promotes cell-associated HIV-1 and transfer of HIV-1 infection to CD4 T cells. KSHV viral protein, v-GPCR which also modulates the mTOR signaling pathway. Besides activating mTOR, v-GPCR can mimic the cellular homolog GPCR and suppress autophagy by blocking the expression of ATG14L. ICP34.5 encoded by HSV-1, binds to Beclin-1 and inhibits autophagy function. And the mutant HSV-1 virus lacking the Beclin-1-binding domain of ICP34.5 cannot block autophagy in neurons. HCMV proteins, TRS1 and IRS1, suppress autophagosome biogenesis by interacting with Beclin-1. In addition, the anti-apoptotic protein, Bcl-2, interacts with the evolutionarily conserved autophagy protein, Beclin-1. The majority of members of the gamma-herpesvirus family encode and express vBcl-2, their cellular counterparts (cBCL-2), and inhibit autophagy by directly interacting with Beclin-1. Like KSHV encodes orf16 and MHV-68 produces M11. There is a checkpoint of the autophagy pathway in which cellular and KSHV FLIPs limit the Atg3-mediated step of LC3 conjugation to regulate autophagosome biogenesis. In addition, KSHV K7 protein interacts with the Rubicon autophagy protein and blocks the autophagosome maturation step by suppressing VPS34 enzymatic activity. Red line, inhibition; green line, promotion

5.3 Autophagy as a Mechanism of Promoting Virus Replication

Double-membrane compartments formed in autophagy serve as an excellent physical platform for viral replication, as they concentrate essential intermediates locally and prevent viral RNAs from detection with innate immune sensors and degradation. This phenomenon was first observed over three decades ago [9, 21, 40]. It is also important to note that RNA viruses are among the most frequent "hackers" of autophagy to promote their own replication.

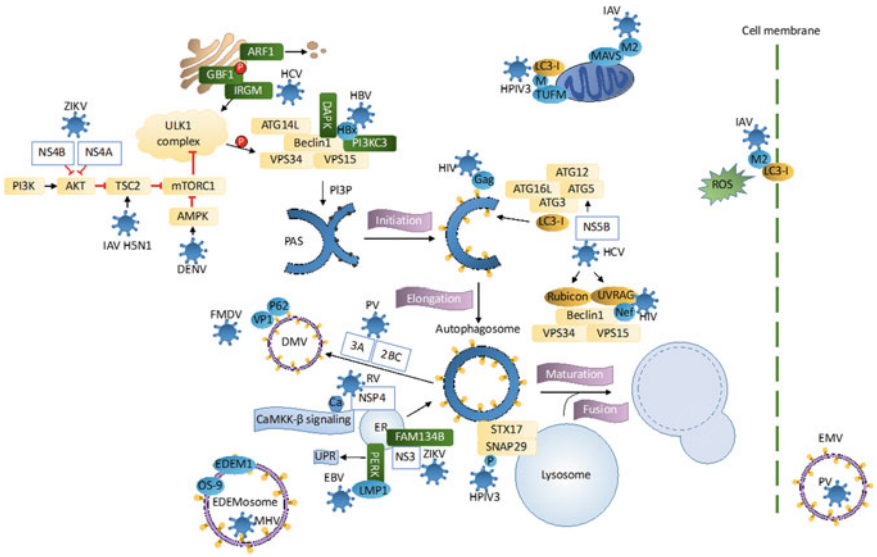
5.3.1 (+) ssRNA Viruses

Studies have illustrated the accumulation of double-membrane vesicles (DMVs) following picornaviral infection. These small RNA viruses take advantage of autophagosomes as membrane scaffolds for their own RNA assembly and replication [2, 55, 148]. Moreover, the role of the autophagy machinery in inducing the non-lytic release of picornaviruses has emerged. Picornaviruses, a group of non-enveloped viruses, are conventionally thought to exit infected cells only through cell rupture. However, growing evidence shows that picornaviruses, including poliovirus (PV) and coxsackievirus, are able to spread in a non-lytic manner among cells via extracellular microvesicles (EMVs), including autophagosome derived EMVs (Fig. 5.3) [11, 18, 41, 113]. These viruses also acquire a defensive advantage by cloaking inside the host-derived vesicles to protect themselves against host immune assaults.

The very first representative to show the benefits that viruses receive from remolding intracellular membranes is PV. Current evidence suggests that rapamycin, which

induces autophagy, up-regulates poliovirus replication, while the silencing of some key genes of autophagosome formation down-regulates it [55]. PV is able to activate the formation of autophagosome-like membranes for RNA replication, virion maturation, and non-lytic viral spread [11, 55, 111]. A further study has shown that the PV protein, 2BC, alone is adequate for inducing the lipidation of LC3 but not for the construction of autophagosomes [139]. Nonetheless, the co-expression of both 2BC and 3A is able to promote the formation of DMVs containing markers of autophagosomes (Fig. 5.3) [55, 111, 131, 139]. Additionally, a recent study revealed that the ULK1 complex is non-essential for PV-induced autophagy [24].

Coxsackievirus B3 (CVB3) is in the same *Picornaviridae* family as PV. The mechanisms by which picornaviruses use to exploit autophagy for their benefits are still unclear. Whether picornaviral infection results in incomplete versus complete autophagy is disputable. Several studies have shown that CVB3 infection restricts the fusion of autophagosomes with lysosomes, leading to the production of giant autophagy-related vesicles during infections [63, 113, 148]. By contrast, another report suggests that CVB3 prompts complete autophagy [121]. A third recently published study showed that CVB3 infection compromises the autophagosome-lysosome/endosome fusion and, at least in part, promotes the accumulation of autophagosomes [94]. A new mechanism has been proposed: synaptosomal-associated protein 29 (SNAP29) and adaptor protein pleckstrin homology domain-containing protein family member 1 (PLEKHM1), known as regulators in autophagosome fusion, are both indispensable to the accumulation of autophagosomes. By cleaving SNAP29 and PLEKHM1 with proteinase 3C, CVB3 curtails



◀**Fig. 5.3 Viruses manipulate autophagy to promote their replication**. Double-membrane vesicles (DMVs), following picornaviral infection, furnish the virus an excellent physical platform for viral RNA assembly and replication. And some picornaviruses, such as PV and coxsackievirus, can spread via extracellular microvesicles (EMVs) in a non-lytic manner between cells. The poliovirus triggered membranes can be specifically induced by the co-expression of two viral proteins, 2BC and 3A. HCV infection prompts the expression of Rubicon and UVRAG, which separately increases and decreases the maturation of autophagosomes. And Rubicon can be triggered by HCV NS4B protein alone. IRGM, known to contribute to autophagy, is localized at the Golgi apparatus and regulates the fragmentation of Golgi membranes in response to HCV infection, resulting in co-localization of Golgi vesicles with replicating HCV. Non-structural viral proteins 2B, 2C and 3A with LC3 and viral structural protein VP1 with Atg5, and LC3 with LAMP-1 co-localize in FMDV-infected cells. DENV stimulates and needs AMPK signaling and AMPK-independent suppression of mTORC1 activity for proviral lipophagy. ZIKV NS4A and NS4B, down-regulate cooperatively the Akt-mTOR pathway and induce cellular dysregulation. ZIKV NS3-mediated cleavage of FAM134B blocks the formation of ER and viral protein enriched autophagosomes, and the reticulophagy pathway further. As for HIV, nondegradative stages of autophagy promote its yields at early stage; HIV Gag-derived proteins bind to and interact with LC3, and autophagy enhances productive Gag processing. And when autophagy progresses to the maturation stages, HIV protein Nef plays as an anti-autophagic maturation factor by the interaction with the autophagy regulatory factor Beclin-1, which protects HIV from degradation. The dual and delicate interaction of HIV with the autophagy pathway enhances viral yields by utilizing the early stages while inhibiting the late stages. SARS-CoV and MHV, activate the formation of DMVs. And MHV utilizes the pathway of EDEMosome formation to generate the DMVs. IAV M2 interacts with LC3 and leads to LC3 re-localization. And a highly pathogenic avian H5N1 strain of IAV is able to block mTOR, activating autophagy. HPIV3 induces incomplete autophagy by blocking autophagosome-lysosome fusion, leading to increased virus production. The viral phosphoprotein binds to SNAP29 and suppresses its interaction with syntaxin17, therefore preventing these two host SNARE proteins from mediating autophagosome-lysome fusion. Matrix protein of HPIV3 shuttles to mitochondria and interacts with TUFM. The interaction between M and the LC3 protein that mediates autophagosome formation. These interactions with both TUFM and LC3 are required for the induction of mitophagy and result in inhibition of the type I interferon response. In RV-infected cells, RV NSP4 co-localized with LC3 in cap-like structures associated with viroplasms. And NSP4 enhances the release of calcium from the ER into the cytoplasm, leading to CaMKK-β signaling to trigger autophagy. HBV HBx maintains interrelationships with PI3KC3 and DAPK, and directly activates Beclin-1 to trigger autophagy. EBV LMP1 up-regulates PERK and the unfolded protein response to drive its own synthesis

autophagic flux and the resulting impaired versions of SNAP29/PLEKHM1 prompt viral replication [94].

Hepatitis C virus (HCV) induces autophagy by promoting the accumulation of autophagosomes and utilizing autophagosomal membranes as the spot for its RNA replication [1, 38, 122]. However, it is still controversial whether HCV is able to efficiently prompt the fusion between autophagosomes and lysosomes. Several studies lean toward the viewpoint that HCV induces autophagosome formation but obstructs the fusion to benefit viral replication and to prevent virion degradation [126, 127, 136]. For example, Sir et al. demonstrated that HCV induces the accumulation of autophagosomes without causing autophagic protein degradation in cells, and this inducement relies on UPR [126]. Dreux et al. suggested that the autophagy pathway is required for the translation of incoming HCV RNA but not for the maintenance of replication [39]. In contrast, Ke et al. found that the entire autophagic process used to

complete autolysosome maturation is essential for supporting HCV RNA replication [62]. Nevertheless, during the early stage of infection, the HCV RNA-dependent RNA polymerase NS5B binds to ATG5, meaning that HCV utilizes ATG5 as a proviral factor at the onset of infection. The resultant downregulation of autophagy via ATG5 silencing obstructs HCV replication and persistence (Fig. 5.3) [47]. Two autophagy regulatory proteins, ultraviolet radiation resistance-associated gene protein (UVRAG), and Rubicon, expressed with different kinetics upon HCV infection activate and suppress the maturation of autophagosomes (Fig. 5.3). HCV is capable of temporally regulating autophagy by inducing the expression of these two proteins differentially to enhance its replication [145]. The early induction of Rubicon by HCV suppresses the fusion between autophagosomes and lysosomes, as a result of the accumulation of autophagosomes and encouragement of HCV replication [145]. Additionally, immunity-related GTPase family M protein (IRGM), an IFN-inducible GTPase, has been reported to regulate autophagy and the development of a variety of intracellular membrane compartments [46]. Upon HCV infection, IRGM interacts with Golgi apparatus-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (GBF1) and facilitates AMPK-mediated GBF1 phosphorylation, thus activating GTPase ADB ribosylation factor 1 (ARF1) for Golgi apparatus fragmentation and coordinating viral replication (Fig. 5.3) [49]. Furthermore, the IRGM-mediated phosphorylation of ULK1 is triggered by HCV infection [16]. The sum of evidence points to the fact that HCV dynamically modulates autophagy to promote viral replication (Fig. 5.3).

Similarly, Foot-and-mouth disease virus (FMDV) leads to ATG5-dependent autophagosome formation as well as the redistribution of LC3 to punctate vesicles. The PI3K activity of VPS34 is non-essential for this induction and occurs very early, as ultraviolet-inactivated FMDV is still able to provoke the autophagosome formation [6]. In addition, co-localization of viral non-structural proteins 2B, 2C, and 3A with LC3 was observed and autophagosomes induced by FMDV contained VP1, the viral capsid protein, which co-localizes with p62, suggesting that autophagosome formation is activated at FMDV entry (Fig. 5.3) [97]. A recent study offered evidence that the expression of FMDV capsid protein VP2 is able to induce autophagy through the EIF2S1-ATF4-AKT-mTOR cascade. VP2 was found to interact with HSPB1 (heat shock protein beta-1) and up-regulate the EIF2S1-ATF4 signaling, leading to autophagy and enhanced FMDV replication [135].

Dengue virus (DENV) has been reported to activate the proliferation of LC3-containing membranes [73, 92]. Using 3-methyladenine or spautin-1, two autophagy inhibitors affect DENV infection [52, 89]. Lipophagy, a form of autophagy, regulates the storage of cellular lipids by lysosomal degradation [84, 124]. Within starving cells, lipophagy breaks down lipid droplets (LDs), in which the eukaryotic cells stock lipids to provide mitochondria with fatty acids, which are oxidized to create acetyl-CoA [84]. Viruses can also take advantage of lipophagy for their own benefits. The number of LDs is increased in DENV-infected cells, and in turn, the inhibition of LD formation remarkably damages DENV replication. Viral capsid proteins are contained in these LDs, which means that these DENV-induced LDs offer a platform for

nucleocapsid formation as well as viral replication [117]. Moreover, lipophagy is activated in DENV-infected cells; and the stored triglycerides are depleted. β-oxidation and energy production are increased in this process, which creates a seedbed of viral replication. If exogenous free fatty acids are added into autophagy-deficient cells, DENV replication will be rescued. Etomoxir, a drug that blocks fatty acid transport into the mitochondria, will prevent it [52]. So, the quantity of free fatty acids and ATP released by lipophagy may be required for DENV replication and persistence. Moreover, DENV induces AMPK kinase activity, which prohibits mTORC1, and this modulation is crucial for virus-induced lipophagy (Fig. 5.3) [58]. Recently, AUP1, a lipid droplet-localized type-III membrane protein with dual localization marks for LDs and ER, was shown to be utilized by DENV to trigger lipophagy. Interaction of unmodified AUP1with the viral non-structural proteins NS4A and NS4B in DENV-infected cells triggers the acyltransferase activity of AUP1, generating phospholipids as the source of membrane components necessary for lipophagy formation and subsequent viral replication [151]. This mechanism seems to be a general phenomenon in flaviviruses and underlies the key role of post-translational modifications during viral infections [151].

Zika virus (ZIKV) has been found to induce the formation of LC3-containing membranes as well [81]. Moreover, the spread of ZIKV might be up-regulated by noncanonical secretory autophagy, as it is for PV and CVB3 [153]. In human neural progenitor cells ER rearrangement and the formation of vesicular clusters in ZIKV infection were thought to be the sites for viral RNA replication and virion assembly [25, 98]. In ZIKV-infected primary fibroblasts, multi-membrane structures are formed resembling autophagic vesicles [48]. In addition, increased lapidated LC3 in ZIKV-infected placentae and decreased viral titers in ATG16-deficient mouse fetuses both work in favor of the proviral role of autophagy [15]. Zika virus (ZIKV) utilizes the ER as a source of membranes to establish their viral replication, assembly and maturation. A selective form of ER degradation by autophagy, or reticulophagy has evolved in the host to restrict DENV and ZIKV, mediated by an ER-resident reticulophagy receptor FAM134B [7]. The virally encoded proteases NS3 in several flaviviruses including ZIKV, DENV, and West Nile Virus (WNV) cleaves FAM134B to suppress the formation of ER and viral protein enriched autophagosomes, as a strategy that viruses manipulate autophagy for their replication (Fig. 5.3) [74]. Furthermore, upon ZIKV infection in fetal neural stem cells AKT phosphorylation and subsequent mTOR activation will be inhibited through the viral protein NS4A and NS4B, which leads to the aberrant activation of autophagy and defective neurogenesis, thus promoting viral replication (Fig. 5.3) [81].

HIV skillfully manipulates the autophagy process by utilizing its two proteins to interact with two different autophagic factors separately. On the one hand, HIV Gag-derived proteins co-localize with and bind to LC3, and autophagy supports productive Gag processing in early and nondegradative stages of autophagy to promote HIV yields (Fig. 5.3). On the other hand, when autophagy enters its maturation stages, HIV protein Nef serves as an anti-autophagic maturation factor through interactions with Beclin-1, thus protecting HIV from degradation. Therefore, the perturbation of the early and late stage of autophagy process promotes HIV survival and replication

[68]. However, during permissive infection, HIV attenuates autophagy in order to avoid proteolytic degradation. Normally, mTOR phosphorylates transcription factor EB (TFEB) and restricts its translocation by favoring its retention within the cytosol. When mTOR is suppressed, TFEB gets dephosphorylated and is allowed to transfer to the nucleus, where it can promote autophagy and lysosomal gene expression. Within infected macrophages, the interplay between HIV and TLR8 activates autophagy, which relies on the dephosphorylation and nuclear translocation of TFEB. During permissive infection, Nef interacts with Beclin-1, leading to mTOR activation, TFEB phosphorylation, and cytosolic sequestration, as well as the suppression of autophagy [14].

5.3.2 (–) ssRNA Viruses

Upon infection, Coronaviruses (CoVs) like the severe acute respiratory syndrome coronavirus (SARS-CoV) and mouse hepatitis virus (MHV) activate the formation of DMVs in host cells and target their replication and transcription complexes (RTCs) on the DMVs-limiting membranes [31, 43, 116]. However, the exact derivation of the DMV lipid bilayers, the host protein content, and the identification of the cellular factors essential for DMVs formation remains unclear [66]. The probable participation of autophagy in the conversion of host membranes into DMVs has been reported [26, 87, 134]. The precise mechanisms that explain why CoVs limits subsequent autophagosome expansion are still a mystery. The non-structural protein 6 (NSP6) has been reported to trigger the autophagic pathway and limit autophagosome expansion to favor CoVs infection [26, 27]. Atg5, according to a study, is non-essential for MHV replication [154]. Contradictory evidence showed either the presence [108, 154] or the absence [31, 129] of LC3/Atg8 on DMVs. Another theory about the origin of virus-induced DMVs suggests that these DMVs are part of a reticulovesicular network of modified ER membranes and contain dsRNA in their interior, which came from a natural and intuitive analysis of SARS-CoV and MHV-infected cells via electron tomography [66]. This idea has been supported by several findings [50, 61, 99]. NSP4, when separately expressed, was shown to localize to the ER and then translocate to the DMVs upon infection [99]. But the deficiency of ER, ER-Golgi intermediate compartment (ERGIC), or Golgi protein markers within CoV-induced DMVs might well mean that their biogenesis does not rely on the traditional pathway [99, 129, 143]. Of special interest is a study that determined MHV hijacks the pathway of EDEMosome (a vesicle involved in ER-associated degradation, ERAD) formation to generate the DMVs (Fig. 5.3). In doing so, MHV trapped two ERAD regulatory proteins into the DMVs, and therefore, exploited the ERAD pathway for viral replication [109]. In addition, this study also revealed an autophagy-independent role for nonlipidated LC3-I [109].

Influenza A virus (IAV) infection also triggers the accumulation of autophagosomes for viral replication [157]. IAV Matrix 2 (M2) ion-channel protein is credited with the manipulation of autophagy, which blocks the fusion of autophagosomes

with lysosomes [42]. Further study showed that M2 hijacks autophagy with its LC3-interacting region [4]. M2 interacts with LC3 and induces LC3 re-localization to the plasma membrane, and disruption of this interaction down-regulates virion budding and stability (Fig. 5.3). Another protein, NS1 triggers autophagy by promoting the synthesis of hemagglutinin (HA) and M2 [155]. Recently, IAV M2 protein was reported to interact with MAVS and positively regulate MAVS-mediated innate immunity. Moreover, ROS production induced by M2 is pivotal for the activation of autophagy and the amplification of the MAVS signaling pathway [146]. In addition, a highly pathogenic avian H5N1 strain of IAV is able to activate autophagy by inhibiting mTOR [85].

Human parainfluenza virus type 3 (HPIV3) suppresses autophagosome maturation as well as triggers the accumulation of autophagosomes [35]. HPIV3 phosphoprotein (P) binds to the SNARE domains of SNAP29 and blocks the interaction between STX17 and SNAP29, which eventually prevents autophagosome-lysosome fusion (Fig. 5.3) [35]. In addition, the matrix protein (M) of HPIV3 interacts with TUFM and binds LC3 to trigger TUFM-mediated mitophagy (Fig. 5.3), a form of autophagy that selectively removes damaged mitochondria and suppresses the subsequent IFN response. These findings suggest that a viral protein is enough to activate mitophagy via bridging autophagosomes and mitochondria [36].

5.3.3 dsRNA Viruses

Within rotavirus (RV)-infected cells, NSP4, whose appearance relies on the intracellular calcium levels co-localizes with LC3 on viroplasms, sites of viral genome replication and immature particle assembly [5]. Further study found that NSP4 activates the release of calcium from the ER into the cytoplasm, inducing calcium/calmodulin-dependent kinase kinase-β (CaMKK-β) signaling to trigger autophagy (Fig. 5.3) [28, 29]. Besides CaMKK-β signaling, a mutually complementary mechanism about a new small RNA was found in RV-initiated autophagy. RV-vsRNA1755 encoded by the NSP4 gene targets the host cell IGF1R which is the part of the PI3K/Akt/mTOR signaling process. In the initial stage of infection RV-vsRNA1755 activates autophagy by obstructing induction of the mTOR pathway [156].

5.3.4 dsDNA Virus

Hepatitis B virus (HBV) has been shown to induce autophagy whether it is in its productive or nonproductive cycles making autophagy vital for its replication [125, 128, 140]. Hepatitis B x protein (HBx) has been linked to an extraordinarily diverse group of pathways, like ones that maintain interrelationships with PI3KC3, or the ones that induce death associated protein kinase (DAPK) in a way that involves Beclin-1 [150], or the ones that directly activate Beclin-1 expression [137] to trigger autophagy

(Fig. 5.3). Another one of its encoded proteins SHBs, can induce autophagy as well [78]. As an intermediate process, the accumulation of autophagosomes mirrors the balance between the rate of their generation and conversion into autolysosomes. Tang et al. suggested the view that HBx, at the initiation stage of autophagic progression triggers autophagy in a Beclin-1-dependent fashion (Fig. 5.3) [137]. Wang et al. suggested that HBV induces autophagy at the initiation stage by the interaction of HBx and c-myc to influence miR-192-3p-XIAP, which in turn regulates Beclin-1 [144]. Meanwhile, Liu et al. revealed that in the late phase of autophagy HBx induces the formation of autophagosomes where HBx evidently damages the lysosomal degradative ability [83]. And they partly supported the conclusion of Sir et al., which stated that HBx is enough to induce autophagosomes [83].

It has also been reported that human gamma-herpesvirus 4 (Epstein–Barr virus, EBV) employ several strategies to interact with autophagic proteins and favor their own survival [23, 90, 123]. Specific autophagy inhibitors are able to encourage EBV lytic replication and might very well influence its oncogenesis [32]. The six-transmembrane spanning domains (6TM) of LMP1 up-regulate PERK, resulting in UPR-mediated autophagy (Fig. 5.3) [69, 70]. Moreover, EBNA1-fragments instead of EBNA3C and EBNA2 are presented via MHC class-II through the autophagy-lysosomal process [138]. And, the accumulation of EBNA1 in autophagosomes suppresses the lysosomal acidification, resulting in a reduction of EBNA1-antigen presentation for $CD4^+$ T lymphocytes recognition [138]. In summary, these findings illustrated that EBV latent antigens hijack autophagy and subsequently influence B-cell lymphomagenesis.

The first study aiming at understanding the implication of autophagy on KSHV replication was performed by Wen et al., who believed that KSHV replication and transcription activator (RTA) enhances autophagy activation to facilitate KSHV lytic replication [147]. Later on, Granato et al. confirmed the function of autophagy in provoking KSHV replication triggered by RTA as well as butyrate combination (T/B), which revealed that the last autophagic steps are suppressed [44].

5.4 Autophagy-Mediated Restriction of Viral Replication

As a piece of vital machinery that responds to environmental stresses rapidly, it is not shocking that autophagy plays a pivotal role in both innate and adaptive immunity to keep cellular homeostasis [114]. But, here, we illustrate that autophagy restricts viral replication by degrading viral components, viral particles or even host factors required for viral replication rather than cooperating with innate and/or adaptive immunity. This process of autophagy targeting individual viral components for degradation is termed virophagy [102]. It's important to note that virophagy targets neosynthesized viral components, while xenophagy targets entire viral particles [86].

Core proteins for HCV virion particles assembly and release are mainly localized within the ER [56]. Overload of HCV in infected cells induces ER stress-associated HPR and subsequent autophagy activation to promote viral replication [62]. The

abilities of HCV to evade autophagic destruction and make use of autophagy for its own benefit have been extensively studied. However, a recent study highlights that an IFN-β-inducible SCOTIN (ER-resident protein, also named SHISA5) recruits HCV non-structural protein 5A (NS5A) to autophagysome for degradation, thereafter suppressing HCV replication (Fig. 5.4) [65].

Upon the Sindbis virus (SINV) infection, Beclin-1 was and Atg5 were reported to protect the host from SINV-mediated encephalitis [82, 101]. Interestingly, knock-down of p62 or other autophagy-related genes up-regulates viral capsid accumulation and progresses virus-induced cell death without influencing virus replication [101]. An E3-ubiquitin ligase, SMURF1 is indispensable for the co-localization of the SINV capsid protein with p62; this interaction advances virophagy by allowing the movement of the SINV viral capsid to autophagosomes [102]. The Fanconi anemia group C protein (FANCC) was also reported to interact with the SINV capsid protein and enhance virophagy [132, 133]. The fact that SMURF1 and FANCC target HSV-1 for virophagy as well and suggests that they often function as virophagic factors (Fig. 5.4) [133].

Picornaviruses are sensed by galectin 8 which restricts viral infection by triggering the autophagic degradation of the viral RNA genome [130]. When poliovirus pierces the endosomal membrane to dump its genome into the cytoplasm, β-galactosides are exposed and activate galectin 8 which results in the detection of punctured endosomes and marks them for further autophagic degradation. Poliovirus, in turn, utilizes the host protein HRAS-like suppressor 3 (PLA2G16) to escape this detection and help genome delivery. Coxsackievirus B3 (CVB3), another picornavirus cleaves p62 and inhibits virophagy by hijacking the viral protease 2A (Fig. 5.4) [120].

HIV-1 is subjected to autophagic degradation as well. In order to surmount innate immunity, the virion infectivity factor (Vif) induces the degradation of an HIV-1 restriction factor APOBEC3G favoring the HIV replication [88]. However, histone deacetylase 6 (HDAC6), in turn, forms a complex with APOBEC3G and provokes autophagy-dependent Vif degradation which down-regulates HIV-1 replication (Fig. 5.4) [141]. Moreover, within $CD4^+$ T cells the transactivator Tat, a protein that promotes viral transcription was selectively degraded by autophagy [115]. In Langerhans cells, which are dendritic immune skin cells, HIV was degraded by the restriction factor tripartite motif-containing protein 5α (TRIM5α) and its ability to regulate the assembly of autophagy activating complexes (Fig. 5.4) [110].

Autophagy also possesses anti-viral capabilities independent of its role in degradation. For mouse norovirus (MNV) infection in vivo, the ATG5/ATG12/ATG16L1 complexplays a key role in autophagosome formation and is essential for IFNγ-mediated anti-viral defense [54]. In ATG16L1 hypomorphic mice, MNV infection induced a phenotype that resembled Crohn's disease [13]. Interestingly, the initiation, fusion, and degradative activities of autophagy were indispensable; while IFNγ-inducible GTPases, which were targeted to MNV replication complexes by LC3 suppressed viral replication [10].

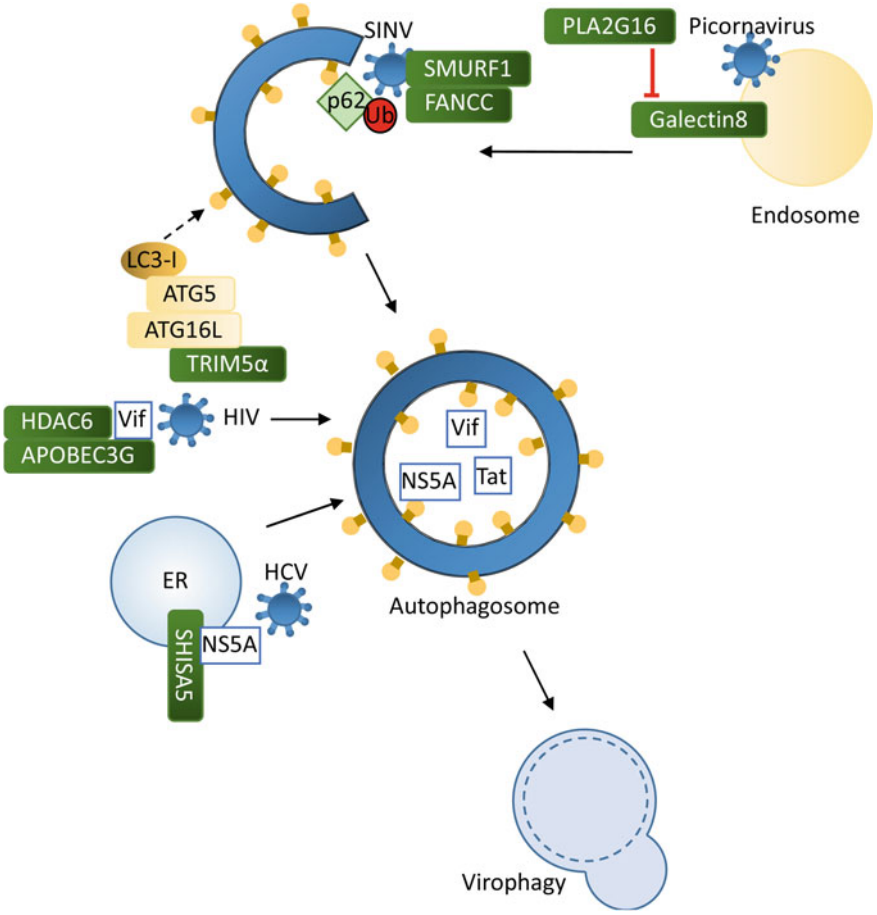


Fig. 5.4 Autophagy-mediated restriction of viral replication. HCV NS5A protein interacts with the IFN-β-inducible protein SHISA5, which transfers NS5A to autophagosomes for further degradation. SMURF1 is indispensable for the co-localization of the SINV capsid protein to p62, which prompts virophagy by shuttling the viral capsid to autophagosomes. FANCC also interacts with SINV capsid protein (not known to be ubiquitinated) and enhance virophagy. Poliovirus breaks the endosomal membrane and releases its genome into the cytoplasm, and Galectin-8 detects the permeated endosomes and marks them for autophagic degradation, but PLA2G16 facilitates viral genome translocation and prevents clearance. Upon HIV viral fusion, TRIM5α induces the recruitment of Atg5 to the TRIM5α–Atg16L1–HIV-1p24 capsid complex, promoting lipidation of LC3 (LC3 II) and thereby mediating autophagosome formation. HIV Vif interacts with the HD6A/APOBEC3G complex to induce its rapid degradation. Autophagy selectively degrades the HIV-1 transactivator Tat, a protein that is essential for HIV-1 transcription and virion production

5.5 Conclusion

The control of viral infection by autophagy is a multi-faceted, dynamic physiological, and pathological process. On one hand, autophagy destructs viruses, regulates inflammatory responses, and provokes antigen presentation. On the other hand, viruses try all means to enhance their immune escape, replication, and release from infected cells by sabotaging or taking advantage of autophagy. Different virus finds its own strategies to survive the autophagic destruction while secures the membrane source provided by autophagy for viral replication. In summary, autophagy and viral infection are highly connected and continuing investigations on the virus autophagy interplays will be a fruitful area of scientific inquiry for many years to come.

References

1. Ait-Goughoulte M, Kanda T, Meyer K, Ryerse JS, Ray RB, Ray R (2008) Hepatitis C virus genotype 1a growth and induction of autophagy. J Virol 82:2241–2249
2. Alirezaei M, Flynn CT, Wood MR, Whitton JL (2012) Pancreatic acinar cell-specific autophagy disruption reduces coxsackievirus replication and pathogenesis in vivo. Cell Host Microbe 11:298–305
3. Altmann M, Hammerschmidt W (2005) Epstein-Barr virus provides a new paradigm: a requirement for the immediate inhibition of apoptosis. PLoS Biol 3:e404
4. Beale R, Wise H, Stuart A, Ravenhill BJ, Digard P, Randow F (2014) A LC3-interacting motif in the influenza A virus M2 protein is required to subvert autophagy and maintain virion stability. Cell Host Microbe 15:239–247
5. Berkova Z, Crawford SE, Trugnan G, Yoshimori T, Morris AP, Estes MK (2006) Rotavirus NSP4 induces a novel vesicular compartment regulated by calcium and associated with viroplasms. J Virol 80:6061–6071
6. Berryman S, Brooks E, Burman A, Hawes P, Roberts R, Netherton C, Monaghan P, Whelband M, Cottam E, Elazar Z et al (2012) Foot-and-mouth disease virus induces autophagosomes during cell entry via a class III phosphatidylinositol 3-kinase-independent pathway. J Virol 86:12940–12953
7. Bhaskara RM, Grumati P, Garcia-Pardo J, Kalayil S, Covarrubias-Pinto A, Chen W, Kudryashev M, Dikic I, Hummer G (2019) Curvature induction and membrane remodeling by FAM134B reticulon homology domain assist selective ER-phagy. Nat Commun 10:2370
8. Bhatt AP, Damania B (2012) AKTivation of PI3K/AKT/mTOR signaling pathway by KSHV. Front Immunol 3:401
9. Bienz K, Egger D, Pasamontes L (1987) Association of polioviral proteins of the P2 genomic region with the viral replication complex and virus-induced membrane synthesis as visualized by electron microscopic immunocytochemistry and autoradiography. Virology 160:220–226
10. Biering SB, Choi J, Halstrom RA, Brown HM, Beatty WL, Lee S, McCune BT, Dominici E, Williams LE, Orchard RC et al (2017) Viral replication complexes are targeted by LC3-guided interferon-inducible GTPases. Cell Host Microbe 22(74–85):e77
11. Bird SW, Maynard ND, Covert MW, Kirkegaard K (2014) Nonlytic viral spread enhanced by autophagy components. Proc Natl Acad Sci USA 111:13081–13086
12. Blanchet FP, Moris A, Nikolic DS, Lehmann M, Cardinaud S, Stalder R, Garcia E, Dinkins C, Leuba F, Wu L et al (2010) Human immunodeficiency virus-1 inhibition of immunoamphisomes in dendritic cells impairs early innate and adaptive immune responses. Immunity 32:654–669

13. Cadwell K, Patel KK, Maloney NS, Liu TC, Ng AC, Storer CE, Head RD, Xavier R, Stappenbeck TS, Virgin HW (2010) Virus-plus-susceptibility gene interaction determines Crohn's disease gene Atg16L1 phenotypes in intestine. Cell 141:1135–1145
14. Campbell GR, Rawat P, Bruckman RS, Spector SA (2015) Human immunodeficiency virus Type 1 Nef Inhibits autophagy through transcription factor EB sequestration. PLoS Pathog 11:e1005018
15. Cao B, Parnell LA, Diamond MS, Mysorekar IU (2017) Inhibition of autophagy limits vertical transmission of Zika virus in pregnant mice. J Exp Med 214:2303–2313
16. Chauhan S, Mandell MA, Deretic V (2015) IRGM governs the core autophagy machinery to conduct antimicrobial defense. Mol Cell 58:507–521
17. Chaumorcel M, Lussignol M, Mouna L, Cavignac Y, Fahie K, Cotte-Laffitte J, Geballe A, Brune W, Beau I, Codogno P et al (2012) The human cytomegalovirus protein TRS1 inhibits autophagy via its interaction with Beclin 1. J Virol 86:2571–2584
18. Chen YH, Du W, Hagemeijer MC, Takvorian PM, Pau C, Cali A, Brantner CA, Stempinski ES, Connelly PS, Ma HC et al (2015) Phosphatidylserine vesicles enable efficient en bloc transmission of enteroviruses. Cell 160:619–630
19. Cheng CY, Chi PI, Liu HJ (2014) Commentary on the regulation of viral proteins in autophagy process. Biomed Res Int 2014:962915
20. Chiramel AI, Brady NR, Bartenschlager R (2013) Divergent roles of autophagy in virus infection. Cells 2:83–104
21. Cho MW, Teterina N, Egger D, Bienz K, Ehrenfeld E (1994) Membrane rearrangement and vesicle induction by recombinant poliovirus 2C and 2BC in human cells. Virology 202:129–145
22. Choi Y, Bowman JW, Jung JU (2018) Autophagy during viral infection—a double-edged sword. Nat Rev Microbiol 16:341–354
23. Cirone M (2018) EBV and KSHV infection dysregulates autophagy to optimize viral replication, prevent immune recognition and promote tumorigenesis. Viruses 10
24. Corona Velazquez A, Corona AK, Klein KA, Jackson WT (2018) Poliovirus induces autophagic signaling independent of the ULK1 complex. Autophagy 14:1201–1213
25. Cortese M, Goellner S, Acosta EG, Neufeldt CJ, Oleksiuk O, Lampe M, Haselmann U, Funaya C, Schieber N, Ronchi P et al (2017) Ultrastructural characterization of Zika virus replication factories. Cell Rep 18:2113–2123
26. Cottam EM, Maier HJ, Manifava M, Vaux LC, Chandra-Schoenfelder P, Gerner W, Britton P, Ktistakis NT, Wileman T (2011) Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. Autophagy 7:1335–1347
27. Cottam EM, Whelband MC, Wileman T (2014) Coronavirus NSP6 restricts autophagosome expansion. Autophagy 10:1426–1441
28. Crawford SE, Estes MK (2013) Viroporin-mediated calcium-activated autophagy. Autophagy 9:797–798
29. Crawford SE, Hyser JM, Utama B, Estes MK (2012) Autophagy hijacked through viroporin-activated calcium/calmodulin-dependent kinase kinase-beta signaling is required for rotavirus replication. Proc Natl Acad Sci USA 109:E3405–E3413
30. Cuconati A, White E (2002) Viral homologs of BCL-2: role of apoptosis in the regulation of virus infection. Genes Dev 16:2465–2478
31. de Haan CA, Reggiori F (2008) Are nidoviruses hijacking the autophagy machinery? Autophagy 4:276–279
32. De Leo A, Colavita F, Ciccosanti F, Fimia GM, Lieberman PM, Mattia E (2015) Inhibition of autophagy in EBV-positive Burkitt's lymphoma cells enhances EBV lytic genes expression and replication. Cell Death Dis 6:e1876
33. Denizot M, Varbanov M, Espert L, Robert-Hebmann V, Sagnier S, Garcia E, Curriu M, Mamoun R, Blanco J, Biard-Piechaczyk M (2008) HIV-1 gp41 fusogenic function triggers autophagy in uninfected cells. Autophagy 4:998–1008
34. Deretic V, Saitoh T, Akira S (2013) Autophagy in infection, inflammation and immunity. Nat Rev Immunol 13:722–737

35. Ding B, Zhang G, Yang X, Zhang S, Chen L, Yan Q, Xu M, Banerjee AK, Chen M (2014) Phosphoprotein of human parainfluenza virus type 3 blocks autophagosome-lysosome fusion to increase virus production. Cell Host Microbe 15:564–577
36. Ding B, Zhang L, Li Z, Zhong Y, Tang Q, Qin Y, Chen M (2017) The matrix protein of Human Parainfluenza virus type 3 induces Mitophagy that suppresses interferon responses. Cell Host Microbe 21(538–547):e534
37. Dong X, Cheng A, Zou Z, Yang YS, Sumpter RM Jr, Huang CL, Bhagat G, Virgin HW, Lira SA, Levine B (2016) Endolysosomal trafficking of viral G protein-coupled receptor functions in innate immunity and control of viral oncogenesis. Proc Natl Acad Sci USA 113:2994–2999
38. Dreux M, Chisari FV (2009) Autophagy proteins promote hepatitis C virus replication. Autophagy 5:1224–1225
39. Dreux M, Gastaminza P, Wieland SF, Chisari FV (2009) The autophagy machinery is required to initiate hepatitis C virus replication. Proc Natl Acad Sci USA 106:14046–14051
40. Egger D, Teterina N, Ehrenfeld E, Bienz K (2000) Formation of the poliovirus replication complex requires coupled viral translation, vesicle production, and viral RNA synthesis. J Virol 74:6570–6580
41. Feng Z, Hensley L, McKnight KL, Hu F, Madden V, Ping L, Jeong SH, Walker C, Lanford RE, Lemon SM (2013) A pathogenic picornavirus acquires an envelope by hijacking cellular membranes. Nature 496:367–371
42. Gannage M, Dormann D, Albrecht R, Dengjel J, Torossi T, Ramer PC, Lee M, Strowig T, Arrey F, Conenello G et al (2009) Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. Cell Host Microbe 6:367–380
43. Gosert R, Kanjanahaluethai A, Egger D, Bienz K, Baker SC (2002) RNA replication of mouse hepatitis virus takes place at double-membrane vesicles. J Virol 76:3697–3708
44. Granato M, Santarelli R, Filardi M, Gonnella R, Farina A, Torrisi MR, Faggioni A, Cirone M (2015) The activation of KSHV lytic cycle blocks autophagy in PEL cells. Autophagy 11:1978–1986
45. Gregoire IP, Rabourdin-Combe C, Faure M (2012) Autophagy and RNA virus interactomes reveal IRGM as a common target. Autophagy 8:1136–1137
46. Gregoire IP, Richetta C, Meyniel-Schicklin L, Borel S, Pradezynski F, Diaz O, Deloire A, Azocar O, Baguet J, Le Breton M et al (2011) IRGM is a common target of RNA viruses that subvert the autophagy network. PLoS Pathog 7:e1002422
47. Guevin C, Manna D, Belanger C, Konan KV, Mak P, Labonte P (2010) Autophagy protein ATG5 interacts transiently with the hepatitis C virus RNA polymerase (NS5B) early during infection. Virology 405:1–7
48. Hamel R, Dejarnac O, Wichit S, Ekchariyawat P, Neyret A, Luplertlop N, Perera-Lecoin M, Surasombatpattana P, Talignani L, Thomas F et al (2015) Biology of Zika virus infection in human skin cells. J Virol 89:8880–8896
49. Hansen MD, Johnsen IB, Stiberg KA, Sherstova T, Wakita T, Richard GM, Kandasamy RK, Meurs EF, Anthonsen MW (2017) Hepatitis C virus triggers Golgi fragmentation and autophagy through the immunity-related GTPase M. Proc Natl Acad Sci USA 114:E3462–E3471
50. Harcourt BH, Jukneliene D, Kanjanahaluethai A, Bechill J, Severson KM, Smith CM, Rota PA, Baker SC (2004) Identification of severe acute respiratory syndrome coronavirus replicase products and characterization of papain-like protease activity. J Virol 78:13600–13612
51. He B (2006) Viruses, endoplasmic reticulum stress, and interferon responses. Cell Death Differ 13:393–403
52. Heaton NS, Randall G (2010) Dengue virus-induced autophagy regulates lipid metabolism. Cell Host Microbe 8:422–432
53. Heaton NS, Randall G (2011) Dengue virus and autophagy. Viruses 3:1332–1341
54. Hwang S, Maloney NS, Bruinsma MW, Goel G, Duan E, Zhang L, Shrestha B, Diamond MS, Dani A, Sosnovtsev SV et al (2012) Nondegradative role of Atg5–Atg12/ Atg16L1 autophagy protein complex in anti-viral activity of interferon gamma. Cell Host Microbe 11:397–409

55. Jackson WT, Giddings TH Jr, Taylor MP, Mulinyawe S, Rabinovitch M, Kopito RR, Kirkegaard K (2005) Subversion of cellular autophagosomal machinery by RNA viruses. PLoS Biol 3:e156
56. Jones DM, McLauchlan J (2010) Hepatitis C virus: assembly and release of virus particles. J Biol Chem 285:22733–22739
57. Jordan TX, Randall G (2012) Manipulation or capitulation: virus interactions with autophagy. Microbes Infect 14:126–139
58. Jordan TX, Randall G (2017) Dengue virus activates the AMP kinase-mTOR axis to stimulate a proviral lipophagy. J Virol 91
59. Joubert PE, Meiffren G, Gregoire IP, Pontini G, Richetta C, Flacher M, Azocar O, Vidalain PO, Vidal M, Lotteau V et al (2009) Autophagy induction by the pathogen receptor CD46. Cell Host Microbe 6:354–366
60. Joubert PE, Werneke SW, de la Calle C, Guivel-Benhassine F, Giodini A, Peduto L, Levine B, Schwartz O, Lenschow DJ, Albert ML (2012) Chikungunya virus-induced autophagy delays caspase-dependent cell death. J Exp Med 209:1029–1047
61. Kanjanahaluethai A, Chen Z, Jukneliene D, Baker SC (2007) Membrane topology of murine coronavirus replicase nonstructural protein 3. Virology 361:391–401
62. Ke PY, Chen SS (2011) Activation of the unfolded protein response and autophagy after hepatitis C virus infection suppresses innate anti-viral immunity in vitro. J Clin Invest 121:37–56
63. Kemball CC, Alirezaei M, Flynn CT, Wood MR, Harkins S, Kiosses WB, Whitton JL (2010) Coxsackievirus infection induces autophagy-like vesicles and megaphagosomes in pancreatic acinar cells in vivo. J Virol 84:12110–12124
64. Kim J, Guan KL (2019) mTOR as a central hub of nutrient signalling and cell growth. Nat Cell Biol 21:63–71
65. Kim N, Kim MJ, Sung PS, Bae YC, Shin EC, Yoo JY (2016) Interferon-inducible protein SCOTIN interferes with HCV replication through the autolysosomal degradation of NS5A. Nat Commun 7:10631
66. Knoops K, Kikkert M, Worm SH, Zevenhoven-Dobbe JC, van der Meer Y, Koster AJ, Mommaas AM, Snijder EJ (2008) SARS-coronavirus replication is supported by a reticulovesicular network of modified endoplasmic reticulum. PLoS Biol 6:e226
67. Ku B, Woo JS, Liang C, Lee KH, Hong HS, Xiaofei E, Kim KS, Jung JU, Oh BH (2008) Structural and biochemical bases for the inhibition of autophagy and apoptosis by viral BCL-2 of murine gamma-herpesvirus 68. PLoS Pathog 4:e25
68. Kyei GB, Dinkins C, Davis AS, Roberts E, Singh SB, Dong C, Wu L, Kominami E, Ueno T, Yamamoto A et al (2009) Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. J Cell Biol 186:255–268
69. Lee DY, Sugden B (2008) The latent membrane protein 1 oncogene modifies B-cell physiology by regulating autophagy. Oncogene 27:2833–2842
70. Lee DY, Sugden B (2008) The LMP1 oncogene of EBV activates PERK and the unfolded protein response to drive its own synthesis. Blood 111:2280–2289
71. Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A (2007) Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science 315:1398–1401
72. Lee JS, Li Q, Lee JY, Lee SH, Jeong JH, Lee HR, Chang H, Zhou FC, Gao SJ, Liang C et al (2009) FLIP-mediated autophagy regulation in cell death control. Nat Cell Biol 11:1355–1362
73. Lee YR, Lei HY, Liu MT, Wang JR, Chen SH, Jiang-Shieh YF, Lin YS, Yeh TM, Liu CC, Liu HS (2008) Autophagic machinery activated by dengue virus enhances virus replication. Virology 374:240–248
74. Lennemann NJ, Coyne CB (2017) Dengue and Zika viruses subvert reticulophagy by NS2B3-mediated cleavage of FAM134B. Autophagy 13:322–332
75. Levine B (2005) Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. Cell 120:159–162
76. Levine B, Kroemer G (2008) Autophagy in the pathogenesis of disease. Cell 132:27–42
77. Levine B, Mizushima N, Virgin HW (2011) Autophagy in immunity and inflammation. Nature 469:323–335

78. Li J, Liu Y, Wang Z, Liu K, Wang Y, Liu J, Ding H, Yuan Z (2011) Subversion of cellular autophagy machinery by hepatitis B virus for viral envelopment. J Virol 85:6319–6333
79. Liang C, Oh BH, Jung JU (2015) Novel functions of viral anti-apoptotic factors. Nat Rev Microbiol 13:7–12
80. Liang Q, Chang B, Brulois KF, Castro K, Min CK, Rodgers MA, Shi M, Ge J, Feng P, Oh BH et al (2013) Kaposi's sarcoma-associated herpesvirus K7 modulates Rubicon-mediated inhibition of autophagosome maturation. J Virol 87:12499–12503
81. Liang Q, Luo Z, Zeng J, Chen W, Foo SS, Lee SA, Ge J, Wang S, Goldman SA, Zlokovic BV et al (2016) Zika virus NS4A and NS4B proteins deregulate Akt-mTOR Signaling in human fetal neural stem cells to inhibit neurogenesis and induce autophagy. Cell Stem Cell 19:663–671
82. Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, Herman B, Levine B (1998) Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. J Virol 72:8586–8596
83. Liu B, Fang M, Hu Y, Huang B, Li N, Chang C, Huang R, Xu X, Yang Z, Chen Z et al (2014) Hepatitis B virus X protein inhibits autophagic degradation by impairing lysosomal maturation. Autophagy 10:416–430
84. Liu K, Czaja MJ (2013) Regulation of lipid stores and metabolism by lipophagy. Cell Death Differ 20:3–11
85. Ma J, Sun Q, Mi R, Zhang H (2011) Avian influenza A virus H5N1 causes autophagy-mediated cell death through suppression of mTOR signaling. J Genet Genomics 38:533–537
86. Ma Y, Galluzzi L, Zitvogel L, Kroemer G (2013) Autophagy and cellular immune responses. Immunity 39:211–227
87. Maier HJ, Britton P (2012) Involvement of autophagy in coronavirus replication. Viruses 4:3440–3451
88. Marin M, Rose KM, Kozak SL, Kabat D (2003) HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat Med 9:1398–1403
89. Mateo R, Nagamine CM, Spagnolo J, Mendez E, Rahe M, Gale M Jr, Yuan J, Kirkegaard K (2013) Inhibition of cellular autophagy deranges dengue virion maturation. J Virol 87:1312–1321
90. McFadden K, Hafez AY, Kishton R, Messinger JE, Nikitin PA, Rathmell JC, Luftig MA (2016) Metabolic stress is a barrier to Epstein-Barr virus-mediated B-cell immortalization. Proc Natl Acad Sci USA 113:E782–E790
91. Meiffren G, Joubert PE, Gregoire IP, Codogno P, Rabourdin-Combe C, Faure M (2010) Pathogen recognition by the cell surface receptor CD46 induces autophagy. Autophagy 6:299–300
92. Miller S, Krijnse-Locker J (2008) Modification of intracellular membrane structures for virus replication. Nat Rev Microbiol 6:363–374
93. Mizushima N, Levine B (2010) Autophagy in mammalian development and differentiation. Nat Cell Biol 12:823–830
94. Mohamud Y, Shi J, Qu J, Poon T, Xue YC, Deng H, Zhang J, Luo H (2018) Enteroviral infection inhibits autophagic flux via disruption of the SNARE complex to enhance viral replication. Cell Rep 22:3292–3303
95. Mouna L, Hernandez E, Bonte D, Brost R, Amazit L, Delgui LR, Brune W, Geballe AP, Beau I, Esclatine A (2016) Analysis of the role of autophagy inhibition by two complementary human cytomegalovirus BECN1/Beclin 1-binding proteins. Autophagy 12:327–342
96. Naniche D, Varior-Krishnan G, Cervoni F, Wild TF, Rossi B, Rabourdin-Combe C, Gerlier D (1993) Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. J Virol 67:6025–6032
97. O'Donnell V, Pacheco JM, LaRocco M, Burrage T, Jackson W, Rodriguez LL, Borca MV, Baxt B (2011) Foot-and-mouth disease virus utilizes an autophagic pathway during viral replication. Virology 410:142–150
98. Offerdahl DK, Dorward DW, Hansen BT, Bloom ME (2017) Cytoarchitecture of Zika virus infection in human neuroblastoma and Aedes albopictus cell lines. Virology 501:54–62

99. Oostra M, Hagemeijer MC, van Gent M, Bekker CP, te Lintelo EG, Rottier PJ, de Haan CA (2008) Topology and membrane anchoring of the coronavirus replication complex: not all hydrophobic domains of nsp3 and nsp6 are membrane spanning. J Virol 82:12392–12405
100. Orvedahl A, Alexander D, Talloczy Z, Sun Q, Wei Y, Zhang W, Burns D, Leib DA, Levine B (2007) HSV-1 ICP34.5 confers neurovirulence by targeting the Beclin 1 autophagy protein. Cell Host Microbe 1:23–35
101. Orvedahl A, MacPherson S, Sumpter R Jr, Talloczy Z, Zou Z, Levine B (2010) Autophagy protects against Sindbis virus infection of the central nervous system. Cell Host Microbe 7:115–127
102. Orvedahl A, Sumpter R Jr, Xiao G, Ng A, Zou Z, Tang Y, Narimatsu M, Gilpin C, Sun Q, Roth M et al (2011) Image-based genome-wide siRNA screen identifies selective autophagy factors. Nature 480:113–117
103. Paludan C, Schmid D, Landthaler M, Vockerodt M, Kube D, Tuschl T, Munz C (2005) Endogenous MHC class II processing of a viral nuclear antigen after autophagy. Science 307:593–596
104. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B (2005) Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 122:927–939
105. Perot BP, Ingersoll MA, Albert ML (2013) The impact of macroautophagy on CD8(+) T-cell-mediated antiviral immunity. Immunol Rev 255:40–56
106. Petkova DS, Verlhac P, Rozieres A, Baguet J, Claviere M, Kretz-Remy C, Mahieux R, Viret C, Faure M (2017) Distinct contributions of autophagy receptors in measles virus replication. Viruses 9
107. Petkova DS, Viret C, Faure M (2012) IRGM in autophagy and viral infections. Front Immunol 3:426
108. Prentice E, Jerome WG, Yoshimori T, Mizushima N, Denison MR (2004) Coronavirus replication complex formation utilizes components of cellular autophagy. J Biol Chem 279:10136–10141
109. Reggiori F, Monastyrska I, Verheije MH, Cali T, Ulasli M, Bianchi S, Bernasconi R, de Haan CA, Molinari M (2010) Coronaviruses Hijack the LC3-I-positive EDEMosomes, ER-derived vesicles exporting short-lived ERAD regulators, for replication. Cell Host Microbe 7:500–508
110. Ribeiro CM, Sarrami-Forooshani R, Setiawan LC, Zijlstra-Willems EM, van Hamme JL, Tigchelaar W, van der Wel NN, Kootstra NA, Gringhuis SI, Geijtenbeek TB (2016) Receptor usage dictates HIV-1 restriction by human TRIM5alpha in dendritic cell subsets. Nature 540:448–452
111. Richards AL, Jackson WT (2012) Intracellular vesicle acidification promotes maturation of infectious poliovirus particles. PLoS Pathog 8:e1003046
112. Richetta C, Gregoire IP, Verlhac P, Azocar O, Baguet J, Flacher M, Tangy F, Rabourdin-Combe C, Faure M (2013) Sustained autophagy contributes to measles virus infectivity. PLoS Pathog 9:e1003599
113. Robinson SM, Tsueng G, Sin J, Mangale V, Rahawi S, McIntyre LL, Williams W, Kha N, Cruz C, Hancock BM et al (2014) Coxsackievirus B exits the host cell in shed microvesicles displaying autophagosomal markers. PLoS Pathog 10:e1004045
114. Romao S, Gannage M, Munz C (2013) Checking the garbage bin for problems in the house, or how autophagy assists in antigen presentation to the immune system. Semin Cancer Biol 23:391–396
115. Sagnier S, Daussy CF, Borel S, Robert-Hebmann V, Faure M, Blanchet FP, Beaumelle B, Biard-Piechaczyk M, Espert L (2015) Autophagy restricts HIV-1 infection by selectively degrading Tat in $CD4^+$ T lymphocytes. J Virol 89:615–625
116. Salonen A, Ahola T, Kaariainen L (2005) Viral RNA replication in association with cellular membranes. Curr Top Microbiol Immunol 285:139–173
117. Samsa MM, Mondotte JA, Iglesias NG, Assuncao-Miranda I, Barbosa-Lima G, Da Poian AT, Bozza PT, Gamarnik AV (2009) Dengue virus capsid protein usurps lipid droplets for viral particle formation. PLoS Pathog 5:e1000632

118. Santiago C, Celma ML, Stehle T, Casasnovas JM (2010) Structure of the measles virus hemagglutinin bound to the CD46 receptor. Nat Struct Mol Biol 17:124–129
119. Sharma V, Verma S, Seranova E, Sarkar S, Kumar D (2018) Selective autophagy and xenophagy in infection and disease. Front Cell Dev Biol 6:147
120. Shi J, Wong J, Piesik P, Fung G, Zhang J, Jagdeo J, Li X, Jan E, Luo H (2013) Cleavage of sequestosome 1/p62 by an enteroviral protease results in disrupted selective autophagy and impaired NFKB signaling. Autophagy 9:1591–1603
121. Shi X, Chen Z, Tang S, Wu F, Xiong S, Dong C (2016) Coxsackievirus B3 infection induces autophagic flux, and autophagosomes are critical for efficient viral replication. Arch Virol 161:2197–2205
122. Shrivastava S, Raychoudhuri A, Steele R, Ray R, Ray RB (2011) Knockdown of autophagy enhances the innate immune response in hepatitis C virus-infected hepatocytes. Hepatology 53:406–414
123. Silva LM, Jung JU (2013) Modulation of the autophagy pathway by human tumor viruses. Semin Cancer Biol 23:323–328
124. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ (2009) Autophagy regulates lipid metabolism. Nature 458:1131–1135
125. Sir D, Ann DK, Ou JH (2010) Autophagy by hepatitis B virus and for hepatitis B virus. Autophagy 6:548–549
126. Sir D, Chen WL, Choi J, Wakita T, Yen TS, Ou JH (2008) Induction of incomplete autophagic response by hepatitis C virus via the unfolded protein response. Hepatology 48:1054–1061
127. Sir D, Liang C, Chen WL, Jung JU, Ou JH (2008) Perturbation of autophagic pathway by hepatitis C virus. Autophagy 4:830–831
128. Sir D, Tian Y, Chen WL, Ann DK, Yen TS, Ou JH (2010) The early autophagic pathway is activated by hepatitis B virus and required for viral DNA replication. Proc Natl Acad Sci USA 107:4383–4388
129. Snijder EJ, van der Meer Y, Zevenhoven-Dobbe J, Onderwater JJ, van der Meulen J, Koerten HK, Mommaas AM (2006) Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. J Virol 80:5927–5940
130. Staring J, von Castelmur E, Blomen VA, van den Hengel LG, Brockmann M, Baggen J, Thibaut HJ, Nieuwenhuis J, Janssen H, van Kuppeveld FJ et al (2017) PLA2G16 represents a switch between entry and clearance of Picornaviridae. Nature 541:412–416
131. Suhy DA, Giddings TH Jr, Kirkegaard K (2000) Remodeling the endoplasmic reticulum by poliovirus infection and by individual viral proteins: an autophagy-like origin for virus-induced vesicles. J Virol 74:8953–8965
132. Sumpter R Jr, Levine B (2011) Selective autophagy and viruses. Autophagy 7:260–265
133. Sumpter R Jr, Sirasanagandla S, Fernandez AF, Wei Y, Dong X, Franco L, Zou Z, Marchal C, Lee MY, Clapp DW et al (2016) Fanconi anemia proteins function in mitophagy and immunity. Cell 165:867–881
134. Sun MX, Huang L, Wang R, Yu YL, Li C, Li PP, Hu XC, Hao HP, Ishag HA, Mao X (2012) Porcine reproductive and respiratory syndrome virus induces autophagy to promote virus replication. Autophagy 8:1434–1447
135. Sun P, Zhang S, Qin X, Chang X, Cui X, Li H, Zhang S, Gao H, Wang P, Zhang Z et al (2018) Foot-and-mouth disease virus capsid protein VP2 activates the cellular EIF2S1-ATF4 pathway and induces autophagy via HSPB1. Autophagy 14:336–346
136. Taguwa S, Kambara H, Fujita N, Noda T, Yoshimori T, Koike K, Moriishi K, Matsuura Y (2011) Dysfunction of autophagy participates in vacuole formation and cell death in cells replicating hepatitis C virus. J Virol 85:13185–13194
137. Tang H, Da L, Mao Y, Li Y, Li D, Xu Z, Li F, Wang Y, Tiollais P, Li T et al (2009) Hepatitis B virus X protein sensitizes cells to starvation-induced autophagy via up-regulation of beclin 1 expression. Hepatology 49:60–71
138. Taylor GS, Long HM, Haigh TA, Larsen M, Brooks J, Rickinson AB (2006) A role for intercellular antigen transfer in the recognition of EBV-transformed B cell lines by EBV nuclear antigen-specific $CD4^+$ T cells. J Immunol 177:3746–3756

139. Taylor MP, Kirkegaard K (2007) Modification of cellular autophagy protein LC3 by poliovirus. J Virol 81:12543–12553
140. Tian Y, Sir D, Kuo CF, Ann DK, Ou JH (2011) Autophagy required for hepatitis B virus replication in transgenic mice. J Virol 85:13453–13456
141. Valera MS, de Armas-Rillo L, Barroso-Gonzalez J, Ziglio S, Batisse J, Dubois N, Marrero-Hernandez S, Borel S, Garcia-Exposito L, Biard-Piechaczyk M et al (2015) The HDAC6/APOBEC3G complex regulates HIV-1 infectiveness by inducing Vif autophagic degradation. Retrovirology 12:53
142. van Gent M, Sparrer KMJ, Gack MU (2018) TRIM proteins and their roles in antiviral host defenses. Annu Rev Virol 5:385–405
143. Verheije MH, Raaben M, Mari M, Te Lintelo EG, Reggiori F, van Kuppeveld FJ, Rottier PJ, de Haan CA (2008) Mouse hepatitis coronavirus RNA replication depends on GBF1-mediated ARF1 activation. PLoS Pathog 4:e1000088
144. Wang J, Chen J, Liu Y, Zeng X, Wei M, Wu S, Xiong Q, Song F, Yuan X, Xiao Y et al (2019) Hepatitis B virus induces autophagy to promote its replication by the axis of miR-192-3p-XIAP through NF kappa B signaling. Hepatology 69:974–992
145. Wang L, Tian Y, Ou JH (2015) HCV induces the expression of Rubicon and UVRAG to temporally regulate the maturation of autophagosomes and viral replication. PLoS Pathog 11:e1004764
146. Wang R, Zhu Y, Lin X, Ren C, Zhao J, Wang F, Gao X, Xiao R, Zhao L, Chen H et al (2019) Influenza M2 protein regulates MAVS-mediated signaling pathway through interacting with MAVS and increasing ROS production. Autophagy 15:1163–1181
147. Wen HJ, Yang Z, Zhou Y, Wood C (2010) Enhancement of autophagy during lytic replication by the Kaposi's sarcoma-associated herpesvirus replication and transcription activator. J Virol 84:7448–7458
148. Wong J, Zhang J, Si X, Gao G, Mao I, McManus BM, Luo H (2008) Autophagosome supports coxsackievirus B3 replication in host cells. J Virol 82:9143–9153
149. Yamamoto K, Ichijo H, Korsmeyer SJ (1999) BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Mol Cell Biol 19:8469–8478
150. Zhang HT, Chen GG, Hu BG, Zhang ZY, Yun JP, He ML, Lai PB (2014) Hepatitis B virus x protein induces autophagy via activating death-associated protein kinase. J Viral Hepat 21:642–649
151. Zhang J, Lan Y, Li MY, Lamers MM, Fusade-Boyer M, Klemm E, Thiele C, Ashour J, Sanyal S (2018) Flaviviruses exploit the lipid droplet protein AUP1 to trigger lipophagy and drive virus production. Cell Host Microbe 23(819–831):e815
152. Zhang T, Dong K, Liang W, Xu D, Xia H, Geng J, Najafov A, Liu M, Li Y, Han X et al (2015) G-protein-coupled receptors regulate autophagy by ZBTB16-mediated ubiquitination and proteasomal degradation of Atg14L. Elife 4:e06734
153. Zhang ZW, Li ZL, Yuan S (2016) The role of secretory autophagy in Zika virus transfer through the placental barrier. Front Cell Infect Microbiol 6:206
154. Zhao Z, Thackray LB, Miller BC, Lynn TM, Becker MM, Ward E, Mizushima NN, Denison MR, Virgin HW (2007). Coronavirus replication does not require the autophagy gene ATG5. Autophagy 3:581–585
155. Zhirnov OP, Klenk HD (2013) Influenza A virus proteins NS1 and hemagglutinin along with M2 are involved in stimulation of autophagy in infected cells. J Virol 87:13107–13114
156. Zhou Y, Geng P, Liu Y, Wu J, Qiao H, Xie Y, Yin N, Chen L, Lin X, Liu Y et al (2018) Rotavirus-encoded virus-like small RNA triggers autophagy by targeting IGF1R via the PI3K/Akt/mTOR pathway. Biochim Biophys Acta Mol Basis Dis 1864:60–68
157. Zhou Z, Jiang X, Liu D, Fan Z, Hu X, Yan J, Wang M, Gao GF (2009) Autophagy is involved in influenza A virus replication. Autophagy 5:321–328

Chapter 6
The Interplay Between Pattern Recognition Receptors and Autophagy in Inflammation

Yun Zhu, Jian Deng, Mei-Ling Nan, Jing Zhang, Akinkunmi Okekunle, Jiang-Yuan Li, Xiao-Qiang Yu and Pei-Hui Wang

Abstract Pattern recognition receptors (PRRs) are sensors of exogenous and endogenous "danger" signals from pathogen-associated molecular patterns (PAMPs), and damage associated molecular patterns (DAMPs), while autophagy can respond to these signals to control homeostasis. Almost all PRRs can induce autophagy directly or indirectly. Toll-like receptors (TLRs), Nod-like receptors (NLRs), retinoic acid-inducible gene-I-like receptors (RLRs), and cyclic guanosine monophosphate–adenosine monophosphate synthase (cGAS)-stimulator of interferon genes (STING) pathway can induce autophagy directly through Beclin-1 or LC3-dependent pathway, while the interactions with the receptor for advanced glycation end products (RAGE)/high mobility group box 1 (HMGB1), CD91/Calreticulin, and TLRs/HSPs are achieved by protein, $Ca2^{+}$, and mitochondrial homeostasis. Autophagy presents antigens to PRRs and helps to clean the pathogens. In addition, the induced autophagy can form a negative feedback regulation of PRRs-mediated inflammation in cell/disease-specific manner to maintain homeostasis and prevent

M.-L. Nan · J. Zhang · P.-H. Wang (✉)
Advanced Medical Research Institute, Shandong University, Jinan 250012, Shandong, China
e-mail: pei-hui.wang@connect.hku.hk

Y. Zhu
Department of Pediatric Surgery,
Guangzhou Women and Children's Medical Center, Guangzhou Institute of Pediatrics,
Guangzhou Medical University, Guangzhou 510623, Guangdong, China

Y. Zhu · J. Deng · P.-H. Wang
School of Biomedical Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China

A. Okekunle
The Postgraduate College, University of Ibadan, Ibadan 200284, Nigeria

Department of Epidemiology and Medical Statistics, College of Medicine, University of Ibadan, Ibadan 200284, Nigeria

J.-Y. Li
Key Laboratory for Cell and Gene Engineering of Zhejiang Province, College of Life Sciences, Zhejiang University, Hangzhou 310058, People's Republic of China

X.-Q. Yu
School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO 64110-2499, USA

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_6

excessive inflammation. Understanding the interaction between PRRs and autophagy in a specific disease will promote drug development for immunotherapy. Here, we focus on the interactions between PRRs and autophagy and how they affect the inflammatory response.

Keywords PRRs · Autophagy · TLRs · NLRs · cGAS-STING · RLRs · RAGE · HMGB1 · Calreticulin · HSPs

6.1 Introduction

6.1.1 Pattern Recognition Receptors

Pattern recognition receptors (PRRs) are host sensors of exogenous and endogenous "danger" signaling existed in cells of innate immune systems, such as dendritic cells (DCs), macrophages, monocytes, neutrophils, and epithelial cells [150]. They can recognize molecules typically from microbial pathogens called pathogen-associated molecular patterns (PAMPs) [83] and components of host cells released during cell damage or death, which are damage associated molecular patterns (DAMPs). PRRs are key components of the innate immune system [104] evolving before adaptive immunity and also distinguish complex molecular architecture to activate down-regulatory signaling to promote homeostasis in immunologic responses [191]. Upon the binding of PRRs with PAMPs or DAMPs, the downstream signaling is activated to release the inflammatory cytokines, thereby initiating an adaptive immune response [83, 160].

6.1.2 Classification of PRRs

Diverse PRR families have been identified as mediators of PAMPs or DAMPs recognition. Toll-like receptors (TLRs) are the most prominent PRRs with the capacity to recognize the widest range of PAMPs or DAMPs. While TLR1, 2, 4, 5, 6, and 10 are located on the cell surface, TLR3, 7, 8, and 9 present in intracellular membranes. TLR signaling can induce pro-inflammatory cytokines and type I interferons (IFNs) depending on the myeloid differentiation factor 88 (MyD88) or the Toll/IFN response factor (TRIF) [68]. In contrast, NOD-like receptors (NLRs) are cytoplasmic PRRs made up of three subfamilies: NODs, NLRPs, and the IPAF [82, 150]. NOD1 and NOD2 can initiate pro-inflammatory signaling by activating NF-κB dependent pathway. NLRP3 induces the formation of the inflammasomes in response to the stimulation from DAMPs including extracellular ATP, hyaluronan, uric acid, and so on, which can activate caspase-1 for the release of IL-1β, and IL-18.

There are also several other PRRs that can recognize more specific types of PAMPs or DAMPs. Cyclic guanosine monophosphate (GMP)-adenosine monophosphate (AMP) synthase (cGAS) can sense the cytoplasm DNA and catalyze the formation of second messenger cGAMP for recruiting the adaptor protein stimulator of IFN gene (STING) [65], which induces the production of IFNs and other pro-inflammatory cytokines through NF-κB, TBK1, and IRF3 dependent pathway [165]. RIG-like receptors (RLRs), including RIG-I, MDA5, and LGP2, can detect cytoplasmic RNA, such as viral RNA and self RNA [98]. RLR signaling induces the production of IFNs through MAVS dependent pathway and can also cross talk with cGAS-STING and inflammasome signaling pathway for the regulation of immune response. Scavenger receptors such as CD36, CD44, CD68, CD91, CXCL16, and the receptor for advanced glycation end products (RAGE) expressed on macrophages or other cell types are mainly responsible for the recognition of DAMPs including HMGB1, Calreticulin, HSP, ATP, S100, and host DNA, as well as some PAMPs, and mediate inflammation, oxidative stress, and apoptosis [135].

6.1.3 PRRs and Inflammatory Disease

PRRs-mediated inflammation contributes to the clearance of microbial infection or tissue damage [2, 9], but also causes autoimmune disease. Overproduction of pro-inflammatory cytokines by immune cells can be fatal and is also critical for the pathogenesis of autoimmune diseases [109]. It is well known that septic shock is caused by acute inflammation from the activation of TLR signaling in response to bacterial components. cGAS-STING can sense self-DNA that is released or leaked from the nucleus and mitochondria into the cytoplasm and trigger autoimmunity in Aicardi-Gourtieres syndrome [46, 102]. TLR9 and non-canonical autophagy also play vital roles in mediating systemic lupus erythematosus [52]. In the mouse model, the lack of negative control of PRRs signaling is associated with autoimmune disease. Loss of TANK, an inhibitor of TLR signaling results in autoimmune glomerular nephritis [77]; while the loss of A20, a negative regulator of NF-κB activator, can result in multi-organ inflammatory disorders [13, 56]. In addition, TLR-mediated inflammation contributes to the pathogenesis of ischemia-reperfusion myocardial injury [6].

In contrast, triggering the PRRs-mediated immune response is a strategy for cancer immunotherapy. One of the ideal targets is the cGAS-STING pathway. STING mediates multiple types of tumor killing effects by recognizing self-DNA from dying tumor cells [24]. The combination of cGAMP with irradiation or immune system-checkpoint inhibitors provides a promising outcome for tumor immunotherapy [25, 27]. In addition, CD91/Calreticulin on plasma membrane promotes an “eat” me signal in tumor cells [119]. Any drug/therapy designed to promote calreticulin escape of ER retention to the plasma membrane can enhance tumor immunity and trigger CD91/calreticulin-mediated tumor killing effects.

6.1.4 Autophagy and Innate Immunity

Autophagy is a conserved "self-eaten" cellular activity in response to starvation. In this activity, cytosolic material is delivered into double-membrane vesicles (autophagosomes) and fused with late endosomes or lysosomes [112]. However, cytosolic bacteria damaged organelles or protein aggregates could also be eliminated through selective autophagy, which indicates the potential of autophagy in regulating innate immunity [180]. Autophagy plays important roles in host cell defense to bacteria invasion including *Shigella flexneri* [121], *Listeria monocytogenes* [136], *Salmonella Typhimurium* [11], and *Mycobacterium tuberculosis* [47]. The importance of autophagy in innate immunity is proven by the fight of bacteria against autophagy [62]. Some bacteria such as *S. Typhimurium*, *M. tuberculosis*, and *Bacillus anthracis* can express some genes or toxins to inhibit autophagy initiation signaling through blocking pro-autophagy signals such as mTOR and reactive oxygen species (ROS) or promoting anti-autophagy signal through a second messenger called cyclic AMP [8, 38, 154]. The bacterium can inactivate autophagy components. For example, *Legionella pneumophila* can produce RAVZ protein, an ATG4B-like cysteine protease to degrade LC3 [20]. *Shigella flexneri* can invade epithelial cells and escape from autophagy by T3SS effector VirA to inactivate RAB GTPases [36]. The most interesting pathway of evasion of autophagy recognition is through masking the bacterial surface by either recruiting host cytoskeleton proteins on their surface in *Listeria monocytogenes* [189] or abolish the binding of ATG5 in *S. flexneri* [121]. Recently, a T3SS effector SopF that potently blocked *Salmonella* autophagy was reported. V-ATPase can recruit ATG16L1 onto bacteria containing vacuole after bacteria caused vacuolar damage, which was blocked by SopF, leading to autophagy inhibition and enhanced *S. Typhimurium* proliferation in vivo. In addition, some bacterium can block autophagosome fusion with the lysosome, although the mechanism remains unclear. Therefore, PRR induced autophagy could be a promising target for drug design in the treatment of bacterial infection.

6.1.5 Autophagy Contributes to Regulate PRRs-Mediated Inflammation

The connection between PRRs and autophagy is well-demonstrated in Crohn's disease. Susceptible genes for Crohn's disease are NOD2 and ATG16L1, which are core autophagy genes [31]. Functional studies show that NOD2 can recruit ATG16L1 to the bacterial entry site for inducement of autophagy [171]. Unexpectedly, the hypomorphic ATG16 allele can enhance the resistance of mice to *Citrobacter rodentium* and uropathogenic *Escherichia coli* [107, 175]. These results might suggest not only the cooperation between PRRs and autophagy but also the negative feedback regulation.

Currently, two different types of autophagy which are LC3- or Beclin 1-dependent autophagy involve the interaction with PRRs. LC3-dependent autophagy is achieved by recognition of 'eat-me' signals of cargoes (e.g., galectin-8 from bacteria, poly-ubiquitin) by cargo receptor p62 and its paralog NBR1, NDP52, T6BP, and optineurin for the association with LC3/GABARAP on phagophores [14, 70]. The generation of ubiquitin "eat-me'"signal is PRR signaling-dependent [64]. More interestingly, Beclin-1/Bcl-2 complex is a toggle switch regulating autophagy/apoptosis. Beclin-1, a Bcl-2 homology 3 (BH3) domain only protein [120], can initiate autophagy through recruiting other autophagic proteins and forming Beclin-1-Vps34-Vps15 core complex [51]. Also, the interaction of Beclin-1 with anti-apoptotic Bcl-2 family members prevents Beclin-1-mediated initiation of autophagy [91].

Most PRRs can affect autophagy directly or indirectly. TLRs, NLRs, RLRs, and cGAS-STING can induce autophagy directly through Beclin-1 or LC3-dependent pathway, but the interactions with RAGE/HMGB1, CD91/Calreticulin, and TLRs/HSPs are achieved by protein, $Ca2^{+}$, and mitochondrial homeostasis. Through the close interaction with PRRs, autophagy presents antigens to PRRs that helps in destroying pathogens. Also, autophagy-mediated PRR inflammation prevents the over-reaction of the immune response. Therefore, the focus of this write-up is to discuss the association between PRRs and autophagy which is a promising therapy in the management of infection concerning the inflammatory response.

6.2 Pathogen-Dependent PRR Signaling and Autophagy

6.2.1 TLRs

The TLRs are integral transmembrane proteins with N-terminal ectodomain of leucine-rich repeats (LRRs) and intracellular Toll/IL-1 receptor (TIR) domain. It contains 6 members (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) located on the plasma membrane and 4 (TLR3, TLR7, TLR8, and TLR9) in the lumen of endocytic compartments that uptake microbial components [161]. Extracellular LRRs of different TLRs with diversity in length, number, and N-linked glycosylation can form a horseshoe-shaped structure for pathogen recognition and shape the recognition specificity. The TLR heterodimers can also regulate the recognition specificity or versatility of PAMPs. TLR4 and TLR5 recognize LPS from Gram-negative bacterial and bacterial flagellin from flagellated bacteria, respectively, but TLR10 can sense the influenza virulence from influenza A virus. However, TLR2/TLR10 heterodimers can sense Listeria proteins. TLR2 combined with TLR1 or TLR6 can recognize most of extracellular PAMPs such as lipopeptides, peptidoglycans, lipoteichoic acid, zymosan, and mannan. In endocytic compartments, endocytic TLRs mainly recognize RNA, TLR3 for viral dsRNA and siRNAs, TLR7/8 for both viral and bacterial single-stranded RNA (ssRNA), TLR9 for unmethylated CpG DNA motif from bacteria [161].

6.2.1.1 TLRs Signaling Pathway

Upon binding with PAMPs, intracellular TIR domain recruits adaptor proteins to activate NF-κB, MAPK signaling for the production of pro-inflammatory cytokines and type I IFNs [118]. Although there are several adaptor proteins reported in TLRs pathways including MyD88, TRIF, MyD88 adapter-like (Mal/TIRAP), Trif-related adaptor molecule (TRAM), and sterile α and armadillo motif-containing protein (SARM). TRIF is recruited by TLR3 and TLR4, and for the rest TLRs, MyD88 is commonly used.

6.2.1.2 TLRs and Autophagy

Cytosolic TLRs such as TLR7/8 and TLR9 exist in the endosome. How endosomal TLRs recognizes cytosolic viral components remains unclear. Endosomal TLRs may "find" and "eat" the viruses through autophagy. For instance, TLR7 recognizes the replicating vesicular stomatitis virus (VSV) in the cytosol of plasmacytoid dendritic cells (pDCs) that are delivered in the lysosomes through autophagy for activating TLR7 signaling [87]. The essential role of autophagy in activating TLR7/9 signaling is confirmed in autophagy-deficient mice or cells. Atg5-deficient mice are susceptible to systemic VSV infection. Without Atg5, pDCs does not secrete IFN-α and IL-12, which are downstream pro-inflammatory cytokines of TLRs signaling [163].

Autophagy does not just passively deliver the PAMPs to TLRs. It also utilizes TLR signaling to promote phagosome maturation, which is observed by the co-occurrence of TLR activation and fusion with lysosomes. Phagocytosis of the fungal cell wall component zymosan can induce Atg proteins-dependent LC3 recruitment to phagosomes and fusion with lysosomes [147].

In the case of extracellular TLRs, they do not need the help of the autophagy to "find" the PAMPs but induce the autophagy for "eating" pathogens. Autophagy inducement is firstly observed in TLR4 [184], then in various TLRs including TLR1-7, which enhanced the microbial clearance. Interestingly, this process is mediated by a relatively conserved mechanism through the TRIF/MyD88 axis. TRIF/MyD88 can be recruited to TLRs after TLR binding with its ligand for the activation of AP-1/IRF3/NF-κB mediated pro-inflammatory cytokines and IFNs as well as regulating Beclin-1/Bcl-2 interaction for the induction of autophagy [152] (Fig. 6.1).

6.2.1.3 TLRs and Autophagy in Autoimmune Disease

The cooperation between autophagy and endosomal TLRs is not always favorable for health but contribute to autoimmune disease. The recruitment of TLR9-containing endosomes to the autophagosomes with DNA-containing antigens through BCR signals can result in B cells hyper-responses [23]. Another study reports different mechanism for TLR9 and non-canonical autophagy-mediated systemic lupus erythematosus, which is caused by uncontrolled production of type I IFNs. Recruitment of TLR9 and LC3 in response to DNA immune complexes results in LC3-associated phagocytosis, thus producing IFN-α [52].

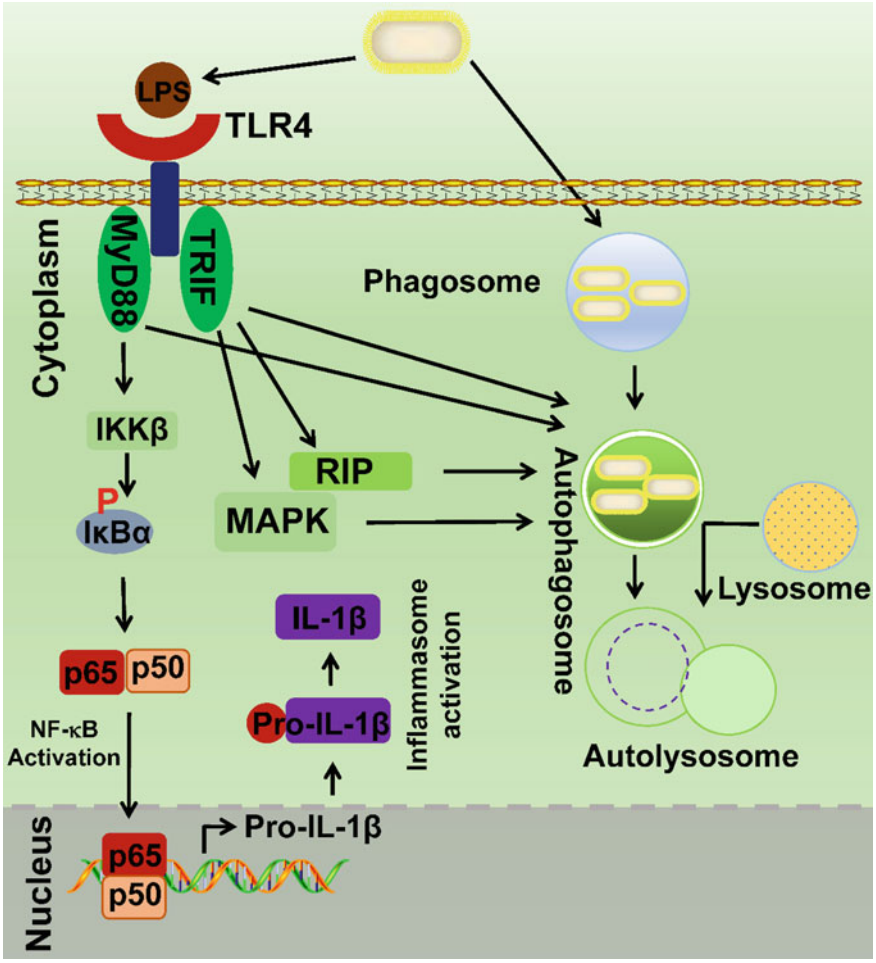


Fig. 6.1 TLRs and autophagy. TLR4 induces autophagosome formation via TRIF-mitogen-activated protein kinase (MAPK)/RIP signaling axis. TLR4 also triggers the myeloid differentiation primary response gene 88 (MyD88)-dependent signaling pathway to activate the transcription factor nuclear factor κB (NF-κB), and promotes pro-IL-1β expression. These processes facilitate fusion of the autophagosomes with the lysosomes, which in turn finally results in the killing of intracellular bacteria

6.2.2 NLRs

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are intracellular sensors of PAMPs and DAMPs including ATP, mtDNA, and ROS that are primarily expressed in macrophages and other professional antigen-presenting cells (APCs). NLRs are signal transduction ATPases with three domains, including a C-terminal leucine-rich repeat (LRR) domain for ligand sensing, a central NATCH,

telomerase-associated protein 1 that mediates self-oligomerization and is essential for activation of NLRs, and an N-terminal effector domain for adapter recruitment [80]. NLRs are classified into three subfamilies according to the nature of the N-terminal domains: NLRC subfamily such as NOD1, NOD2, NLRC3, NLRC4, and NLRC5 with caspase activation and recruitment domain (CARD), NLRP subfamily with pyrin domain including NLRP1-10 for inflammasome assembly, and NAIP subfamily with three baculovirus inhibitors (BIRs) of the apoptosis protein repeat domain such as NAIPs [79, 115].

6.2.2.1 NLR Signaling Pathway

NLR signaling can regulate the production of pro-inflammatory cytokines, and assembly of inflammasomes. NOD1 and NOD2 can sense bacterial peptidoglycan in epithelial cells of the gastrointestinal tract for recruitment of receptor-interacting protein kinase 2 (RIP2), leading to activation of NF-κB and AP-1 signaling for the production of type I INFs and pro-inflammatory cytokines [129]. Unexpectedly, some NLR members such as NLRP4, NLRP6, and NLRP12 can also inhibit NF-κB signaling cascades, although the mechanism is unclear. NLRC3 are reported to inhibit NF-κB signaling through cross talk with TLR4-mediated NF-κB signaling [149]. In addition, NLRs are essential for the assembly of inflammasomes, which can mediate the activation of caspase-1 for the maturation of inactive cytokine from pro-inflammatory stimuli [108, 150]. Upon the detection of PAMPs with NLRs, self-oligomerization occurs followed by recruitment of apoptosis-associated speck-like protein containing a CARD (ASC) as an adaptor for caspase-1 activation. Currently, NLR inflammasomes are reported to mediate the inflammatory response of different DAMPs or PAMPs, e.g., NLRC4 inflammasomes for flagellin, T3SS and T4SS [45, 155, 195], NLRP1 for anthrax lethal toxin [113] and NLRP3 for ATP, amyloid, monosodium urate crystals and silica [16]. Interestingly, these cytosol PAMPs might be generated by IFN inducible protein IRGB10, which can damage the bacterial membrane for the release of DNA and LPS [106].

6.2.2.2 NLRs and Autophagy

NLRs are very important to clear the cytosol-dwelling bacteria. NLRs can also directly induce autophagy in response to bacterial invasion [23, 171]. Different from TLRs, RIP2, and ATG16L1 play an essential role in the inducement of autophagy. Atg16L1 is recruited to the plasma membrane by NOD2 at the first site of bacterial invasion for bacterial trafficking to the autophagosomes and fusion with the lysosomes [171]. NOD2 mutation and Atg16L1 SNPs are associated with Crohn's disease, which is likely to be inflammatory bowel disease due to immunodeficiency. RIP2 is essential for NOD2-dependent autophagy through recognition of muramyl dipeptide from Gram-positive bacteria [57].

NLR inflammasomes can reciprocally regulate autophagy in cell context and antigen-dependent manner. NLRP4 signalosome can induce autophagy in phagosomes in response to group A *Streptococcus* infection through dissociation from Beclin-1 [71]. NLRP3 inflammasome can trigger autophagy for the promotion of *Pseudomo aeruginosa* phagocyte destruction [29]. However, TLR2/TLR4 can activate autophagy but degrade NLRP3 and reduce IL-1β production [21] (Fig. 6.2).

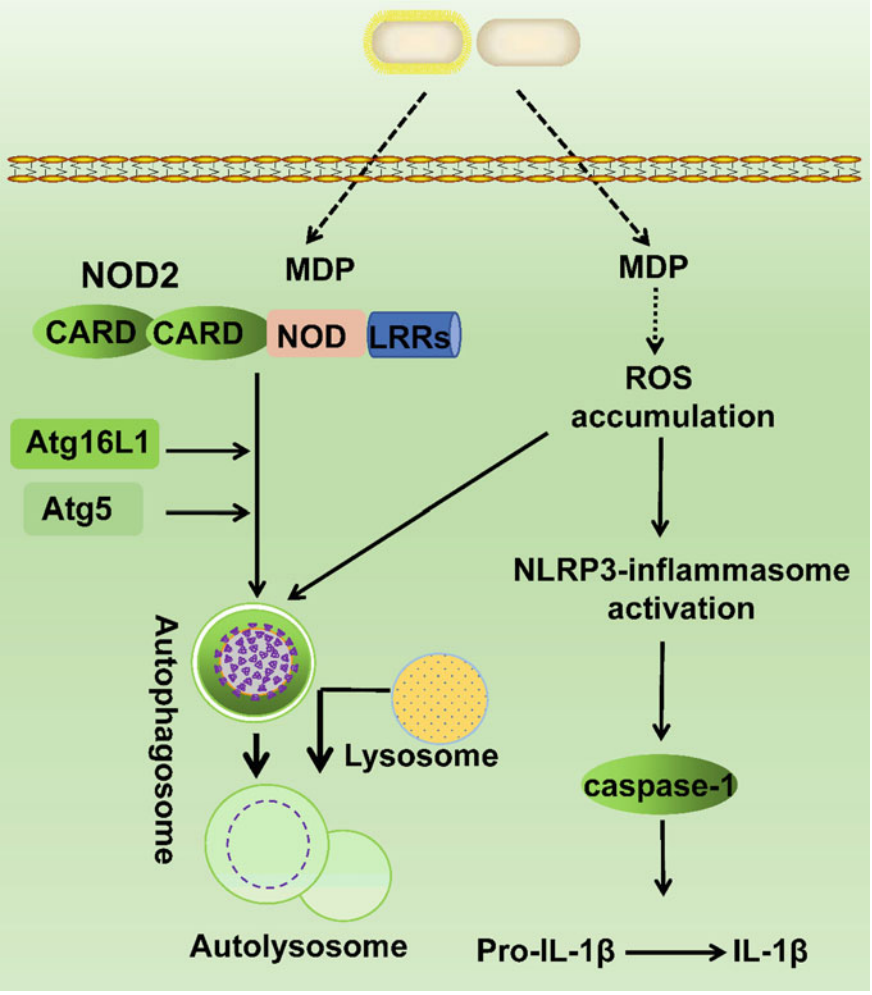


Fig. 6.2 NLRs and autophagy. Activation of NOD2 by bacteria induces autophagosome formation. NOD2 is activated by muramyl dipeptide (MDP) which is found in both Gram-negative and Gram-positive bacteria. In this process, autophagy proteins such as Atg5 and Atg16L1 are required. Autophagy also regulates NLRP3 inflammasome-induced inflammatory responses. MDP released by bacterial infection regenerates ROS, leading to NLRP3 inflammasome activation, which finally activates caspase-1 and results in the maturation and secretion of pro-IL-1β and pro-IL-18

6.2.3 cGAS-STING Pathway

6.2.3.1 The cGAS-STING Pathway of Cytosolic DNA Sensing

cGAS-STING is a cytosolic DNA sensing pathway for triggering immune responses. cGAS can sense the cytosolic DNA and catalyze the formation of second messenger cGAMP which is an endogenous high-affinity ligand for the adaptor protein STING [65]. Upon the binding with cGAMP, the conformation of STING changes and translocates from the endoplasmic reticulum (ER) to the Golgi apparatus for protein modification [145]. The modified STING can recruit and activate TANK-binding kinase 1 (TBK1) and IFN regulatory factor 3 (IRF3) [165]. In addition, STING also activates NF-κB for the production of type I IFNs and other pro-inflammatory cytokines.

6.2.3.2 cGAS Activation

cGAS can bind to the sugar–phosphate backbone of dsDNA and is activated upon binding with dsDNA [159]. Therefore, oxidation of DNA bases caused by ultraviolet irradiation does not change the binding ability with cGAS [43]. ssDNA can also activate cGAS through the formation of internal duplex or Y-shaped structure [53]. Although short dsDNA with 15 bp can activate cGAS, long DNA is more essential for cGAS activation. dsRNA can not activate cGAS although it can bind with cGAS [193].

6.2.3.3 Functions of the GAS-STING Pathway

The cGAS-STING pathway can sense DNA released from microbial pathogens including DNA viruses, retroviruses, endogenous retroviruses and retroelements, and DNA producing bacteria. In cGAS-deficient mice, IFN induction is absent in response to the infection of several DNA viruses including herpes simplex virus, vaccinia virus, adenovirus, cytomegalovirus and Kaposi's sarcoma-associated herpesvirus (KSHV) [92, 101, 125, 182, 192]. Retroviruses HIV-1 and HIV-2 might also be detected by cGAS [85]. HIV can reversely transcribe viral RNA into cDNA and inject the cDNA into the nucleus for the integration to the host genome. However, if the cDNA leak into the cytoplasm due to broken viral capsid, the cDNA can be sensed by cGAS for triggering the induction of IFNs and other inflammatory cytokines. cGAS-deficient mice also cannot respond to multivalent antigens such as bacterial capsular polysaccharides that activate the transcription of endogenous retroviral RNA and is reversely transcribed to DNA by the RNA helicase RIG-I for cGAS sensing [190]. Many intracellular bacteria including *Mycobacteria*, *Legionella*, *Listeria*, *Shigella*, *Francisella*, *Chlamydia*, *Neisseria*, group B *Streptococcus* and so on induce IFNs through the cGAS-STING pathway although it is unclear how bacterial DNA might gain access to the cytoplasm [4, 5, 22, 48, 157, 194].

6.2.3.4 cGAS-STING Pathway in Autoimmune Diseases

Self-DNA that released or leaked from the nucleus and mitochondria into cytoplasm can activate cGAS and trigger autoimmunity. In Aicardi-Gourtieres syndrome, a collection of monogenic autoimmune disease, the mutation in TREX1 which is an exonuclease of dsDNA and ssDNA or RNase H2 which degrades RNA in RNA–DNA hybrid leads to elevated expression of type I IFNs. Mice lacking TREX1 or RNase H2 activates cGAS-STING pathway and exhibits elevated expression IFNs [46, 102]. In patients with early-onset vasculopathy and pulmonary inflammation, gain-of-function mutations of the gene encoding STING are identified, which can render the protein constitutively active and result in IFN production [95]. These genetic studies support the role of the cGAS-STING pathway in autoimmune diseases.

6.2.3.5 cGAS-STING Pathway in Cancer

Self-DNA from dying tumor cells can also trigger the cGAS-STING pathway to induce IFNs [24]. Interestingly, STING mediates multiple types of tumor killing effects. STING is required for priming $CD8^+$ T cells against tumor-associated antigens and is also essential for the anti-tumor effects of radiation. CD47 antibody, a phagocytosis-inhibitory protein, exerts STING-dependent anti-tumor effects [28, 94]. The anti-tumor effect mediated by STING probably through tumor-derived DNA, which delivers to the cytoplasm of DCs and facilitates the activation of $CD8^+$ T cells. Therefore, activation of the cGAS-STING pathway is applied in anti-tumor therapy. One common strategy is the combination of cGAMP with irradiation or immune system-checkpoint inhibitors [25, 27]. However, in some cases, activation of STING facilitates tolerogenic response and metastasis [18, 63]. Optimal combination of different treatments may be essential to achieve a good clinical outcome.

6.2.3.6 cGAS-STING and Autophagy

The cytosolic DNA sensor cGAS can sense cytosolic DNA from bacteria or virus and activate ubiquitin-mediated autophagy for microbe clearance. During *M. tuberculosis* infection, the STING-dependent cytosolic pathway can recognize mycobacterial DNA, which exposed to the host through extra-embryonic spermatogenic homeobox 1 (ESX-1) secretion system, resulting in the recruitment of ubiquitin chains, LC3-binding autophagic adaptors p62 and NDP52 for targeting the mycobacteria to the selective autophagy pathway [179]. Also, cGAS-STING can interact with Beclin-1 to promote PI3 KC3-induced autophagy [159]. Autophagy can also repress STING-dependent IFN responses through Atg9a [145]. Lack of Atg9a induces overactivation of type I IFN through promoting the interaction between STING and TBK1. In the cGAS sensing pathway, the negative feedback control by autophagy occurs through the release of Rubicon, which enhances the autophagy-mediated degradation of pathogen DNA [90] (Fig. 6.3).

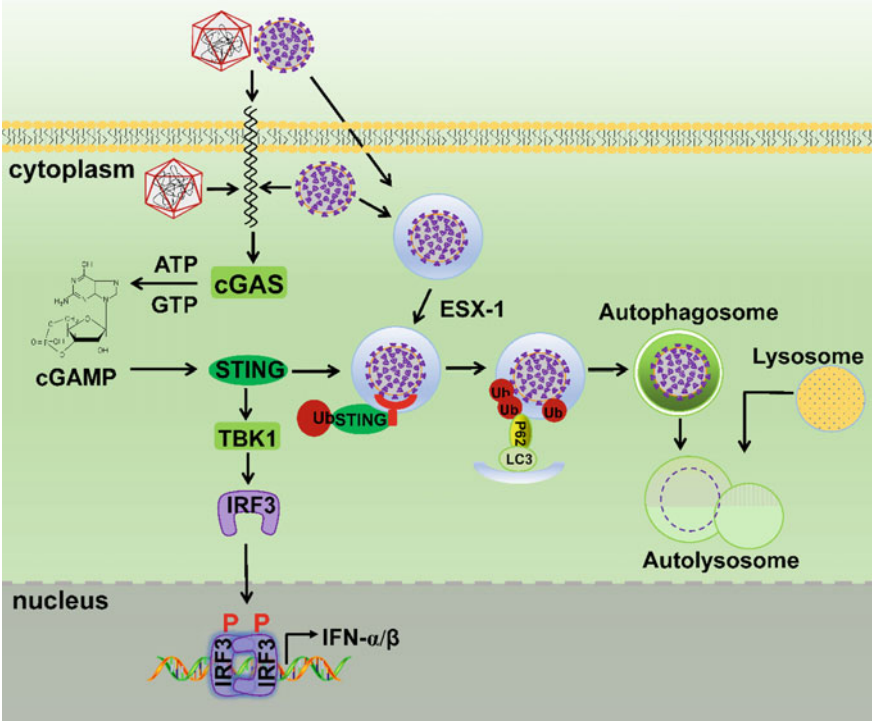


Fig. 6.3 cGAS-STING signaling and autophagy. cGAS-STING pathway mediates anti-microbial innate immunity by inducing the production of type I IFNs and inflammatory cytokines upon recognition of microbial DNA. During bacterial clearance, bacterial extracellular DNA, which is exposed to the host through ESX-1-mediated permeabilization of the phagosomal membrane, is recognized by the STING-dependent cytosolic pathway. The ubiquitinated bacterial DNA, which binds to the autophagosome-associated protein LC3 via adaptor protein p62 and NDP52, is targeted to the selective autophagy pathway

6.2.4 RIG-I-like Receptors (RLRs)

Retinoic acid-inducible gene (RIG-I)-I-like receptors (RLRs) are cytoplasmic sensors of viral RNA [122]. RLRs includes three members: RIG-I, melanoma differentiation associated factor 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2). They are a family of DExD/H box RNA helicases with the capacity to hydrolyze ATP, bind and possibly unwind RNA. RIG-I and MDA5 have two additional domains, an N-terminal region consisting of tandem CARDs, and a C-terminal repressor domain embedded within the C-terminal domain for autoregulation. However, LGP2 can only work as a regulator of RIG-I and MDA5 signaling due to lack of N-terminal CARDs [188].

6.2.4.1 RLRs Signaling Pathway

Upon the detection of viral RNA, RLRs are recruited to a membrane-associated CARD-containing adaptor protein MAVS through homotypic CARD interactions [78]. The interaction with MAVS can accumulate the downstream signaling molecules to form a MAVS signalosome, which can drive IRF3, IRF7, and NF-κB mediated IFN production [55]. In another study, IRF3, IRF7, NF-κB, ATF-2, c-Jun, and transcriptional enhancer CBP-p300 can form a complex to enhance the expression of IFN-β [127]. However, IRF3 and NF-κB might play an essential role in inducing the complex formation, as in most cases, IRF3 and components of the NF-κB activation program constitute MAVS signalosome [55]. The secreted IFN-β can amplify the IFN response by inducing the ISGF3-dependent expression of IFN-stimulated genes for increasing the expression of IFN-α subtypes in a positive feedback loop [97, 133]. In addition to IFN-α/β, RLRs also induce the expression of IFN-λ following the infection of a paramyxovirus Newcastle disease virus through IRFs and NF-κB binding [123]. RIG-1 also associates with ASC protein and triggers caspase-1-dependent inflammasome activation for promoting the mature of pro-IL-1β and pro-IL-18, which involves a MAVS independent signaling [133].

6.2.4.2 Cross talk with Other PRRs Signaling

RLR signaling intersects with TLR signaling probably through the shared components such as IRF3, IRF7, and NF-κB. RLRs can also apply STING as a cofactor, which passes RLR-mediated immune response to virus RNA and STING-mediated immune response to virus DNA [65]. RLR and NLR signaling have direct interactions in terms of regulation of inflammasome signaling. NLRX1 can disrupt the interaction between MAVS and RLR and inhibit the RLR-mediated IFN induction. NLRX1 depletion can rescue the RLR-mediated elimination of virus [114]. Another NLR member, NLRC5 can directly interact with RIG-I and MDA5 and disrupt the RLR-mediated activation of NF-κB. Knockdown of NLRC5 gene can enhance IFN production and antiviral response [26].

6.2.4.3 RLRs and Autophagy

RLR signaling is also reported to induce autophagy but through cross talk with STING [141] (Fig. 6.4). Autophagy can also inhibit RLR signaling [37, 69, 183]. Atg5 deficiency leads to the overproduction of type I IFNs in RIG-I/MDA-5-mediated pro-inflammatory response to VSV infection. On the other hand, with the overexpression of Atg5, the IFN signaling is also suppressed [72]. There are different explanations for how Atg5 can inhibit RLR signaling. One study showed that Atg5 can interact directly with RIG-I for repression of RLR signaling. Another study has emphasized the indirect role of autophagy in the regulation of RLR signaling. Atg5 deficiency causes more dysfunctional mitochondria and ROS production, which can enhance RLR signaling [164].

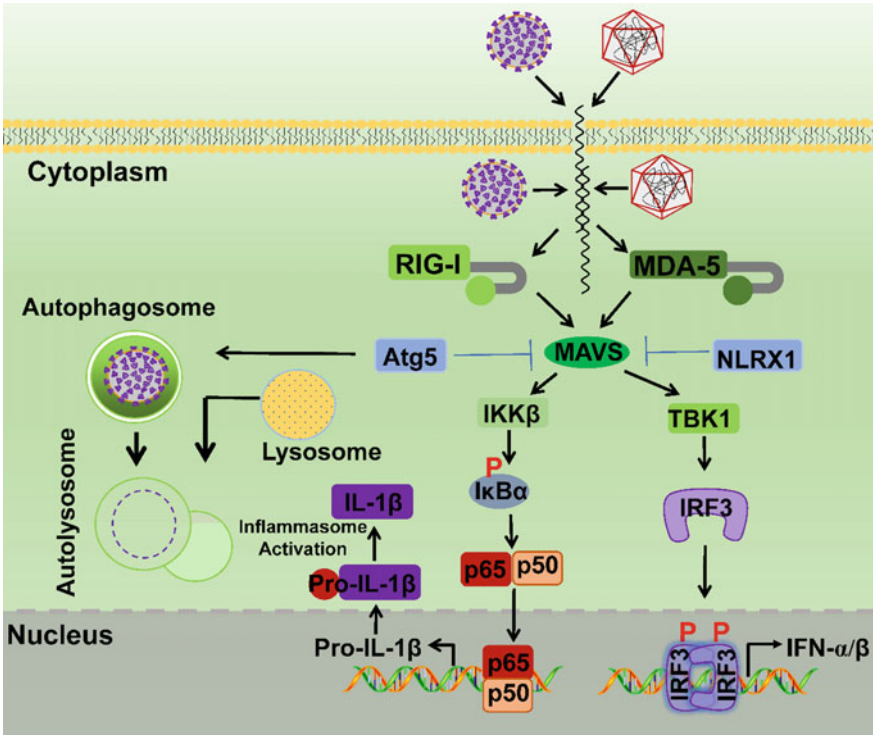


Fig. 6.4 RLRs and autophagy. RIG-1and MDA5 can be activated by bacteria and virus via the adaptor protein MAVS located at mitochondria, leading to the activation of IRF-3 and NF-κB, and promoting pro-IL-1β and IFN expression. Atg5, the autophagy protein, can interact with RIG-I for repression of RLR signaling

6.3 Non-pathogens-Associated PRRs and Autophagy

In addition to PAMPs from pathogens, DAMPs has been identified as ligands of PRRs which induce inflammatory response and autophagy. DAMPs are cell-derived molecules that can initiate non-pathogen-driven immunity such as in response to trauma, ischemia, cancer or other tissue damage. These DAMPs come from various cell components, including cell-derived HMGB1 and S100, heat shock proteins (HSP) from exosomes, hyaluronic acid from the extracellular matrix, in plasma components such as complement, and non-protein ATP, heparin sulfate, RNA and DNA. DAMPs can interact with TLRs and RAGE for activating downstream inflammation through mitogen-activated protein kinases (MAPKs), NF-κB, and PI3K/AKT. Increased serum levels of these DAMPs are associated with inflammatory diseases, including sepsis, arthritis, atherosclerosis, systemic lupus erythematosus, Crohn's disease, and cancer. Several DAMPs such as HMGB1, ATP, S100, and host DNA have been well-characterized [12, 15, 44, 162].

6.3.1 RAGE/HMGB1

RAGE is a multiligand member of the immunoglobulin superfamily. It is first described as a receptor for the products of non-enzymatic glycation and oxidation of proteins or advanced glycation end products (AGE) [67]. However, in addition to AGEs, RAGE can bind with a variety of DAMPs such as HMGB1, S100 [156] as well as in vitro dsDNA and dsRNA [128]. Ligands-RAGE interaction activates MAPK, p38, JNK signaling [89, 186], JAK/STAT pathways, rho and rac GTPases, and p21ras [3, 88, 116].

HMGB1 is chromatin-associated proteins, which is released from the nucleus or the cell in response to various stress, such as bacterial products [66, 177], virus infection [17], inflammatory stimuli [169] or apoptotic cells [139], necrotic cells [148]. Release of HMGB1 is closely associated with autophagy induction through positive feedback regulation. On one hand, HMGB1 localization and release can be regulated by autophagy through ROS [166, 167]. On the other hand, HMGB1 is a direct regulator of Bcl2-Beclin-1 complex for competing Bcl2 or promoting Bcl2 phosphorylation for induction of Beclin-1-dependent autophagy [167]. In colorectal cancer, cytosolic p53 or HMGB1 competes to regulate apoptosis or autophagy [96]. In contrast, in the nucleus, histone deacetylase (HDACs) regulate the nuclear location of HMGB1, which might suggest HMGB1 is linked to HDAC-autophagy pathway [143]. HMGB1 may also regulate mitophagy [168] through HSPB1 as well as mediating PAMP-induced autophagy [10]. These pieces of evidence suggest HMGB1 acts as a universal factor for inducement of autophagy.

6.3.1.1 RAGE/HMGB1 and Autophagy

RAGE/HMGB1 can activate autophagy through increasing Beclin-1-PI3KC3 interaction and decreasing mTOR phosphorylation, which limits apoptosis thus promoting tumor cell survival [73, 74]. Knockdown of RAGE diminishes HMGB1-induced autophagy in cancer cells [166]. In Lung ischemia-reperfusion injury, HMGB1 and HSP60 aggravate lung tissue damage through triggering inflammatory cytokine production and activation of the autophagy flux. However, autophagy inhibition by knockdown of Atg7 or Beclin-1 can markedly reduce the inflammatory cytokine production, which dependents on ubiquitination of TRAF6 [93].

Another mechanism for RAGE/HMGB1-mediated autophagy is through the modulation of mitochondrial activity. Knockdown of RAGE decreases mitochondrial respiratory chain complex I activity and ATP production through IL-6/STAT3 [74]. It is also reported that HSPB1, a cytoskeleton regulator critical for dynamic intracellular trafficking during autophagy and mitophagy, is required for HMGB1-dependent mitochondrial homeostasis [168]. In addition to HMGB1, other ligands, S100 are also reported to induce autophagy [44]. In macrophages, RAGE can enhance phagocytosis-dependent clearance of apoptosis through binding with phosphatidylserine receptor.

In addition to RAGE, other DAMPs receptors are also reported to induce pro-inflammation response but whether the mediated innate immune response depends on autophagy remains unclear. For instance, AIM2-like receptors (ALR) can sense the vaccinia virus and induce the processing of pro-IL-1β into the mature IL-1β form [58].

6.3.2 CD91/Calreticulin

Endoplasmic reticulum (ER) chaperones such as calreticulin and oxidoreductases can be exposed on the plasma membrane in stressed, damaged or dying cells and tumor cells, thus work as one type of DAMP [42, 144]. Calreticulin on plasma membrane promotes an "eat" me signal in tumor cells [119]. In the tumor, chemotherapeutic stimuli, e.g., cisplatin and anthracyclines doxorubicin, idarubicin, and mitoxantrone can trigger calreticulin exposure on plasma membrane [119, 185]. The calreticulin receptor CD91 on DCs and other APCs can recognize calreticulin and induce phagocytotic signal [119]. The Calreticulin-CD91 complex activity could be interfered by CD47 [39, 140]. Injection of Calreticulin coated cancer cells can activate tumor-specific immune response [181]. Interestingly, overexpression of calreticulin is observed in tumor tissue and is associated with the development and progression of pancreatic cancer [151]. In hepatocellular carcinoma, high levels of circulating anti-calreticulin antibodies have been found [132]. In bladder cancer, serum anti-calreticulin autoantibodies can mark cancer progression [111]. These pieces of evidence suggest calreticulin can be an indicator of tumor immunogenicity and may provide avenues of cancer treatment.

In addition to calreticulin, immunoglobulin binding protein (BiP/GRP78), a major ER-lumenal chaperone, can regulate protein folding and ER stress by triggering the unfolded protein response (UPR) to activate the transcription of other ER chaperones and oxidoreductases [40]. Therefore, surface BiP/GRP78 indicates inhibition of tumor cell apoptosis and immunorecognition. High expression of BiP/GRP78 inhibits apoptosis not only through repression of UPR [99] but also through sequestering pro-apoptotic Bcl2 family proteins [196]. In addition, pro-apoptotic Ca^{2+} transfer from ER to mitochondria is also inhibited by BiP/GRP78 [50, 124]. Overexpression of BiP/GRP78 is observed in various cancers and associated with tumor proliferation and invasion as well as therapeutic resistance [86].

6.3.2.1 Cell Surface Calreticulin-Mediated Autophagy

Cell surface calreticulin can induce "eat" me signal for phagocytic uptake and immunogenicity of cells [119]. Several mechanisms have been proposed to explain the escape of calreticulin from ER and exposure on the cell surface. One mechanism suggests that calreticulin needs to bind with phosphatidylserine to expose in a calcium-dependent manner [1, 39, 170]. In cells exposed to anthracycline chemotherapeutics, calreticulin-ERp57 complexes are exposed on cell surface with activation of

pancreatic ER kinase (PERK), leading to the induction of ROS, pro-apoptotic cleavage of caspase-8, activation of pro-apoptotic molecules such as Bcl-2-associated X protein (BAX) and Bcl-2-homologous antagonist/killer (BAK), and ER calcium efflux [126]. In addition, ERp57-independent secretory pathway might contribute to calreticulin cell surface expression in cells through the inactivation of SERCA-2 and disruption of ER calcium homeostasis [41]. Therefore, ER stress can perturb Ca^{2+} homeostasis and glucose transport and may be a key factor for cell surface calreticulin-mediated autophagy [75].

6.3.2.2 ER Chaperones in Cancer Treatment

Cell surface ER chaperones as critical hallmarks of cancer cells which insights the development of new drugs for cancer treatment. Modulation of UPR is a common approach with a diversity of drugs attempting to prevent the pro-survival role of UPR in preclinical studies [54]. These drugs can prevent cancer growth in the myeloma xenograft model [158], especially combined with bortezomib, an inducer of ER stress through blocking proteasome [110]. In addition to UPR, interference of ER Ca^{2+} content by SERCA inhibitor can promote calreticulin escape of ER retention for enhanced tumor immunity, and at the same time promote tumor growth through increasing surface BIP/GRP78 [34, 137, 178]. Mitoxantrone, an anthracycline with promising potential to promote calreticulin plasma membrane exposure, is currently in clinical trials against lymphoma [174]. In contrast, the oncogenic role of BiP/GRP78 makes it an inhibitory target for testing cancer drug. Antibody-based experimental therapies targeting BiP/GRP78 is under development [142, 173]. In addition, inhibitory agents targeting BiP/GRP78 has been developed. One strategy is the application of bacterial toxin subAb to selective destruction of surface BiP/GRP78 [131], which inhibits the cancer xenografts in mice [7]. BiP/GRP78 ATPase inhibitor such as epigallocatechin gallate can also work as tumor repressor [30]. BiP/GRP78-binding peptides also obstruct Xenograft growth of tumors [103].

Autophagy is a basic catabolic process, serving as an internal engine during responses to various cellular stresses. As regards cancer, autophagy may play a tumor-suppressive role by preserving cellular integrity during tumor development and by possible contribution to cell death. However, autophagy may also exert oncogenic effects by promoting tumor cell survival thereby preventing cell death. Autophagy modulation might be promising in anticancer therapies, however, it is a context-dependent matter if inhibition or activation of autophagy leads to tumor cell death.

6.3.3 TLRs/HSPs

HSPs are functional molecular chaperones which facilitate the synthesis and folding of proteins, induce proteasomal degradation, and prevent apoptosis. In addition to maintaining protein homeostasis, HSPs can be released in response to cell stress

and injury for promoting pro-inflammatory cytokine and APC activation. HSPs is upregulated in various tumors and correlated with tumor proliferation, lymph node metastases, and drug resistance to chemotherapies. Conversely, knockdown of HSPs inhibit tumor growth and increase drug response. HSPs are recognized by TLR4, TLR2, CD40, CD91, and CCR5, facilitating intracellular antigen processing and presentation with the exertion of immunoregulatory effects [59].

6.3.3.1 TLRs/HSPs and Autophagy

More importantly, HSPs can cooperate with autophagy to protect protein homeostasis, which can be disrupted by intracellular problems such as translational errors as well as extracellular stressors such as radiation, toxic chemicals, endotoxins, and osmotic pressure through altering the folding capacity [105]. Dysregulation of homeostasis is associated with diseases such as Huntington, amyloidosis, Alzheimer, Parkinson, and cancers [134, 60, 100]. Cells employ several systems to ensure protein homeostasis, including cellular chaperones, the ubiquitin-proteasome system, and autophagy. Interestingly, autophagy is ubiquitous in eukaryotic cells, but the HSP chaperone system is available for both prokaryotes and eukaryotes [49, 81]. Although HSPs and autophagy represent the distinct system of protein homeostasis, the cooperation between HSP and autophagy can be shown in a type of autophagy called chaperone-mediated autophagy [19, 33]. In chaperone-mediated autophagy, HSPA8/HSC70 can recognize cytosolic proteins with signature exposed pentapeptide motif (KFERQ) and target them to undergo unfolding and translocation into lysosomal lumen for degradation [76]. In mouse embryonic fibroblasts, HSP70 is required for panobinostat-induced autophagosomes formation [187]. HSF1 knockdown prevents a chemotherapeutic agent carboplatin-induced autophagy [32]. In addition, rapamycin activated autophagy also accompany with activation of HSF1 and HSP expression in brain and improvement of protein homeostasis [32].

Some recent studies provide evidence that in certain stress conditions, HSP inhibits autophagy, which might reflect the priority of the HSP response over autophagy by certain conditions. For instance, overexpression of the HSP70 protein inhibits starvation- or rapamycin-induced autophagy [35], heat-induced autophagy [61], and pro-apoptotic agent OSU-03012-induced autophagy in colorectal cancer cells [130]. In contrast, inhibition of the HSP70-dependent proteasomal pathway induces autophagy [176]. Mechanism study reveals that downregulation of AKT-TSC-mTOR pathway may be responsible for the repression role of HSPs system in the regulation of autophagy [138].

6.4 Conclusions and Perspectives

In the light of the afore evidence, PRRs are sensors of "danger" signals, while autophagy responds to these signals to maintain homeostasis. Therefore, PRRs and autophagy have close cooperation to enhance mycobacterial clearance as autophagy presents antigens to PRRs and helps to clean the pathogens while PRRs promote autophagy mature. Their roles in innate immunity are quite distinctive and best illustrated by the negative feedback regulation of PRR-mediated inflammation.

Autophagy deficiency such as Atg16L1 deficiency results in enhanced production of pro-inflammatory cytokine secretion [146]. In Crohn's disease, Atg16L1-deficiency is present with endotoxin-induced inflammasome activation suggesting the negative feedback regulation of inflammasomes by autophagy. Increasing studies reveal the mechanism of how autophagy regulates cytokine secretion. A study reveals blocking mitophagy leads to the activation of NLRP3 inflammasome with the accumulation of ROS but the inhibition of mitochondrial activity suppresses inflammasome activation [197]. In another report, depletion of autophagic proteins increases dysfunctionality of mitochondria, cytosolic translocation of mitochondrial DNA (mtDNA) and LPS, thereby activating NLRP3 inflammasome [117]. From these studies, autophagy can regulate inflammasome in a quantitively negative feedback mechanism via the manipulation of mitochondrial integrity.

Another way that autophagy limits the inflammatory response is ubiquitination of inflammasomes. Autophagic adaptor p62 can recognize ubiquitinated inflammasomes into autophagy pathways, which limits IL-1β production [153]. In RIG-I/MDA5-mediated pro-inflammatory response to VSV infection, Atg5 deficiency results in overproduction of type I IFNs following VSV infection, while IFN signaling is suppressed when overexpressing Atg5 [72]. There are different explanations for how Atg5 inhibits RLR signaling. A study revealed that Atg5 can interact directly with RIG-I for repression of RLR signaling. Another study emphasized the indirect role of autophagy in the regulation of RLR signaling. With Atg5 deficiency, more dysfunctional mitochondria and ROS production are generated which may enhance RLR signaling [164]. In addition, autophagy can also repress STING-dependent IFN responses through Atg9a [145]. Also, lack of Atg9a induces overactivation of type I IFN through the promotion of STING and TBK1 assembly. In cGAS-mediated DNA sensing signaling, Rubicon is released to establish negative feedback controlled by autophagy by enhancing the autophagy-mediated degradation of pathogen DNA [90].

Autophagy induced by PRR activation can negatively regulate the PRR signaling through various mechanisms depending on the configuration of PRRs and cell contexts. The activation of the negative feedback regulation of inflammation helps to maintain homeostasis and prevent excessive inflammation. There are also other reports which are inconsistent with the above regulatory relation between PRRs and autophagy. Exploring the distinctive roles of PRRs and autophagy will promote the understanding of microenvironment-dependent regulatory relations between PRRs

and autophagy. Also, investigating interactions between PRRs and autophagy in specific disease microenvironments, e.g., specific cancers will benefit drug development for immunotherapy.

As a key to maintain homeostasis, autophagy can respond to almost all the "danger" signals from PAMPs or DAMPs, with the aid of various types of PRRs. Almost all PRRs can induce autophagy directly for PRRs/PAMPs, but indirectly for PRRs/DAMPs. TLRs, NLRs, RLRs, and cGAS-STING can induce autophagy directly through Beclin 1 or LC3-dependent pathway, while the interactions with RAGE/HMGB1, CD91/Calreticulin, and TLRs/HSPs are achieved by protein, Ca^{2+}, mitochondrial or ROS homeostasis. As autophagy already existed before the emergence of PRRs/PAMPs, it might regulate PRRs/DAMPs through indirect response to "host-danger" signals more efficiently than the direct interaction response to PRRs/PAMPs. However, before the presence of PRRs/PAMPs, autophagy may already attend to host defense against pathogens, which contributes to the formation of the interaction with PRRs/DAMPs. Mitochondria is supposed to derive from autophagy of a *Rickettsia*-like α-*Protobacterium* [84]. In addition, mitochondria are prime autophagic targets and eliminating the damaged or dysfunctional mitochondrial occurs in all cells at all times [172]. This hypothesis also suggests that although autophagy is the oldest and conserved system, it may be easily regulated by various environmental signals probably due to its role in maintaining homeostasis, which is also supported by the increasing number of new types of autophagy identified. However, a more difficult question is how the autophagy coordinate various signals for its action. Further studies should be conducted to investigate these mechanisms, which can ultimately unravel the rebuilding of homeostasis to cure the disease and therapeutic purposes.

Acknowledgements The P.-H. Wang laboratory was supported by Cheeloo Young Scholar Program of Shandong University.

References

1. Afshar N, Black BE, Paschal BM (2005) Retrotranslocation of the chaperone calreticulin from the endoplasmic reticulum lumen to the cytosol. Mol Cell Biol 25:8844–8853
2. Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124:783–801
3. Altman BJ, Jacobs SR, Mason EF, Michalek RD, MacIntyre AN, Coloff JL, Ilkayeva O, Jia W, He Y-W, Rathmell JC (2011) Autophagy is essential to suppress cell stress and to allow BCR-Abl-mediated leukemogenesis. Oncogene 30:1855
4. Andrade WA, Agarwal S, Mo S, Shaffer SA, Dillard JP, Schmidt T, Hornung V, Fitzgerald KA, Kurt-Jones EA, Golenbock DT (2016) Type I interferon induction by Neisseria gonorrhoeae: dual requirement of cyclic GMP-AMP synthase and Toll-like receptor 4. Cell reports 15:2438–2448
5. Andrade WA, Firon A, Schmidt T, Hornung V, Fitzgerald KA, Kurt-Jones EA, Trieu-Cuot P, Golenbock DT, Kaminski P-A (2016) Group B Streptococcus degrades cyclic-di-AMP to modulate STING-dependent type I interferon production. Cell Host Microbe 20:49–59

6. Arumugam TV, Okun E, Tang S-C, Thundyil J, Taylor SM, Woodruff TM (2009) Toll-like receptors in ischemia-reperfusion injury. Shock 32
7. Backer JM, Krivoshein AV, Hamby CV, Pizzonia J, Gilbert KS, Ray YS, Brand H, Paton AW, Paton JC, Backer MV (2009) Chaperone-targeting cytotoxin and endoplasmic reticulum stress-inducing drug synergize to kill cancer cells. Neoplasia 11:1165–1173
8. Beebe SJ (1994) The cAMP-dependent protein kinases and cAMP signal transduction. Semin Cancer Biol 5:285–294
9. Beutler B, Jiang Z, Georgel P, Crozat K, Croker B, Rutschmann S, Du X, Hoebe K (2006) Genetic analysis of host resistance: toll-like receptor signaling and immunity at large. Annu Rev Immunol 24:353–389
10. Bianchi ME (2009) HMGB1 loves company. J Leukoc Biol 86:573–576
11. Birmingham CL, Smith AC, Bakowski MA, Yoshimori T, Brumell JH (2006) Autophagy controls Salmonella infection in response to damage to the Salmonella-containing vacuole. J Biol Chem 281:11374–11383
12. Biswas D, Qureshi OS, Lee W-Y, Croudace JE, Mura M, Lammas DA (2008) ATP-induced autophagy is associated with rapid killing of intracellular mycobacteria within human monocytes/macrophages. BMC Immunol 9:35
13. Boone DL, Turer EE, Lee EG, Ahmad R-C, Wheeler MT, Tsui C, Hurley P, Chien M, Chai S, Hitotsumatsu O (2004) The ubiquitin-modifying enzyme A20 is required for termination of toll-like receptor responses. Nat Immunol 5:1052
14. Boyle KB, Randow F (2013) The role of 'eat-me'signals and autophagy cargo receptors in innate immunity. Curr Opin Microbiol 16:339–348
15. Chaturvedi A, Dorward D, Pierce SK (2008) The B cell receptor governs the subcellular location of toll-like receptor 9 leading to hyperresponses to DNA-containing antigens. Immunity 28:799–809
16. Chen G, Shaw MH, Kim Y-G, Nuñez G (2009) NOD-like receptors: role in innate immunity and inflammatory disease. Ann Rev Pathol Mech Dis 4:365–398
17. Chen L-C, Yeh T-M, Wu H-N, Lin Y-Y, Shyu H-W (2008) Dengue virus infection induces passive release of high mobility group box 1 protein by epithelial cells. J Infect 56:143–150
18. Chen Q, Boire A, Jin X, Valiente M, Er EE, Lopez-Soto A, Jacob LS, Patwa R, Shah H, Xu K (2016) Carcinoma–astrocyte gap junctions promote brain metastasis by cGAMP transfer. Nature 533:493
19. Chiang H-L, Plant CP, Dice JF (1989) A role for a 70-kilodalton heat shock protein in lysosomal degradation of intracellular proteins. Science 246:382–385
20. Choy A, Dancourt J, Mugo B, O'Connor TJ, Isberg RR, Melia TJ, Roy CR (2012) The Legionella effector RavZ inhibits host autophagy through irreversible Atg8 deconjugation. Science 338:1072–1076
21. Chuang S-Y, Yang C-H, Chou C-C, Chiang Y-P, Chuang T-H, Hsu L-C (2013) TLR-induced PAI-2 expression suppresses IL-1β processing via increasing autophagy and NLRP3 degradation. In: Proceedings of the national academy of sciences, 201306556
22. Collins AC, Cai H, Li T, Franco LH, Li X-D, Nair VR, Scharn CR, Stamm CE, Levine B, Chen ZJ (2015) Cyclic GMP-AMP synthase is an innate immune DNA sensor for Mycobacterium tuberculosis. Cell Host Microbe 17:820–828
23. Cooney R, Baker J, Brain O, Danis B, Pichulik T, Allan P, Ferguson DJP, Campbell BJ, Jewell D, Simmons A (2010) NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. Nat Med 16:90
24. Corrales L, Gajewski TF (2016) Endogenous and pharmacologic targeting of the STING pathway in cancer immunotherapy. Cytokine 77:245
25. Corrales L, Glickman LH, McWhirter SM, Kanne DB, Sivick KE, Katibah GE, Woo S-R, Lemmens E, Banda T, Leong JJ (2015) Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. Cell reports 11:1018–1030
26. Cui J, Zhu L, Xia X, Wang HY, Legras X, Hong J, Ji J, Shen P, Zheng S, Chen ZJ (2010) NLRC5 negatively regulates the NF-κB and type I interferon signaling pathways. Cell 141:483–496

27. Demaria O, De Gassart A, Coso S, Gestermann N, Di Domizio J, Flatz L, Gaide O, Michielin O, Hwu P, Petrova TV (2015) STING activation of tumor endothelial cells initiates spontaneous and therapeutic antitumor immunity. Proc Natl Acad Sci 112:15408–15413
28. Deng L, Liang H, Xu M, Yang X, Burnette B, Arina A, Li X-D, Mauceri H, Beckett M, Darga T (2014) STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity 41:843–852
29. Deng Q, Wang Y, Zhang Y, Li M, Li D, Huang X, Wu Y, Pu J, Wu M (2016) Pseudomonas aeruginosa triggers macrophage autophagy to escape intracellular killing by activation of the NLRP3 inflammasome. Infect Immun 84:56–66
30. Deng W-G, Ruan K-H, Du MIN, Saunders MA, Wu KK (2001) Aspirin and salicylate bind to immunoglobulin heavy chain binding protein (BiP) and inhibit its ATPase activity in human fibroblasts. FASEB J 15:2463–2470
31. Deretic V, Saitoh T, Akira S (2013) Autophagy in infection, inflammation and immunity. Nat Rev Immunol 13:722
32. Desai S, Liu Z, Yao J, Patel N, Chen J, Wu Y, Ahn EE-Y, Fodstad O, Tan M (2013) Heat shock factor 1 (HSF1) controls chemoresistance and autophagy through transcriptional regulation of autophagy-related protein 7 (ATG7). J Biol Chem 288:9165–9176
33. Dice JF (1990) Peptide sequences that target cytosolic proteins for lysosomal proteolysis. Trends Biochem Sci 15:305–309
34. Doan NTQ, Paulsen ES, Sehgal P, Møller JV, Nissen P, Denmeade SR, Isaacs JT, Dionne CA, Christensen SB (2015) Targeting thapsigargin towards tumors. Steroids 97:2–7
35. Dokladny K, Zuhl MN, Mandell M, Bhattacharya D, Schneider S, Deretic V, Moseley PL (2013) Regulatory coordination between two major intracellular homeostatic systems: heat shock response and autophagy. J Biol Chem jbc-M113
36. Dong N, Zhu Y, Lu Q, Hu L, Zheng Y, Shao F (2012) Structurally distinct bacterial TBC-like GAPs link Arf GTPase to Rab1 inactivation to counteract host defenses. Cell 150:1029–1041
37. Du Y, Duan T, Feng Y, Liu Q, Lin M, Cui J, Wang RF (2018) LRRC25 inhibits type I IFN signaling by targeting ISG15-associated RIG-I for autophagic degradation. EMBO J 37:351–366
38. Eriksson S, Lucchini S, Thompson A, Rhen M, Hinton JCD (2003) Unravelling the biology of macrophage infection by gene expression profiling of intracellular Salmonella enterica. Mol Microbiol 47:103–118
39. Gardai SJ, McPhillips KA, Frasch SC, Janssen WJ, Starefeldt A, Murphy-Ullrich JE, Bratton DL, Oldenborg P-A, Michalak M, Henson PM (2005) Cell-surface calreticulin initiates clearance of viable or apoptotic cells through trans-activation of LRP on the phagocyte. Cell 123:321–334
40. Gardner BM, Pincus D, Gotthardt K, Gallagher CM, Walter P (2013) Endoplasmic reticulum stress sensing in the unfolded protein response. Cold Spring Harb Perspect Biol a013169
41. Garg AD, Krysko DV, Verfaillie T, Kaczmarek A, Ferreira GB, Marysael T, Rubio N, Firczuk M, Mathieu C, Roebroek AJM (2012) A novel pathway combining calreticulin exposure and ATP secretion in immunogenic cancer cell death. EMBO J 31:1062–1079
42. Garg AD, Martin S, Golab J, Agostinis P (2014) Danger signalling during cancer cell death: origins, plasticity and regulation. Cell Death Differ 21:26
43. Gehrke N, Mertens C, Zillinger T, Wenzel J, Bald T, Zahn S, Tüting T, Hartmann G, Barchet W (2013) Oxidative damage of DNA confers resistance to cytosolic nuclease TREX1 degradation and potentiates STING-dependent immune sensing. Immunity 39:482–495
44. Ghavami S, Eshragi M, Ande SR, Chazin WJ, Klonisch T, Halayko AJ, McNeill KD, Hashemi M, Kerkhoff C, Los M (2010) S100A8/A9 induces autophagy and apoptosis via ROS-mediated cross-talk between mitochondria and lysosomes that involves BNIP3. Cell Res 20:314
45. Gomes MTR, Campos PC, Oliveira FS, Corsetti PP, Bortoluci KR, Cunha LD, Zamboni DS, Oliveira SC (2013) Critical role of ASC inflammasomes and bacterial type IV secretion system in caspase-1 activation and host innate resistance to Brucella abortus infection. J Immunol 1202817

46. Gray EE, Treuting PM, Woodward JJ, Stetson DB (2015) Cutting edge: cGAS is required for lethal autoimmune disease in the Trex1-deficient mouse model of Aicardi–Goutieres syndrome. J Immunol 1500969
47. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V (2004) Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell 119:753–766
48. Hansen K, Prabakaran T, Laustsen A, Jørgensen SE, Rahbæk SH, Jensen SB, Nielsen R, Leber JH, Decker T, Horan KA (2014) Listeria monocytogenes induces IFNβ expression through an IFI16-, cGAS-and STING-dependent pathway. EMBO J 33:1654–1666
49. Hartl FU, Hayer-Hartl M (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. Science 295:1852–1858
50. Hayashi T, Su T-P (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca^{2+} signaling and cell survival. Cell 131:596–610
51. He C, Levine B (2010) The beclin 1 interactome. Curr Opin Cell Biol 22:140–149
52. Henault J, Martinez J, Riggs JM, Tian J, Mehta P, Clarke L, Sasai M, Latz E, Brinkmann MM, Iwasaki A (2012) Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. Immunity 37:986–997
53. Herzner A-M, Hagmann CA, Goldeck M, Wolter S, Kübler K, Wittmann S, Gramberg T, Andreeva L, Hopfner K-P, Mertens C (2015) Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA. Nat Immunol 16:1025
54. Hetz C, Chevet E, Harding HP (2013) Targeting the unfolded protein response in disease. Nat Rev Drug Discovery 12:703
55. Hiscott J, Lacoste J, Lin R (2006) Recruitment of an interferon molecular signaling complex to the mitochondrial membrane: disruption by hepatitis C virus NS3-4A protease. Biochem Pharmacol 72:1477–1484
56. Hitotsumatsu O, Ahmad R-C, Tavares R, Wang M, Philpott D, Turer EE, Lee BL, Shiffin N, Advincula R, Malynn BA (2008) The ubiquitin-editing enzyme A20 restricts nucleotide-binding oligomerization domain containing 2-triggered signals. Immunity 28:381–390
57. Homer CR, Kabi A, Marina-García N, Sreekumar A, Nesvizhskii AI, Nickerson KP, Chinnaiyan AM, Nuñez G, McDonald C (2012) A dual role for receptor-interacting protein kinase 2 (RIP2) kinase activity in nucleotide-binding oligomerization domain 2 (NOD2)-dependent autophagy. J Biol Chem 287:25565–25576
58. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA (2009) AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 458:514
59. Horváth I, Glatz A, Nakamoto H, Mishkind ML, Munnik T, Saidi Y, Goloubinoff P, Harwood JL, Vigh L (2012) Heat shock response in photosynthetic organisms: membrane and lipid connections. Prog Lipid Res 51:208–220
60. Howlett DR, Jennings KH, Lee DC, Clark MSG, Brown F, Wetzel R, Wood SJ, Camilleri P, Roberts GW (1995) Aggregation state and neurotoxic properties of Alzheimer beta-amyloid peptide. Neurodegeneration 4:23–32
61. Hsu S-F, Chao C-M, Huang W-T, Lin M-T, Cheng B-C (2013) Attenuating heat-induced cellular autophagy, apoptosis and damage in H9c2 cardiomyocytes by pre-inducing HSP70 with heat shock preconditioning. Int J Hyperth 29:239–247
62. Huang J, Brumell JH (2014) Bacteria–autophagy interplay: a battle for survival. Nat Rev Microbiol 12:101
63. Huang L, Li L, Lemos H, Chandler PR, Pacholczyk G, Baban B, Barber GN, Hayakawa Y, McGaha TL, Ravishankar B (2013) Cutting edge: DNA sensing via the STING adaptor in myeloid dendritic cells induces potent tolerogenic responses. J Immunol 1301419
64. Huett A, Heath RJ, Begun J, Sassi SO, Baxt LA, Vyas JM, Goldberg MB, Xavier RJ (2012) The LRR and RING domain protein LRSAM1 is an E3 ligase crucial for ubiquitin-dependent autophagy of intracellular Salmonella Typhimurium. Cell Host Microbe 12:778–790
65. Ishikawa H, Barber GN (2008) STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455:674

66. Ivanov S, Dragoi A-M, Wang X, Dallacosta C, Louten J, Musco G, Sitia G, Yap GS, Wan Y, Biron CA (2007) A novel role for HMGB1 in TLR9-mediated inflammatory responses to CpG-DNA. Blood 110:1970–1981
67. Janeway C (1989) Immunogenecity signals 1, 2, 3… and 0. Immunol Today 10:283–286
68. Jiménez-Dalmaroni MJ, Gerswhin ME, Adamopoulos IE (2016) The critical role of toll-like receptors—from microbial recognition to autoimmunity: a comprehensive review. Autoimmun Rev 15:1–8
69. Jin S, Tian S, Luo M, Xie W, Liu T, Duan T, Wu Y, Cui J (2017) Tetherin suppresses Type I interferon signaling by targeting MAVS for NDP52-mediated selective autophagic degradation in human cells. Mol Cell 68(308–322):e304
70. Johansen T, Lamark T (2011) Selective autophagy mediated by autophagic adapter proteins. Autophagy 7:279–296
71. Jounai N, Kobiyama K, Shiina M, Ogata K, Ishii KJ, Takeshita F (2011) NLRP4 negatively regulates autophagic processes through an association with beclin1. J Immunol 1001654
72. Jounai N, Takeshita F, Kobiyama K, Sawano A, Miyawaki A, Xin K-Q, Ishii KJ, Kawai T, Akira S, Suzuki K (2007) The Atg5–Atg12 conjugate associates with innate antiviral immune responses. Proc Natl Acad Sci 104:14050–14055
73. Kang R, Tang D, Livesey KM, Schapiro NE, Lotze MT, Zeh Iii HJ (2011) The Receptor for Advanced Glycation End-products (RAGE) protects pancreatic tumor cells against oxidative injury. Antioxid Redox Signal 15:2175–2184
74. Kang R, Tang D, Schapiro NE, Livesey KM, Farkas A, Loughran P, Bierhaus A, Lotze MT, Zeh HJ (2010) The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. Cell Death Differ 17:666
75. Kania E, Pająk B, Orzechowski A (2015) Calcium homeostasis and ER stress in control of autophagy in cancer cells. BioMed Res Int 2015
76. Kaushik S, Cuervo AM (2012) Chaperone-mediated autophagy: a unique way to enter the lysosome world. Trends Cell Biol 22:407–417
77. Kawagoe T, Takeuchi O, Takabatake Y, Kato H, Isaka Y, Tsujimura T, Akira S (2009) TANK is a negative regulator of toll-like receptor signaling and is critical for the prevention of autoimmune nephritis. Nat Immunol 10:965
78. Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O, Akira S (2005) IPS-1, an adaptor triggering RIG-I-and Mda5-mediated type I interferon induction. Nat Immunol 6:981
79. Kersse K, Bertrand MJM, Lamkanfi M, Vandenabeele P (2011) NOD-like receptors and the innate immune system: coping with danger, damage and death. Cytokine Growth Factor Rev 22:257–276
80. Koonin EV, Aravind L (2000) The NACHT family—a new group of predicted NTPases implicated in apoptosis and MHC transcription activation. Trends Biochem Sci 25:223–224
81. Kroemer G, Mariño G, Levine B (2010) Autophagy and the integrated stress response. Mol Cell 40:280–293
82. Kufer TA, Banks DJ, Philpott DJ (2006) Innate immune sensing of microbes by Nod proteins. Ann NY Acad Sci 1072:19–27
83. Kumar H, Kawai T, Akira S (2011) Pathogen recognition by the innate immune system. Int Rev Immunol 30:16–34
84. Kurland CG, Andersson SGE (2000) Origin and evolution of the mitochondrial proteome. Microbiol Mol Biol Rev 64:786–820
85. Lahaye X, Satoh T, Gentili M, Cerboni S, Conrad C, Hurbain I, El Marjou A, Lacabaratz C, Lelièvre J-D, Manel N (2013) The capsids of HIV-1 and HIV-2 determine immune detection of the viral cDNA by the innate sensor cGAS in dendritic cells. Immunity 39:1132–1142
86. Lee AS (2014) Glucose-regulated proteins in cancer: molecular mechanisms and therapeutic potential. Nat Rev Cancer 14:263
87. Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A (2007) Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science 315:1398–1401

88. Levine B, Mizushima N, Virgin HW (2011) Autophagy in immunity and inflammation. Nature 469:323
89. Liang C, Feng P, Ku B, Dotan I, Canaani D, Oh B-H, Jung JU (2006) Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. Nat Cell Biol 8:688
90. Liang Q, Seo GJ, Choi YJ, Kwak M-J, Ge J, Rodgers MA, Shi M, Leslie BJ, Hopfner K-P, Ha T (2014) Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. Cell Host Microbe 15:228–238
91. Liang XH, Kleeman LK, Jiang HH, Gordon G, Goldman JE, Berry G, Herman B, Levine B (1998) Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. J Virol 72:8586–8596
92. Lio C-WJ, McDonald B, Takahashi M, Dhanwani R, Sharma N, Huang J, Pham E, Benedict CA, Sharma S (2016) cGAS-STING signaling regulates initial innate control of cytomegalovirus infection. J Virol JVI-01040
93. Liu X, Cao H, Li J, Wang B, Zhang P, Zhang XD, Liu Z, Yuan H, Zhan Z (2017) Autophagy induced by DAMPs facilitates the inflammation response in lungs undergoing ischemia-reperfusion injury through promoting TRAF6 ubiquitination. Cell Death Differ 24:683
94. Liu X, Pu Y, Cron K, Deng L, Kline J, Frazier WA, Xu H, Peng H, Fu Y-X, Xu MM (2015) CD47 blockade triggers T cell-mediated destruction of immunogenic tumors. Nat Med 21:1209
95. Liu Y, Jesus AA, Marrero B, Yang D, Ramsey SE, Montealegre Sanchez GA, Tenbrock K, Wittkowski H, Jones OY, Kuehn HS (2014) Activated STING in a vascular and pulmonary syndrome. N Engl J Med 371:507–518
96. Livesey K, Kang R, Vernon P, Buchser W, Loughran P, Watkins SC, Zhang L, Manfredi JJ, Zeh HJ, Li L (2012) p 53/HMGB1 complexes regulate autophagy and apoptosis. Cancer Res, canres-2291
97. Loo Y-M, Fornek J, Crochet N, Bajwa G, Perwitasari O, Martinez-Sobrido L, Akira S, Gill MA, García-Sastre A, Katze MG (2008) Distinct RIG-I and MDA5 signaling by RNA viruses in innate immunity. J Virol 82:335–345
98. Loo Y-M, Gale M Jr (2011) Immune signaling by RIG-I-like receptors. Immunity 34:680–692
99. Luo B, Lee AS (2013) The critical roles of endoplasmic reticulum chaperones and unfolded protein response in tumorigenesis and anticancer therapies. Oncogene 32:805
100. Lynch-Day MA, Mao K, Wang K, Zhao M, Klionsky DJ (2012) The role of autophagy in Parkinson's disease. Cold Spring Harb Perspect Med a009357
101. Ma Z, Jacobs SR, West JA, Stopford C, Zhang Z, Davis Z, Barber GN, Glaunsinger BA, Dittmer DP, Damania B (2015) Modulation of the cGAS-STING DNA sensing pathway by gammaherpesviruses. Proc Natl Acad Sci 112:E4306–E4315
102. Mackenzie KJ, Carroll P, Lettice L, Tarnauskaitė Ž, Reddy K, Dix F, Revuelta A, Abbondati E, Rigby RE, Rabe B (2016) Ribonuclease H2 mutations induce a cGAS/STING-dependent innate immune response. EMBO J 35:831–844
103. Maddalo D, Neeb A, Jehle K, Schmitz K, Muhle-Goll C, Shatkina L, Walther TV, Bruchmann A, Gopal SM, Wenzel W (2012) A peptidic unconjugated GRP78/BiP ligand modulates the unfolded protein response and induces prostate cancer cell death. PLoS One 7:e45690
104. Mahla RS, Reddy CM, Prasad D, Kumar H (2013) Sweeten PAMPs: role of sugar complexed PAMPs in innate immunity and vaccine biology. Front Immunol 4:248
105. Mailhos C, Howard MK, Latchman DS (1993) Heat shock protects neuronal cells from programmed cell death by apoptosis. Neuroscience 55:621–627
106. Man SM, Karki R, Sasai M, Place DE, Kesavardhana S, Temirov J, Frase S, Zhu Q, Malireddi RKS, Kuriakose T (2016) IRGB10 liberates bacterial ligands for sensing by the AIM2 and caspase-11-NLRP3 inflammasomes. Cell 167:382–396
107. Marchiando AM, Ramanan D, Ding Y, Gomez LE, Hubbard-Lucey VM, Maurer K, Wang C, Ziel JW, van Rooijen N, Nuñez G (2013) A deficiency in the autophagy gene Atg16L1 enhances resistance to enteric bacterial infection. Cell Host Microbe 14:216–224
108. Martinon F, Burns K, Tschopp J (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-β. Mol Cell 10:417–426

109. Medzhitov R (2008) Origin and physiological roles of inflammation. Nature 454:428
110. Mimura N, Fulciniti M, Gorgun G, Tai Y-T, Cirstea D, Santo L, Hu Y, Fabre C, Minami J, Ohguchi H (2012) Blockade of XBP1 splicing by inhibition of IRE1α is a promising therapeutic option in multiple myeloma. Blood, blood-2011
111. Minami S, Nagashio R, Ueda J, Matsumoto K, Goshima N, Hattori M, Hachimura K, Iwamura M, Sato Y (2014) Detection of tumor-associated antigens in culture supernatants using autoantibodiesin sera from patients with bladder cancer. Biomed Res 35:25–35
112. Mizushima N, Komatsu M (2011) Autophagy: renovation of cells and tissues. Cell 147:728–741
113. Moayeri M, Sastalla I, Leppla SH (2012) Anthrax and the inflammasome. Microbes Infect 14:392–400
114. Moore CB, Bergstralh DT, Duncan JA, Lei Y, Morrison TE, Zimmermann AG, Accavitti-Loper MA, Madden VJ, Sun L, Ye Z (2008) NLRX1 is a regulator of mitochondrial antiviral immunity. Nature 451:573
115. Motta V, Soares F, Sun T, Philpott DJ (2015) NOD-like receptors: versatile cytosolic sentinels. Physiol Rev 95:149–178
116. Münz C (2009) Enhancing immunity through autophagy. Annu Rev Immunol 27:423–449
117. Nakahira K, Haspel JA, Rathinam VAK, Lee S-J, Dolinay T, Lam HC, Englert JA, Rabinovitch M, Cernadas M, Kim HP (2011) Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome. Nat Immunol 12:222
118. O'Neill LAJ, Bowie AG (2007) The family of five: TIR-domain-containing adaptors in toll-like receptor signalling. Nat Rev Immunol 7:353
119. Obeid M, Tesniere A, Ghiringhelli F, Fimia GM, Apetoh L, Perfettini J-L, Castedo M, Mignot G, Panaretakis T, Casares N (2007) Calreticulin exposure dictates the immunogenicity of cancer cell death. Nat Med 13:54
120. Oberstein A, Jeffrey PD, Shi Y (2007) Crystal structure of the Bcl-XL-Beclin 1 peptide complex Beclin 1 is a novel BH3-only protein. J Biol Chem 282:13123–13132
121. Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C (2005) Escape of intracellular Shigella from autophagy. Science 307:727–731
122. Onoguchi K, Yoneyama M, Fujita T (2011) Retinoic acid-inducible gene-I-like receptors. J Interferon Cytokine Res 31:27–31
123. Onoguchi K, Yoneyama M, Takemura A, Akira S, Taniguchi T, Namiki H, Fujita T (2007) Virus infections activate type I and type III interferon genes through a common mechanism. J Biol Chem 282:7576–7581
124. Ouyang Y-B, Xu L-J, Emery JF, Lee AS, Giffard RG (2011) Overexpressing GRP78 influences $Ca2^{+}$ handling and function of mitochondria in astrocytes after ischemia-like stress. Mitochondrion 11:279–286
125. Paijo J, Döring M, Spanier J, Grabski E, Nooruzzaman M, Schmidt T, Witte G, Messerle M, Hornung V, Kaever V (2016) cGAS senses human cytomegalovirus and induces type I interferon responses in human monocyte-derived cells. PLoS Pathog 12:e1005546
126. Panaretakis T, Kepp O, Brockmeier U, Tesniere A, Bjorklund AC, Chapman DC, Durchschlag M, Joza N, Pierron G, Van Endert P (2009) Mechanisms of pre-apoptotic calreticulin exposure in immunogenic cell death. EMBO J 28:578–590
127. Panne D (2008) The enhanceosome. Curr Opin Struct Biol 18:236–242
128. Park H, Boyington JC (2010) The 1.5 Å crystal structure of human receptor for advanced glycation endproducts (RAGE) ectodomains reveals unique features determining ligand binding. J Biol Chem jbc-M110
129. Park J-H, Kim Y-G, McDonald C, Kanneganti T-D, Hasegawa M, Body-Malapel M, Inohara N, Nunez G (2007) RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. J Immunol 178:2380–2386
130. Park MA, Yacoub A, Rahmani M, Zhang G, Hart L, Hagan MP, Calderwood SK, Sherman MY, Koumenis C, Spiegel S (2008) OSU-03012 stimulates PKR-like endoplasmic reticulum-dependent increases in 70-kDa heat shock protein expression, attenuating its lethal actions in transformed cells. Mol Pharmacol 73:1168–1184

131. Paton AW, Beddoe T, Thorpe CM, Whisstock JC, Wilce MCJ, Rossjohn J, Talbot UM, Paton JC (2006) AB 5 subtilase cytotoxin inactivates the endoplasmic reticulum chaperone BiP. Nature 443:548
132. Pekáriková A, Sánchez D, Palová-Jelínková L, Šimšová M, Beneš Z, Hoffmanová I, Drastich P, Janatková I, Mothes T, Tlaskalová-Hogenová H (2010) Calreticulin is a B cell molecular target in some gastrointestinal malignancies. Clin Exp Immunol 160:215–222
133. Poeck H, Bscheider M, Gross O, Finger K, Roth S, Rebsamen M, Hannesschläger N, Schlee M, Rothenfusser S, Barchet W (2010) Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1β production. Nat Immunol 11:63
134. Polling S, Hill AF, Hatters DM (2012) Polyglutamine aggregation in Huntington and related diseases. In: Tandem repeat polymorphisms. Springer, pp 125–140
135. PrabhuDas MR, Baldwin CL, Bollyky PL, Bowdish DME, Drickamer K, Febbraio M, Herz J, Kobzik L, Krieger M, Loike J (2017) A consensus definitive classification of scavenger receptors and their roles in health and disease. J Immunol 198:3775–3789
136. Py BF, Lipinski MM, Yuan J (2007) Autophagy limits Listeria monocytogenes intracellular growth in the early phase of primary infection. Autophagy 3:117–125
137. Pyrko P, Kardosh A, Liu Y-T, Soriano N, Xiong W, Chow RH, Uddin J, Petasis NA, Mircheff AK, Farley RA (2007) Calcium-activated endoplasmic reticulum stress as a major component of tumor cell death induced by 2, 5-dimethyl-celecoxib, a non-coxib analogue of celecoxib. Mol Cancer Ther 6:1262–1275
138. Qin L, Wang Z, Tao L, Wang Y (2010) ER stress negatively regulates AKT/TSC/mTOR pathway to enhance autophagy. Autophagy 6:239–247
139. Qin S, Wang H, Yuan R, Li H, Ochani M, Ochani K, Rosas-Ballina M, Czura CJ, Huston JM, Miller E (2006) Role of HMGB1 in apoptosis-mediated sepsis lethality. J Exp Med 203:1637–1642
140. Raghavan M, Wijeyesakere SJ, Peters LR, Del Cid N (2013) Calreticulin in the immune system: ins and outs. Trends Immunol 34:13–21
141. Rasmussen SB, Horan KA, Holm CK, Stranks AJ, Mettenleiter TC, Simon AK, Jensen SB, Rixon FJ, He B, Paludan SR (2011) Activation of autophagy by α-herpesviruses in myeloid cells is mediated by cytoplasmic viral DNA through a mechanism dependent on stimulator of IFN genes. J Immunol 1100949
142. Rauschert N, Brändlein S, Holzinger E, Hensel F, Müller-Hermelink H-K, Vollmers HP (2008) A new tumor-specific variant of GRP78 as target for antibody-based therapy. Lab Invest 88:375
143. Robert T, Vanoli F, Chiolo I, Shubassi G, Bernstein KA, Rothstein R, Botrugno OA, Parazzoli D, Oldani A, Minucci S (2011) HDACs link the DNA damage response, processing of double-strand breaks and autophagy. Nature 471:74
144. Rubartelli A, Lotze MT (2007) Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. Trends Immunol 28:429–436
145. Saitoh T, Fujita N, Hayashi T, Takahara K, Satoh T, Lee H, Matsunaga K, Kageyama S, Omori H, Noda T (2009) Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. Proc Natl Acad Sci 106:20842–20846
146. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang B-G, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M (2008) Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production. Nature 456:264
147. Sanjuan MA, Dillon CP, Tait SWG, Moshiach S, Dorsey F, Connell S, Komatsu M, Tanaka K, Cleveland JL, Withoff S (2007) Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature 450:1253
148. Scaffidi P, Misteli T, Bianchi ME (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418:191
149. Schneider M, Zimmermann AG, Roberts RA, Zhang L, Swanson KV, Wen H, Davis BK, Allen IC, Holl EK, Ye Z (2012) The innate immune sensor NLRC3 attenuates toll-like receptor signaling via modification of the signaling adaptor TRAF6 and transcription factor NF-κB. Nat Immunol 13:823
150. Schroder K, Tschopp J (2010) The inflammasomes. Cell 140:821–832

151. Sheng W, Chen C, Dong M, Zhou J, Liu Q, Dong Q, Li F (2014) Overexpression of calreticulin contributes to the development and progression of pancreatic cancer. J Cell Physiol 229:887–897
152. Shi C-S, Kehrl JH (2008) MyD88 and trif target Beclin 1 to trigger autophagy in macrophages. J Biol Chem 283:33175–33182
153. Shi C-S, Shenderov K, Huang N-N, Kabat J, Abu-Asab M, Fitzgerald KA, Sher A, Kehrl JH (2012) Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. Nat Immunol 13:255
154. Shin D-M, Jeon B-Y, Lee H-M, Jin HS, Yuk J-M, Song C-H, Lee S-H, Lee Z-W, Cho S-N, Kim J-M (2010) Mycobacterium tuberculosis Eis regulates autophagy, inflammation, and cell death through redox-dependent signaling. PLoS Pathog 6:e1001230
155. Silveira TN, Zamboni DS (2010) Pore formation triggered by Legionella spp. is an Nlrc4 inflammasome-dependent host cell response that precedes pyroptosis. Infect Immun 78:1403–1413
156. Sparvero LJ, Asafu-Adjei D, Kang R, Tang D, Amin N, Im J, Rutledge R, Lin B, Amoscato AA, Zeh HJ (2009) RAGE (Receptor for Advanced Glycation Endproducts), RAGE ligands, and their role in cancer and inflammation. J Transl Med 7:17
157. Storek KM, Gertsvolf NA, Ohlson MB, Monack DM (2015) cGAS and Ifi204 cooperate to produce type I IFNs in response to Francisella infection. J Immunol 1402764
158. Suh DH, Kim MK, Kim HS, Chung HH, Song YS (2012) Unfolded protein response to autophagy as a promising druggable target for anticancer therapy. Ann NY Acad Sci 1271:20–32
159. Sun L, Wu J, Du F, Chen X, Chen ZJ (2013) Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339:786–791
160. Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. Annu Rev Immunol 21:335–376
161. Takemura N, Kawasaki T, Kunisawa J, Sato S, Lamichhane A, Kobiyama K, Aoshi T, Ito J, Mizuguchi K, Karuppuchamy T (2014) Blockade of TLR3 protects mice from lethal radiation-induced gastrointestinal syndrome. Nat Commun 5:3492
162. Takenouchi T, Nakai M, Iwamaru Y, Sugama S, Tsukimoto M, Fujita M, Wei J, Sekigawa A, Sato M, Kojima S (2009) The activation of P2X7 receptor impairs lysosomal functions and stimulates the release of autophagolysosomes in microglial cells. J Immunol 182:2051–2062
163. Tal MC, Iwasaki A (2009) Autophagy and innate recognition systems. In: Autophagy in infection and immunity. Springer, pp 107–121
164. Tal MC, Sasai M, Lee HK, Yordy B, Shadel GS, Iwasaki A (2009) Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. Proc Natl Acad Sci 106:2770–2775
165. Tanaka Y, Chen ZJ (2012) STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. Sci Signal 5:ra20
166. Tang D, Kang R, Cheh C-W, Livesey KM, Liang X, Schapiro NE, Benschop R, Sparvero LJ, Amoscato AA, Tracey KJ (2010) HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. Oncogene 29:5299
167. Tang D, Kang R, Livesey KM, Cheh C-W, Farkas A, Loughran P, Hoppe G, Bianchi ME, Tracey KJ, Zeh HJ (2010) Endogenous HMGB1 regulates autophagy. J Cell Biol 190:881–892
168. Tang D, Kang R, Livesey KM, Kroemer G, Billiar TR, Van Houten B, Zeh HJ, Lotze MT (2011) High-mobility group box 1 is essential for mitochondrial quality control. Cell Metab 13:701–711
169. Tang D, Shi Y, Kang R, Li T, Xiao W, Wang H, Xiao X (2007) Hydrogen peroxide stimulates macrophages and monocytes to actively release HMGB1. J Leukoc Biol 81:741–747
170. Tarr JM, Young PJ, Morse R, Shaw DJ, Haigh R, Petrov PG, Johnson SJ, Winyard PG, Eggleton P (2010) A mechanism of release of calreticulin from cells during apoptosis. J Mol Biol 401:799–812
171. Travassos LH, Carneiro LAM, Ramjeet M, Hussey S, Kim Y-G, Magalhães JG, Yuan L, Soares F, Chea E, Le Bourhis L (2010) Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat Immunol 11:55

172. Twig G, Elorza A, Molina AJA, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G (2008) Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. EMBO J 27:433–446
173. Uckun FM, Qazi S, Ozer Z, Garner AL, Pitt J, Ma H, Janda KD (2011) Inducing apoptosis in chemotherapy-resistant B-lineage acute lymphoblastic leukaemia cells by targeting HSPA5, a master regulator of the anti-apoptotic unfolded protein response signalling network. Br J Haematol 153:741–752
174. Vacchelli E, Aranda F, Eggermont A, Galon J, Sautès-Fridman C, Cremer I, Zitvogel L, Kroemer G, Galluzzi L (2014) Trial watch: chemotherapy with immunogenic cell death inducers. Oncoimmunology 3:e27878
175. Wang-Sattler R, Yu Z, Herder C, Messias AC, Floegel A, He Y, Heim K, Campillos M, Holzapfel C, Thorand B et al (2012) Novel biomarkers for pre-diabetes identified by metabolomics. Mol Syst Biol 8:615
176. Wang AM, Morishima Y, Clapp KM, Peng H-M, Pratt WB, Gestwicki JE, Osawa Y, Lieberman AP (2010) Inhibition of hsp70 by methylene blue affects signaling protein function and ubiquitination and modulates polyglutamine protein degradation. J Biol Chem 285:15714–15723
177. Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, Frazier A, Yang H, Ivanova S, Borovikova L (1999) HMG-1 as a late mediator of endotoxin lethality in mice. Science 285:248–251
178. Wang J-L, Lin K-L, Chou C-T, Kuo C-C, Cheng J-S, Hsu S-S, Chang H-T, Tsai J-Y, Liao W-C, Lu Y-C (2012) Effect of celecoxib on Ca^{2+} handling and viability in human prostate cancer cells (PC3). Drug Chem Toxicol 35:456–462
179. Watson RO, Manzanillo PS, Cox JS (2012) Extracellular M. tuberculosis DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell 150:803–815
180. Weidberg H, Shvets E, Elazar Z (2011) Biogenesis and cargo selectivity of autophagosomes. Annu Rev Biochem 80:125–156
181. Wu H, Han Y, Qin Y, Cao C, Xia Y, Liu C, Wang Y (2013) Whole-cell vaccine coated with recombinant calreticulin enhances activation of dendritic cells and induces tumour-specific immune responses. Oncol Rep 29:529–534
182. Wu J-J, Li W, Shao Y, Avey D, Fu B, Gillen J, Hand T, Ma S, Liu X, Miley W (2015) Inhibition of cGAS DNA sensing by a herpesvirus virion protein. Cell Host Microbe 18:333–344
183. Xian H, Yang S, Jin S, Zhang Y, Cui J (2019) LRRC59 modulates type I interferon signaling by restraining the SQSTM1/p 62-mediated autophagic degradation of pattern recognition receptor DDX58/RIG-I. Autophagy 1–11
184. Xu YI, Jagannath C, Liu X-D, Sharafkhaneh A, Kolodziejska KE, Eissa NT (2007) Toll-like receptor 4 is a sensor for autophagy associated with innate immunity. Immunity 27:135–144
185. Yamamura Y, Tsuchikawa T, Miyauchi K, Takeuchi S, Wada M, Kuwatani T, Kyogoku N, Kuroda A, Maki T, Shichinohe T (2015) The key role of calreticulin in immunomodulation induced by chemotherapeutic agents. Int J Clin Oncol 20:386–394
186. Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, Bause A, Li Y, Stommel JM, Dell'Antonio G (2011) Pancreatic cancers require autophagy for tumor growth. Genes Dev 25:717–729
187. Yang Y, Fiskus W, Yong B, Atadja P, Takahashi Y, Pandita TK, Wang H-G, Bhalla KN (2013) Acetylated hsp70 and KAP1-mediated Vps34 SUMOylation is required for autophagosome creation in autophagy. In: Proceedings of the national academy of sciences, 201217692
188. Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, Taira K, Foy E, Loo Y-M, Gale M, Akira S (2005) Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J Immunol 175:2851–2858
189. Yoshikawa Y, Ogawa M, Hain T, Yoshida M, Fukumatsu M, Kim M, Mimuro H, Nakagawa I, Yanagawa T, Ishii T (2009) Listeria monocytogenes ActA-mediated escape from autophagic recognition. Nat Cell Biol 11:1233
190. Zeng M, Hu Z, Shi X, Li X, Zhan X, Li X-D, Wang J, Choi JH, Wang K-W, Purrington T (2014) MAVS, cGAS, and endogenous retroviruses in T-independent B cell responses. Science 346:1486–1492

191. Zhan Z, Xie X, Cao H, Zhou X, Zhang XD, Fan H, Liu Z (2014) Autophagy facilitates TLR4- and TLR3-triggered migration and invasion of lung cancer cells through the promotion of TRAF6 ubiquitination. Autophagy 10:257–268
192. Zhang G, Chan B, Samarina N, Abere B, Weidner-Glunde M, Buch A, Pich A, Brinkmann MM, Schulz TF (2016) Cytoplasmic isoforms of Kaposi sarcoma herpesvirus LANA recruit and antagonize the innate immune DNA sensor cGAS. Proc Natl Acad Sci 113:E1034–E1043
193. Zhang X, Wu J, Du F, Xu H, Sun L, Chen Z, Brautigam CA, Zhang X, Chen ZJ (2014a) The cytosolic DNA sensor cGAS forms an oligomeric complex with DNA and undergoes switch-like conformational changes in the activation loop. Cell Rep 6:421–430
194. Zhang Y, Yeruva L, Marinov A, Prantner D, Wyrick PB, Lupashin V, Nagarajan UM (2014b) The DNA sensor, cyclic GMP–AMP synthase, is essential for induction of IFN-β during Chlamydia trachomatis infection. J Immunol 1302718
195. Zhao Y, Yang J, Shi J, Gong Y-N, Lu Q, Xu H, Liu L, Shao F (2011) The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature 477:596
196. Zhou H, Zhang Y, Fu Y, Chan L, Lee AS (2011a) A novel mechanism of anti-apoptotic function of 78 kDa glucose-regulated protein (GRP78), an endocrine resistance factor in breast cancer, through release of B-cell lymphoma 2 (BCL-2) from BCL-2-interacting killer (BIK). J Biol Chem jbc-M110
197. Zhou R, Yazdi AS, Menu P, Tschopp J (2011b) A role for mitochondria in NLRP3 inflammasome activation. Nature 469:221

Chapter 7
Regulation of Inflammasome by Autophagy

Tao Liu

Abstract Inflammasome is a molecular platform that mediates the activation of caspases, maturation of interleukin-1 (IL-1) family members, and leads to inflammatory cell death called pyroptosis. It is vital for innate immune responses, providing protection against infectious agents, sterile environmental insults, and host cell damages. Aberrant activation of inflammasome is closely correlated with numerous hereditary and acquired inflammatory disorders. Therefore, a better understanding of how inflammasome is regulated may provide more promising therapeutics for controlling inflammasome-associated diseases. In recent years, it becomes apparent that autophagy, a cellular machinery essential for the recycling of intracellular components and maintenance of cellular homeostasis, acts as a key player in the activation and regulation of inflammasome, and ameliorates symptoms of inflammasome-related diseases. This review will discuss the recent insights into inflammasome activation and regulation mediated by autophagy.

Keywords Inflammasome · Autophagy · Pyroptosis · Autophagic degradation · Inflammasome-related disorders

7.1 Introduction

In the early 1980s, attention had been paid to interleukin-1 beta (IL-1β), which plays a critical role in host defense and has a determining effect on the inflammatory process. IL-1β is a 17.4-kilodalton hormone derived from a 33-kilodalton inactive precursor cleaved by caspase-1 in various cell types [43]. In 2002, Emad S. Alnemri and his colleagues observed apoptosis-associated speck-like protein (ASC), an activating adaptor, which interacts specifically with pro-caspase-1 via caspase activation and recruitment domain (CARD)–CARD interactions and induces caspase-1 activation for IL-1β processing [60]. At the same year, Jürg Tschopp and colleagues identified the caspase-activating complex and first coined the concept of the inflammasome that

T. Liu (✉)
Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA
e-mail: retao@channing.harvard.edu

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_7

comprises caspase-1, caspase-5, ASC, and Nod-like receptors (NODs) to mediate IL-1β production. Depletion of ASC in differentiated THP-1 cells showed a loss of pro-inflammatory caspase activation and pro-IL-1β processing [39]. Since then, inflammasome has been proved very fruitful which largely expand our knowledge for the molecular basis of innate immune responses and inflammation.

7.2 Mechanisms of Inflammasome Activation

To date, at least 11 receptor proteins have been identified to assemble inflammasome, including the nucleotide-binding oligomerization domain (NOD), leucine-rich repeat (LRR)-containing protein (NLR) family members such as NLRP1, NLRP3, NLRP6, NLRP7, NLRP9b, NLRP12, and NLRC4, as well as the proteins absent in melanoma 2 (AIM2), retinoic acid-inducible gene I (RIG-I), interferon-γ (IFNγ)-inducible protein 16 (IFI16), and pyrin. Besides, noncanonical signaling, which consists of caspase-11 in mice and caspase-4 and/or caspase-5 in human cells, acts as a complement for inflammasome signaling [3, 46]. In respect to activating stimuli, these different receptors trigger a distinct mechanism of inflammasome activation: (1) the maturation of inflammatory cytokines including IL-1β and IL-18 and (2) pyroptosis (Fig. 7.1).

7.2.1 NLRP1 Inflammasome

NLRP1 is the first described receptor for inflammasome activation [39]. It is present in diverse mammalian species including primates, rodents, ungulates, and even marsupials. Within these species, NLRP1 has evolved with extensive diversity [5]. For instance, humans harbor a single NLRP1 gene, whereas mice express three NLRP1 paralogs (*Nlrp1a, b, c*) [41]. For humans, NLRP1 protein contains an amino-terminal pyrin domain (PYD), a nucleotide-binding oligomerization domain (NACHT) domain, a leucine-rich repeat domain (LRR) domain, a function to find domain (FIIND), and a CARD domain on the C-terminus [34]. Among them, FIIND domain undergoes posttranslational auto-cleavage and CARD domain bypasses the requirement for ASC and mediates homotypic interactions with other downstream CARD-containing proteins, leading to activation of the NLRP1 inflammasome. Nevertheless, ASC association in the complex still enhances human NLRP1 inflammasome activity [5, 8]. Compared with human NLRP1, murine Nlrp1 ortholog, especially Nlrp1b, lacks functional PYD domain and is not dispensable to interact with ASC to activate caspase-1. Nlrp1b is activated via proteasome-mediated degradation upon the stimulation of lethal factor (LF) protease secreted by *Bacillus anthracis*. Thus, proteasome inhibitors stabilize cleaved Nlrp1b and prevent NLRP1 inflammasome activation [53]. Dysfunctions of NLRP1 have been associated with increased risk of many diseases. In mice, increased activity of NLRP1 accelerates

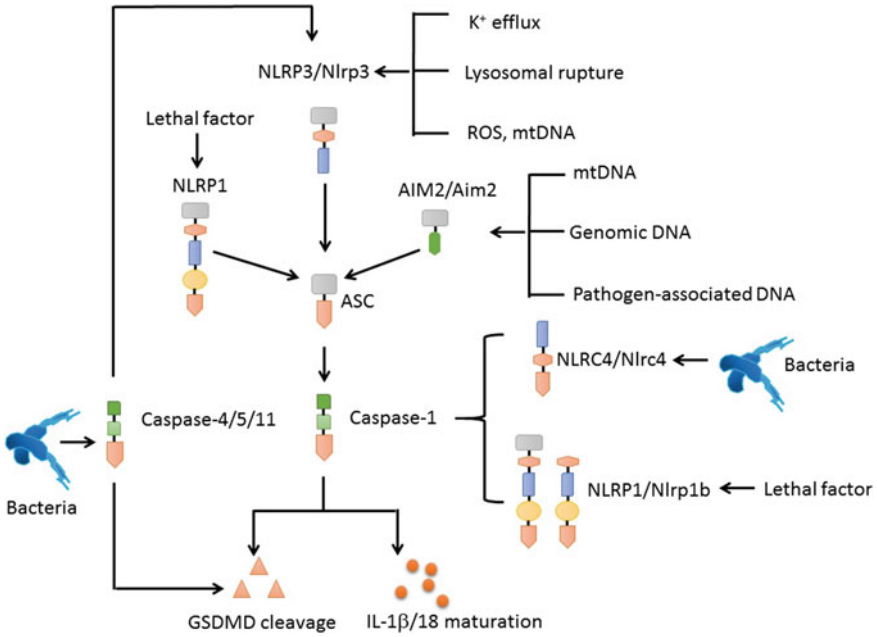


Fig. 7.1 Mechanisms of inflammasome activation. Different inflammasomes respond to various stimulations. K^+ efflux, lysosomal rupture, ROS, and mtDNA are thought to activate NLRP3 inflammasome. The presence of bacterial might be detected by caspase-4/5/11 and NLRC4. AIM2 is activated by various DNA, such as mtDNA, genomic DNA, and pathogen-associated DNA. NLRP1 is activated by the presence of lethal factor. Upon stimulation, NLRP3 (human and murine) and AIM2 (human and murine) interact with ASC to mediate caspase-1 processing, IL-1β/18 maturation, and cleavage of GSDMD. NLRC4 (human and murine) and NLRP1 (murine) bypass ASC and directly interact with caspase-1 for downstream cascade activation. However, for human NLRP1, ASC is not required for inflammasome activation. Nevertheless, ASC association in the complex still enhances human NLRP1 inflammasome activity. Additionally, caspase-4/5/11 work dependent or independent on caspase-1. It directly cleaves GSDMD for pyroptosis and also targets NLRP3 for inflammasome activation

DSS-induced experimental mouse colitis by limiting beneficial, butyrate-producing *Clostridiales* in the gut [68]; In humans, upregulation of NLRP1 inflammasome in primary keratinocytes from patients shows a severe skin inflammation and epidermal hyperplasia [80]. However, for obesity and metabolic syndrome, NLRP1 plays a protective role as deletion of Nlrp1 in mice leads to obesity and metabolic syndrome. In contrast, mice with an activating mutation in Nlrp1 have a higher IL-18 secretion level and are resistant to diet-induced metabolic disorder [42].

7.2.2 NLRP3 Inflammasome

Among the NLR family, pyrin domain-containing 3 (NLRP3) inflammasome is the most extensively characterized inflammasome which consists of a sensor (NLRP3), an adaptor (ASC), and an effector (caspase-1) that promote the maturation of pro-inflammatory cytokines as well as inflammatory cell death called pyroptosis [1]. NLRP3 belongs to NLR family that also contains a PYD, a central NACHT domain, and a carboxy-terminal LRR domain [54]. It can be activated by a broad range of danger signals that derive not only from microorganisms but also from endogenous danger signals and environmental irritants [67]. Upon stimulation, NLRP3 interacts with NEK7 and then NACHT domain binds to ATP to undergo conformational transition and become activated. Next, activated NLRP3 forms oligomerization through interactions between NACHT domains and thereby recruits ASC through homotypic PYD–PYD interactions, triggering nucleates helical ASC filament formation. Assembled ASC filament interacts with caspase-1 through CARD–CARD interactions and mediates caspase-1 auto-cleavage and activation [55, 66]. Furthermore, the recruitment of NLRP3 to dispersed trans-Golgi network (dTGN) via phosphatidylinositol-4-phosphate (PtdIns4P) is also required for ASC polymerization and downstream signaling cascade activation [6]. Nevertheless, mechanisms of NLRP3 inflammasome activation are largely unknown. For instance, what's the role of NEK7 for NLRP3's function? Is it a direct sensor for inflammasome activator?

To activate NLRP3 inflammasome, two steps are required. The first step, also called priming step, nuclear factor-kappa B (NF-κB) signaling activation induces the expression of NLRP3 and pro-IL-1β. The second step, also called activating step. For this step, at least three working models of NLRP3 inflammasome activation are proposed: (1) The phagocytosis of crystals such as MSU, silica, asbestos, amyloid-β, and alum, leading to lysosomal rupture and release of the proteinase cathepsin B, which activates NLRP3 through an unclear mechanism [22]; (2) Loss of mitochondrial integrity leads to NLRP3 inflammasome activation. Mitochondrial damage induces mitochondrial reactive oxygen species (mtROS) release. Then, the increased production of mtROS converts mtDNA to an oxidized form and thereby the cytosolic oxidized mtDNA associates with NLRP3 for its activation [81]; (3) ATP triggers the efflux of K^+, resulting in NLRP3 inflammasome activation. With ATP stimulation, the purinergic receptor P2X7 cooperates with TWIK2, an ATP-responsive K^+ efflux channel protein, triggering NLRP3 inflammasome activation [9, 54]. Nonetheless, whether NLRP3 acts as a direct sensor for cathepsin B and K^+ is unclear. Thus, future studies are required to determine detailed mechanisms of NLRP3 inflammasome activation.

7.2.3 NLRC4 Inflammasome

NLRC4 inflammasome is a critical player of the host to defense against gram-negative bacteria including *Legionella pneumophila*, *Salmonella enterica serovar*, *Typhimurium* (*Salmonella*), and *Shigella flexneri* [15]. With bacteria stimulation, NLR family apoptosis inhibitory proteins (NAIPs) bind to bacterial protein and undergo conformation changes. Then, the ligand-bound NAIPs use its nucleating surface to associate with the inactive NLRC4 and overcome NLRC4's auto-inhibition. The active NLRC4 utilizes the catalytic surface to catalyze more inactive NLRC4, self-propagating its active conformation to form the wheel-like architecture for downstream signaling [25, 78]. Like NLRP1, NLRC4 share a common three-domain structure: an N-terminal CARD, a central nucleotide-binding domain, and a series of C-terminal LRRs. Among them, CARD helps NLRC4 directly to activate caspase-1 without ASC recruitment [11].

7.2.4 AIM2 Inflammasome

Absent in melanoma 2 (AIM2) is a critical sensor for inflammasome activation which mediates the recognition of cytoplasmic and nuclear double-strand DNA including pathogen-associated DNA as well as the aberrant presence of host DNA including genomic and mitochondrial DNA [4, 14, 21, 24]. It is structurally defined with hematopoietic interferon-inducible nuclear antigens with 200-amino acid repeat (HIN200) domain and pyrin domain (PYD) [4]. HIN domain is responsible for DNA recognition and PYD mediates the signaling transduction. In quiescent conditions, HIN domain associates with PYD in an auto-inhibition state. Upon DNA stimulation, AIM2's conformation changes lead to the liberation of its auto-inhibited state. Subsequently, PYD interacts with ASC for pro-caspase-1 processing and inflammatory cytokines production [28].

7.2.5 Noncanonical Inflammasome

Canonical inflammasomes are mediated by sensors (NLRP1, NLRP3, NLRC4, AIM2, and so on), and mediator (caspase-1) with or without the adaptor (ASC) for pro-inflammatory cytokines IL-1β and IL-18 secretion during the innate immune responses. However, facing with infection of bacteria such as *Escherichia coli*, *Citrobacter rodentium*, *Shigella Flexneri*, *Salmonella typhimurium*, *Legionella pneumophila*, and *Burkholderia thailandensis*, caspase-4, 5, and 11 directly bind to bacterial lipopolysaccharide (LPS) and orchestrate both caspase-1-dependent and independent pathway for inflammasome activation. In mice, loss of caspase-11 protects host from bacteria-induced lethal sepsis [30, 58, 62].

Besides, in intestine, NLRP6 and NLRP9b have been reported to interact with ASC and caspase-1 to form inflammasome, regulating colonic microbial ecology and restricting rotavirus infection, respectively [13, 83]. In endothelial cells, interferon gamma-inducible protein 16 (IFI16) interacts with the ASC and pro-caspase-1 to form inflammasome in the nucleus to mediate Kaposi sarcoma-associated herpesvirus (KSHV) genome recognition [31]. In macrophages, NLRP7, NLRP12, and pyrin are identified to assemble inflammasome to specifically mediate bacterial recognition and clearance [32, 70, 75]. Furthermore, RNA helicase RIG-I, a key antiviral sensor for RNA-mediated interferon signaling, engages the CARD9–Bcl-10 module for NF-κB activation and triggers ASC for inflammasome activation in an NLRP3-independent manner [47].

7.3 Autophagy Regulates Inflammasome Activation

Numerous studies have shown that inflammasome activation is critical for homeostasis. The aberrations of inflammasome activation lead to a variety of diseases such as infectious disease, metabolic syndrome, neurodegenerative disease, liver injury, and autoinflammatory disease [63, 69]. Hence, the precise activation of inflammasome is definitely important.

As we described in our previous chapter, autophagy is a ubiquitous cellular mechanism for a host to mediate superfluous or potentially dangerous cytosolic entities degradation. It has been implicated in inflammasome inactivation from the clearance of inflammasome inducers to the sequestration and removal of inflammasome components. Loss of the components of the autophagic machinery, such as Atg16L1, Atg7, and Beclin-1, results in increased production of IL-1β and pyroptosis, consistent with enhanced inflammasome activation and tissue damage [23, 48, 52]. This section will review the involvement of autophagy in inflammasome activation and regulation (Fig. 7.2).

7.3.1 Autophagy Degrades Inflammasome Components for Inhibition

Autophagy has been considered as an unselective nutrient recycling process. It is essential for the degradation of long-lived proteins and organelles [16]. Emerging evidence shows that inflammasome especially NLRP3 inflammasome could be delivered to autophagosomes for degradation. For example, Tim4, a phosphatidylserine (PS) receptor, induces the degradation of NLRP3 inflammasome components through activating AMPKα mediated autophagy, hereby ameliorating the release of IL-1β and IL-18 [37]. TNFAIP3, also named A20, induces autophagy to restrict

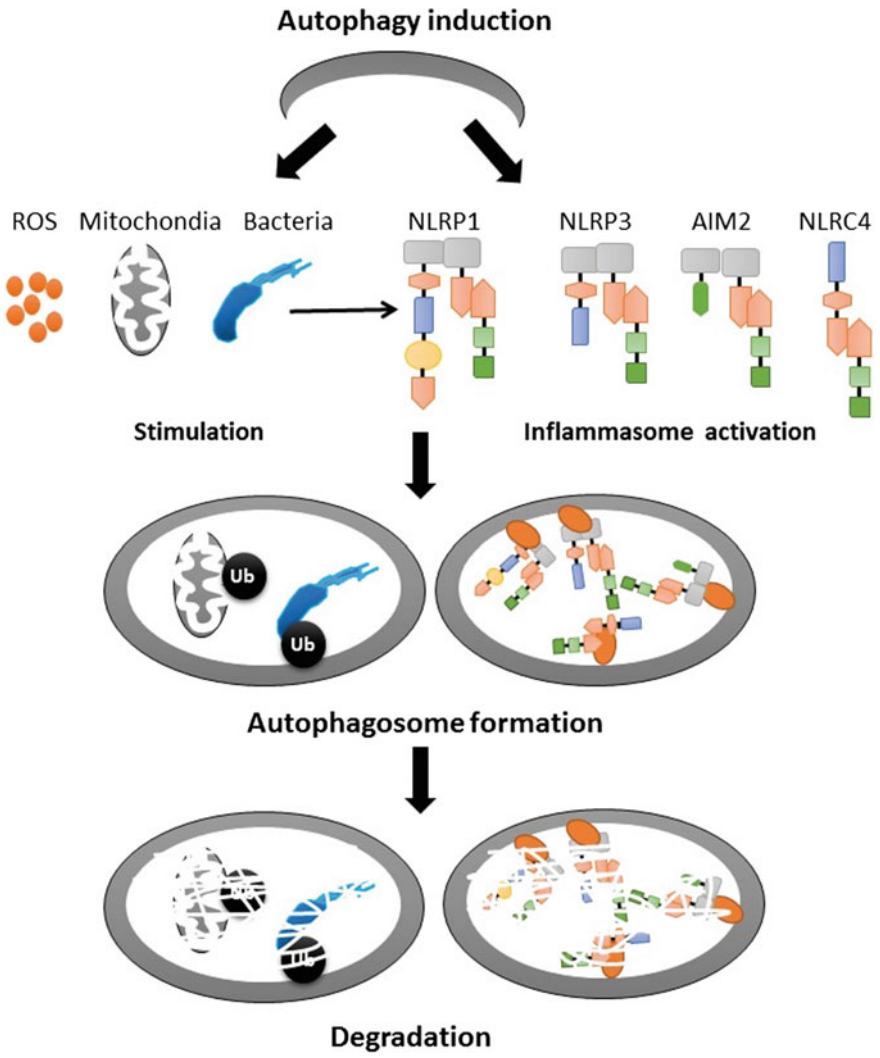


Fig. 7.2 Regulation of inflammasome via autophagy. Autophagy suppresses inflammasome activation by two strategies: (1) Stimuli such as ROS, damaged mitochondria, and bacteria could be delivered to autophagosome for degradation. Ubiquitination of damaged mitochondria and bacteria is required for its recognition (left). (2) The over-activated components of inflammasome could be recognized by selective autophagic receptors and recruited to autophagosome for degradation (right)

NLRP3 inflammasome activation to the early stage of LPS stimulation [77]. Death-associated protein kinase 1 (DAPK1) co-localizes with the Beclin-1 and Atg7 to induce noncanonical fungal autophagy and restrains NLRP3 inflammasome activation in response to IFN-γ [44]. In contrast, Glycogen synthase kinase 3β (GSK-3β) inhibits autophagic activity (ratio of LC3B-II/LC3B-I and p62/SQSTM1) to upregulate NLRP3 inflammasome activation [71]. Furthermore, the autophagic degradation of NLRP3 depends on NLRP3's phosphorylation. Only phosphorylated, but not dephosphorylated NLRP3, is found in autophagosomes. Therefore, protein tyrosine phosphatase, nonreceptor type 22 (PTPN22), an important phosphatase responsible for NLRP3's dephosphorylation, promotes inflammatory response by blocking NLRP3's autophagic degradation. In cells lacking PTPN22, more NLRP3 proteins are present in the autophagosome-enriched fraction upon NLRP3 activation [59]. Besides, Parkinson's disease-associated mitochondrial serine protease HTRA2 restricts the activation of ASC-dependent NLRP3 and AIM2 inflammasomes by upregulating autophagy to prevent prolonged accumulation of the inflammasome adaptor ASC [51].

At present, autophagy is thought to be a highly regulated and specific degradation pathway for removal of aggregate-prone or misfolded proteins. Its selectivity is achieved through autophagy receptors such as p62, NBR1 (next to BRCA1 gene 1 protein), NDP52, optineurin (OPTN), and TAX1BP1/CALCOCO3. They associate with autophagy modifier proteins of the LC3/GABARAP family, delivering cargoes to autophagosomal membrane through LC3-interacting regions (LIRs) [38, 56, 61]. Among those five known autophagy receptors, p62 is the only receptor reported to regulate the stability of inflammasome components. Upon stimulation, inflammasome sensors like AIM2 and NLRP3 induce G protein RalB and autophagosome formation. Then, ASC undergoes ubiquitination and p62 recognizes ubiquitinated ASC, assisting inflammasome delivery to the autophagy pathway for degradation [57]. In autophagy-deficient ATG4B mutant (ATG4B C74A) or ATG5 knockout cells, inflammasome components and p62 accumulate on membrane remnants, leading to exacerbation of cytokine response [12]. As the expression level of p62 can be upregulated upon NF-κB activation in LPS-primed macrophages, NF-κB signaling can promote autophagy and negatively regulate NLRP3-inflammasome activation to orchestrate a self-limiting host response that maintains homeostasis of immunity and favors tissue repair [82]. Although we have known that p62 is critical for various cargoes recognition, the mechanism for specificity and selectivity of p62-mediated autophagic degradation in mammalian cells is unknown. Jun Cui and his colleagues have identified a series of secondary receptors for cargoes recognition in innate immune responses. For example, LRRC25 acts as a secondary receptor to assist RIG-I delivery to autophagosomes for degradation in a p62-dependent manner. Furthermore, LRRC59 specifically interacts with ISG15-associated RIG-I and blocks its association with LRRC25, restraining the p62-mediated autophagic degradation of pattern recognition receptor RIG-I [10, 73]. For inflammasome regulation, they also identified that E3 ligase TRIM11 inhibits AIM2 inflammasome by degrading AIM2 via p62-dependent selective autophagy [36]. Recently, Santosh Chauhan and

his colleagues also have identified a Crohn disease (CD) risk factor, IRGM, directly interacts with NLRP3 and ASC, restraining NLRP3 inflammasome activation by mediating its p62-dependent selective autophagy [40].

7.3.2 *Autophagy Inhibits Inflammasome Activation by Targeting Damaged Mitochondria Clearance*

Mitophagy is an essential quality control process, mediating engulfment and destruction of damaged mitochondria by the cellular autophagy apparatus for autophagic degradation [16]. The phosphatase and tensin homologue (PTEN)-induced putative kinase 1 (PINK1)-parkin pathway is mainly responsible for mitophagy to ensure elimination of defective organelles [45]. Following membrane potential dissipation, PINK1 transports from inner mitochondrial membrane (IMM) to the outer mitochondrial membrane and activates the E3 ligase parkin and poly-ubiquitin substrates via phosphorylation. Then, parkin localizes on mitochondria and cooperates with PINK1 to amplify ubiquitination of mitochondria, mediating ubiquitin-labeled mitochondria into autophagosomes through selective receptors like OPTN, p52, and NDP52 [19, 45]. As discussed in the section of inflammasome activation, mitochondrial dysfunction is the main inducer for inflammasome activation. Thus, mitophagy might be a key player for control of inflammasome activation. Indeed, depletion of PINK1 and parkin upregulates mitochondrial iron accumulation, leading to inflammasome activation [29]. In addition, several regulators of mitophagy are reported to control inflammasome activation such as ESN2 (sestrin 2), a stress-inducible protein which induces mitophagy in macrophages by facilitating aggregation of p62 and its binding to lysine 63 (Lys63)-linked ubiquitin chains on the mitochondrial surface, suppresses prolonged NLRP3 inflammasome activation [33]. In addition, FUN14 domain-containing 1 (FUNDC1), a well-characterized mitophagy receptor, suppresses inflammasome activation by promoting dysfunctional mitochondria clearance in response to hypoxia and mitochondrial stresses. What's more, Src kinase is able to phosphorylate FUNDC1 to prevent its binding with light chain 3 (LC3) and subsequent mitophagy, thus upregulating inflammasome activation [35].

7.3.3 *Autophagy Manipulates ROS Clearance to Regulate Inflammasome Activation*

As we know, mitochondrial dysfunction also induces ROS production for inflammasome activation. Thus, the clearance of ROS could largely affect inflammasome activation. For instance, Fanconi anemia complementation group C (FANCC) protein is reported to interact with parkin, for mtROS clearance and inflammasome inhibition [64]. General controlled nonrepressed (GCN2), a modulator for cellular metabolism

in response to amino acid starvation, suppresses inflammasome activation by targeting ROS for autophagic degradation. Knockout of GCN2 in $CD11c^+$APCs or intestinal epithelial cells results in enhanced intestinal inflammation. Consistently, the blockade of ROS and IL-1β results in reduced inflammation in $GCN2^{-/-}$ mice [50]. Furthermore, the potential therapeutic intervention has been implicated against inflammatory diseases by targeting GCN2. Small molecule halofuginone (HF) results in significant inhibition of production of IL-1β dependent on general control of GCN2 [2]. In addition, Atp6v0d2, xanthine oxidoreductase, and prohibitin 2 are also reported to regulate inflammasome activation by manipulating autophagy [26, 72, 74].

Other than damaged mitochondria and ROS, autophagy is also functional for the clearance of other activators to regulate inflammasome activation. For example, mitochondrial DNA binds specifically to NLRC4 which contributes to *Pseudomonas aeruginosa* activation of the inflammasome and is downregulated by autophagy [27]. Additionally, phagocytosis of extracellular β-amyloid fibrils by microglia acting through its receptor OPTN decreases the secretion of IL-1β, thereby affecting neuronal survival [7].

7.4 The Pharmacological Regulation of Inflammasome by Autophagy

Upon activation, inflammasome produces highly pro-inflammatory cytokines and accelerates various inflammatory diseases. Fortunately, our host system develops a great tool of autophagy to control the over-activation of inflammasome. Notably, at present, several small molecules have been implicated to manipulate autophagy to inhibit inflammasome activation. For instance, kaempferol (KA) promotes autophagy in microglia, leading to reduced NLRP3 protein expression, which inhibits LPS- and SNCA-induced neurodegeneration in mice [20]. Andrographolide attenuates chronic unpredictable mild stress (CUMS)-induced depressive-like behavior in mice by the upregulating autophagy [18]. Metformin treatment improves NLRP3 inflammasome-induced diabetic cardiomyopathy via AMPK/mTOR-dependent effects [76]. Similarly, α-Mangostin, astragaloside IV, kynurenic acid, purple sweet potato color, and melatonin ameliorate NLRP3 inflammasome-induced sepsis, liver and kidney injury, colitis, endothelial senescence, and atherosclerosis by targeting autophagy, respectively [17, 38, 49, 65, 79].

7.5 Conclusion

In response to distinct activators, various receptors cooperate with or without adaptor ASC to mediate inflammatory caspases processing and produce inflammatory cytokines, which in turn induces macrophages activation and neutrophils recruitment for stimulation clearance. However, over-activation of inflammasome leads to severe inflammatory diseases. Thus, inflammasome activity should be stringently controlled. Autophagy is a fundamental and conserved degradation system in events of nutrient starvation or metabolic stress. It regulates inflammasome activation by manipulating multiple precise mechanisms, targeting inflammasome components, damaged mitochondrial, ROS, and diverse stimulations for clearance. Previously, autophagic degradation is thought to be nonspecific. However, recent studies have offered a new notion that autophagy is selective. Moreover, the selectivity of autophagic degradation is achieved through cargo receptors and secondary cargo receptors. Autophagy-deficient cells show robust inflammasome activation in immune or nonimmune cells, leading to development of inflammatory diseases. Therefore, deployment of small molecules to target autophagy for inflammasome inhibition is a potential approach to control inflammation and inflammation-related diseases. Although it is just a beginning for clinic trial, it would be a promising therapeutic strategy for inflammatory diseases which are associated with aberrant inflammasome activation in future.

References

1. Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J (2004) NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. Immunity 20:319–325
2. Battu S, Afroz S, Giddaluru J, Naz S, Huang W, Khumukcham SS, Khan RA, Bhat SY, Qureshi IA, Manavathi B et al (2018) Amino acid starvation sensing dampens IL-1beta production by activating riboclustering and autophagy. PLoS Biol 16:e2005317
3. Broz P, Dixit VM (2016) Inflammasomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol 16:407–420
4. Burckstummer T, Baumann C, Bluml S, Dixit E, Durnberger G, Jahn H, Planyavsky M, Bilban M, Colinge J, Bennett KL, Superti-Furga G (2009) An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. Nat Immunol 10:266–272
5. Chavarria-Smith J, Vance RE (2015) The NLRP1 inflammasomes. Immunol Rev 265:22–34
6. Chen J, Chen ZJ (2018) PtdIns4P on dispersed trans-Golgi network mediates NLRP3 inflammasome activation. Nature 564:71–76
7. Cho MH, Cho K, Kang HJ, Jeon EY, Kim HS, Kwon HJ, Kim HM, Kim DH, Yoon SY (2014) Autophagy in microglia degrades extracellular beta-amyloid fibrils and regulates the NLRP3 inflammasome. Autophagy 10:1761–1775
8. D'Osualdo A, Weichenberger CX, Wagner RN, Godzik A, Wooley J, Reed JC (2011) CARD8 and NLRP1 undergo autoproteolytic processing through a ZU5-like domain. PLoS One 6:e27396
9. Di A, Xiong S, Ye Z, Malireddi RKS, Kometani S, Zhong M, Mittal M, Hong Z, Kanneganti TD, Rehman J, Malik AB (2018) The TWIK2 potassium efflux channel in macrophages mediates NLRP3 inflammasome-induced inflammation. Immunity 49(56–65):e54

10. Du Y, Duan T, Feng Y, Liu Q, Lin M, Cui J, Wang RF (2018) LRRC25 inhibits type I IFN signaling by targeting ISG15-associated RIG-I for autophagic degradation. EMBO J 37:351–366
11. Duncan JA, Canna SW (2018) The NLRC4 inflammasome. Immunol Rev 281:115–123
12. Dupont N, Lacas-Gervais S, Bertout J, Paz I, Freche B, Van Nhieu GT, van der Goot FG, Sansonetti PJ, Lafont F (2009) Shigella phagocytic vacuolar membrane remnants participate in the cellular response to pathogen invasion and are regulated by autophagy. Cell Host Microbe 6:137–149
13. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, Flavell RA (2011) NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell 145:745–757
14. Fernandes-Alnemri T, Yu JW, Datta P, Wu J, Alnemri ES (2009) AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature 458:509–513
15. Franchi L, Nunez G (2012) Immunology. Orchestrating inflammasomes. Science 337:1299–1300
16. Gatica D, Lahiri V, Klionsky DJ (2018) Cargo recognition and degradation by selective autophagy. Nat Cell Biol 20:233–242
17. Ge Y, Xu X, Liang Q, Xu Y, Huang M (2019) Alpha-Mangostin suppresses NLRP3 inflammasome activation via promoting autophagy in LPS-stimulated murine macrophages and protects against CLP-induced sepsis in mice. Inflamm Res: Off J Eur Histamine Res Soc … [et al.] 68:471–479
18. Geng J, Liu J, Yuan X, Liu W, Guo W (2019) Andrographolide triggers autophagy-mediated inflammation inhibition and attenuates chronic unpredictable mild stress (CUMS)-induced depressive-like behavior in mice. Toxicol Appl Pharmacol 379:114688
19. Georgakopoulos ND, Wells G, Campanella M (2017) The pharmacological regulation of cellular mitophagy. Nat Chem Biol 13:136–146
20. Han X, Sun S, Sun Y, Song Q, Zhu J, Song N, Chen M, Sun T, Xia M, Ding J et al (2019) Small molecule-driven NLRP3 inflammation inhibition via interplay between ubiquitination and autophagy: implications for Parkinson disease. Autophagy 1–22
21. Hornung V, Ablasser A, Charrel-Dennis M, Bauernfeind F, Horvath G, Caffrey DR, Latz E, Fitzgerald KA (2009) AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 458:514–518
22. Hornung V, Bauernfeind F, Halle A, Samstad EO, Kono H, Rock KL, Fitzgerald KA, Latz E (2008) Silica crystals and aluminum salts activate the NALP3 inflammasome through phagosomal destabilization. Nat Immunol 9:847–856
23. Houtman J, Freitag K, Gimber N, Schmoranzer J, Heppner FL, Jendrach M (2019) Beclin1-driven autophagy modulates the inflammatory response of microglia via NLRP3. EMBO J 38
24. Hu B, Jin C, Li HB, Tong J, Ouyang X, Cetinbas NM, Zhu S, Strowig T, Lam FC, Zhao C et al (2016) The DNA-sensing AIM2 inflammasome controls radiation-induced cell death and tissue injury. Science 354:765–768
25. Hu Z, Zhou Q, Zhang C, Fan S, Cheng W, Zhao Y, Shao F, Wang HW, Sui SF, Chai J (2015) Structural and biochemical basis for induced self-propagation of NLRC4. Science 350:399–404
26. Ives A, Nomura J, Martinon F, Roger T, LeRoy D, Miner JN, Simon G, Busso N, So A (2015) Xanthine oxidoreductase regulates macrophage IL1beta secretion upon NLRP3 inflammasome activation. Nat Commun 6:6555
27. Jabir MS, Hopkins L, Ritchie ND, Ullah I, Bayes HK, Li D, Tourlomousis P, Lupton A, Puleston D, Simon AK et al (2015) Mitochondrial damage contributes to Pseudomonas aeruginosa activation of the inflammasome and is downregulated by autophagy. Autophagy 11:166–182
28. Jin T, Perry A, Jiang J, Smith P, Curry JA, Unterholzner L, Jiang Z, Horvath G, Rathinam VA, Johnstone RW et al (2012) Structures of the HIN domain: DNA complexes reveal ligand binding and activation mechanisms of the AIM2 inflammasome and IFI16 receptor. Immunity 36:561–571
29. Kang R, Xie Y, Zeh HJ, Klionsky DJ, Tang D (2019) Mitochondrial quality control mediated by PINK1 and PRKN: links to iron metabolism and tumor immunity. Autophagy 15:172–173

30. Kayagaki N, Warming S, Lamkanfi M, Vande Walle L, Louie S, Dong J, Newton K, Qu Y, Liu J, Heldens S et al (2011) Non-canonical inflammasome activation targets caspase-11. Nature 479:117–121
31. Kerur N, Veettil MV, Sharma-Walia N, Bottero V, Sadagopan S, Otageri P, Chandran B (2011) IFI16 acts as a nuclear pathogen sensor to induce the inflammasome in response to Kaposi Sarcoma-associated herpesvirus infection. Cell Host Microbe 9:363–375
32. Khare S, Dorfleutner A, Bryan NB, Yun C, Radian AD, de Almeida L, Rojanasakul Y, Stehlik C (2012) An NLRP7-containing inflammasome mediates recognition of microbial lipopeptides in human macrophages. Immunity 36:464–476
33. Kim MJ, Bae SH, Ryu JC, Kwon Y, Oh JH, Kwon J, Moon JS, Kim K, Miyawaki A, Lee MG et al (2016) SESN2/sestrin2 suppresses sepsis by inducing mitophagy and inhibiting NLRP3 activation in macrophages. Autophagy 12:1272–1291
34. Lacey CA, Miao EA (2019) NLRP1—One NLR to guard them all. EMBO J 38:e102494
35. Li W, Li Y, Siraj S, Jin H, Fan Y, Yang X, Huang X, Wang X, Wang J, Liu L et al (2019) FUN14 domain-containing 1-mediated mitophagy suppresses hepatocarcinogenesis by inhibition of inflammasome activation in mice. Hepatology 69:604–621
36. Liu T, Tang Q, Liu K, Xie W, Liu X, Wang H, Wang RF, Cui J (2016) TRIM11 suppresses AIM2 inflammasome by degrading AIM2 via p62-dependent selective autophagy. Cell Rep 16:1988–2002
37. Liu W, Bai F, Wang H, Liang Y, Du X, Liu C, Cai D, Peng J, Zhong G, Liang X et al (2019) Tim-4 Inhibits NLRP3 inflammasome via the LKB1/AMPKalpha pathway in macrophages. J Immunol 203:990–1000
38. Ma S, Chen J, Feng J, Zhang R, Fan M, Han D, Li X, Li C, Ren J, Wang Y, Cao F (2018) Melatonin ameliorates the progression of atherosclerosis via mitophagy activation and NLRP3 inflammasome inhibition. Oxidative Med Celllo Longev 2018:9286458
39. Martinon F, Burns K, Tschopp J (2002) The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-beta. Mol Cell 10:417–426
40. Mehto S, Chauhan S, Jena KK, Chauhan NR, Nath P, Sahu R, Dhar K, Das SK, Chauhan S (2019) IRGM restrains NLRP3 inflammasome activation by mediating its SQSTM1/p62-dependent selective autophagy. Autophagy 15:1645–1647
41. Mitchell PS, Sandstrom A, Vance RE (2019) The NLRP1 inflammasome: new mechanistic insights and unresolved mysteries. Curr Opin Immunol 60:37–45
42. Murphy AJ, Kraakman MJ, Kammoun HL, Dragoljevic D, Lee MK, Lawlor KE, Wentworth JM, Vasanthakumar A, Gerlic M, Whitehead LW et al (2016) IL-18 production from the NLRP1 inflammasome prevents obesity and metabolic syndrome. Cell Metab 23:155–164
43. O'Neill LA (2008) The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev 226:10–18
44. Oikonomou V, Moretti S, Renga G, Galosi C, Borghi M, Pariano M, Puccetti M, Palmerini CA, Amico L, Carotti A et al (2016) Noncanonical fungal autophagy inhibits inflammation in response to IFN-gamma via DAPK1. Cell Host Microbe 20:744–757
45. Palikaras K, Lionaki E, Tavernarakis N (2018) Mechanisms of mitophagy in cellular homeostasis, physiology and pathology. Nat Cell Biol 20:1013–1022
46. Place DE, Kanneganti TD (2018) Recent advances in inflammasome biology. Curr Opin Immunol 50:32–38
47. Poeck H, Bscheider M, Gross O, Finger K, Roth S, Rebsamen M, Hannesschlager N, Schlee M, Rothenfusser S, Barchet W et al (2010) Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production. Nat Immunol 11:63–69
48. Pu Q, Gan C, Li R, Li Y, Tan S, Li X, Wei Y, Lan L, Deng X, Liang H et al (2017) Atg7 deficiency intensifies inflammasome activation and pyroptosis in pseudomonas sepsis. J Immunol 198:3205–3213
49. Qu X, Gao H, Tao L, Zhang Y, Zhai J, Sun J, Song Y, Zhang S (2019) Astragaloside IV protects against cisplatin-induced liver and kidney injury via autophagy-mediated inhibition of NLRP3 in rats. J Toxicol Sci 44:167–175

50. Ravindran R, Loebbermann J, Nakaya HI, Khan N, Ma H, Gama L, Machiah DK, Lawson B, Hakimpour P, Wang YC et al (2016) The amino acid sensor GCN2 controls gut inflammation by inhibiting inflammasome activation. Nature 531:523–527
51. Rodrigue-Gervais IG, Doiron K, Champagne C, Mayes L, Leiva-Torres GA, Vanie P Jr, Douglas T, Vidal SM, Alnemri ES, Saleh M (2018) The mitochondrial protease HtrA2 restricts the NLRP3 and AIM2 inflammasomes. Sci Rep 8:8446
52. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M et al (2008) Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1beta production. Nature 456:264–268
53. Sandstrom A, Mitchell PS, Goers L, Mu EW, Lesser CF, Vance RE (2019) Functional degradation: a mechanism of NLRP1 inflammasome activation by diverse pathogen enzymes. Science 364
54. Schroder K, Zhou R, Tschopp J (2010) The NLRP3 inflammasome: a sensor for metabolic danger? Science 327:296–300
55. Sharif H, Wang L, Wang WL, Magupalli VG, Andreeva L, Qiao Q, Hauenstein AV, Wu Z, Nunez G, Mao Y, Wu H (2019) Structural mechanism for NEK7-licensed activation of NLRP3 inflammasome. Nature 570:338–343
56. Sharma V, Verma S, Seranova E, Sarkar S, Kumar D (2018) Selective autophagy and xenophagy in infection and disease. Front Cell Dev Biol 6:147
57. Shi CS, Shenderov K, Huang NN, Kabat J, Abu-Asab M, Fitzgerald KA, Sher A, Kehrl JH (2012) Activation of autophagy by inflammatory signals limits IL-1beta production by targeting ubiquitinated inflammasomes for destruction. Nat Immunol 13:255–263
58. Shi J, Zhao Y, Wang Y, Gao W, Ding J, Li P, Hu L, Shao F (2014) Inflammatory caspases are innate immune receptors for intracellular LPS. Nature 514:187–192
59. Spalinger MR, Lang S, Gottier C, Dai X, Rawlings DJ, Chan AC, Rogler G, Scharl M (2017) PTPN22 regulates NLRP3-mediated IL1B secretion in an autophagy-dependent manner. Autophagy 13:1590–1601
60. Srinivasula SM, Poyet JL, Razmara M, Datta P, Zhang Z, Alnemri ES (2002) The PYRIN-CARD protein ASC is an activating adaptor for caspase-1. J Biol Chem 277:21119–21122
61. Stolz A, Ernst A, Dikic I (2014) Cargo recognition and trafficking in selective autophagy. Nat Cell Biol 16:495–501
62. Stowe I, Lee B, Kayagaki N (2015) Caspase-11: arming the guards against bacterial infection. Immunol Rev 265:75–84
63. Strowig T, Henao-Mejia J, Elinav E, Flavell R (2012) Inflammasomes in health and disease. Nature 481:278–286
64. Sumpter R Jr, Sirasanagandla S, Fernandez AF, Wei Y, Dong X, Franco L, Zou Z, Marchal C, Lee MY, Clapp DW et al (2016) Fanconi anemia proteins function in mitophagy and immunity. Cell 165:867–881
65. Sun C, Diao Q, Lu J, Zhang Z, Wu D, Wang X, Xie J, Zheng G, Shan Q, Fan S et al (2019) Purple sweet potato color attenuated NLRP3 inflammasome by inducing autophagy to delay endothelial senescence. J Cell Physiol 234:5926–5939
66. Swanson KV, Deng M, Ting JP (2019) The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol 19:477–489
67. Tschopp J, Schroder K (2010) NLRP3 inflammasome activation: the convergence of multiple signalling pathways on ROS production? Nature reviews. Immunology 10:210–215
68. Tye H, Yu CH, Simms LA, de Zoete MR, Kim ML, Zakrzewski M, Penington JS, Harapas CR, Souza-Fonseca-Guimaraes F, Wockner LF et al (2018) NLRP1 restricts butyrate producing commensals to exacerbate inflammatory bowel disease. Nat Commun 9:3728
69. Van Opdenbosch N, Lamkanfi M (2019) Caspases in cell death, inflammation, and disease. Immunity 50:1352–1364
70. Vladimer GI, Weng D, Paquette SW, Vanaja SK, Rathinam VA, Aune MH, Conlon JE, Burbage JJ, Proulx MK, Liu Q et al (2012) The NLRP12 inflammasome recognizes Yersinia pestis. Immunity 37:96–107

71. Wang Y, Meng C, Zhang J, Wu J, Zhao J (2019) Inhibition of GSK-3beta alleviates cerebral ischemia/reperfusion injury in rats by suppressing NLRP3 inflammasome activation through autophagy. Int Immunopharmacol 68:234–241
72. Xia Y, Liu N, Xie X, Bi G, Ba H, Li L, Zhang J, Deng X, Yao Y, Tang Z et al (2019) The macrophage-specific V-ATPase subunit ATP6V0D2 restricts inflammasome activation and bacterial infection by facilitating autophagosome-lysosome fusion. Autophagy 15:960–975
73. Xian H, Yang S, Jin S, Zhang Y, Cui J (2019) LRRC59 modulates type I interferon signaling by restraining the SQSTM1/p62-mediated autophagic degradation of pattern recognition receptor DDX58/RIG-I. Autophagy 1–11
74. Xu Y, Wang J, Xu W, Ding F, Ding W (2019) Prohibitin 2-mediated mitophagy attenuates renal tubular epithelial cells injury by regulating mitochondrial dysfunction and NLRP3 inflammasome activation. Am J Physiol Renal Physiol 316:F396–F407
75. Xu H, Yang J, Gao W, Li L, Li P, Zhang L, Gong YN, Peng X, Xi JJ, Chen S et al (2014) Innate immune sensing of bacterial modifications of Rho GTPases by the Pyrin inflammasome. Nature 513:237–241
76. Yang F, Qin Y, Wang Y, Meng S, Xian H, Che H, Lv J, Li Y, Yu Y, Bai Y, Wang L (2019) Metformin inhibits the NLRP3 inflammasome via AMPK/mTOR-dependent effects in diabetic cardiomyopathy. Int J Biol Sci 15:1010–1019
77. Zhai Y, Lin P, Feng Z, Lu H, Han Q, Chen J, Zhang Y, He Q, Nan G, Luo X et al (2018) TNFAIP3-DEPTOR complex regulates inflammasome secretion through autophagy in ankylosing spondylitis monocytes. Autophagy 14:1629–1643
78. Zhang L, Chen S, Ruan J, Wu J, Tong AB, Yin Q, Li Y, David L, Lu A, Wang WL et al (2015) Cryo-EM structure of the activated NAIP2-NLRC4 inflammasome reveals nucleated polymerization. Science 350:404–409
79. Zheng X, Hu M, Zang X, Fan Q, Liu Y, Che Y, Guan X, Hou Y, Wang G, Hao H (2019) Kynurenic acid/GPR35 axis restricts NLRP3 inflammasome activation and exacerbates colitis in mice with social stress. Brain Behav Immun 79:244–255
80. Zhong FL, Mamai O, Sborgi L, Boussofara L, Hopkins R, Robinson K, Szeverenyi I, Takeichi T, Balaji R, Lau A et al (2016) Germline NLRP1 mutations cause skin inflammatory and cancer susceptibility syndromes via inflammasome activation. Cell 167(187–202):e117
81. Zhong Z, Liang S, Sanchez-Lopez E, He F, Shalapour S, Lin XJ, Wong J, Ding S, Seki E, Schnabl B et al (2018) New mitochondrial DNA synthesis enables NLRP3 inflammasome activation. Nature 560:198–203
82. Zhong Z, Umemura A, Sanchez-Lopez E, Liang S, Shalapour S, Wong J, He F, Boassa D, Perkins G, Ali SR et al (2016) NF-kappaB restricts inflammasome activation via elimination of damaged mitochondria. Cell 164:896–910
83. Zhu S, Ding S, Wang P, Wei Z, Pan W, Palm NW, Yang Y, Yu H, Li HB, Wang G et al (2017) Nlrp9b inflammasome restricts rotavirus infection in intestinal epithelial cells. Nature 546:667–670

Chapter 8
The Cross-Regulation Between Autophagy and Type I Interferon Signaling in Host Defense

Shouheng Jin

Abstract The production of type I interferons (IFNs) is one of the hallmarks of intracellular antimicrobial program. Typical type I IFN response activates the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, which results in the transcription of plentiful IFN-stimulated genes (ISGs) to establish the comprehensive antiviral states. Type I IFN signaling should initiate timely to provoke innate and adaptive immune responses for effective elimination of the invading pathogens. Meanwhile, a precise control must come on the stage to restrain the persistent activation of type I IFN responses to avoid attendant toxicity. Autophagy, a conserved eukaryotic degradation system, mediated by a number of autophagy-related (ATG) proteins, plays an essential role in the clearance of invading microorganism and manipulation of type I responses. Autophagy modulates type I IFN responses through regulatory integration with innate immune signaling pathways, and by removing endogenous ligands of innate immune sensors. Moreover, selective autophagy governs the choice of innate immune factors as specific cargoes for degradation, thus tightly monitoring the type I IFN responses. This review will focus on the cross-regulation between autophagy and type I IFN signaling in host defense.

Keywords Type I interferon · Autophagy · ISG · Selective autophagic degradation

8.1 Introduction

Innate immune responses, initiated by germline-encoded pattern-recognition receptors (PRRs), provides the first line of host defense against invading microorganisms [62]. PRRs, including Toll-like receptors (TLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs), Nod-like receptors (NLRs), and several nucleic acid sensors, detect microbial components termed as pathogen-associated molecular pattern (PAMPs) to provoke the innate immune responses [100]. Upon recognition of

S. Jin (✉)
MOE Key Laboratory of Gene Function and Regulation, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou 510006, Guangdong, China
e-mail: jinshh3@mail.sysu.edu.cn

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_8

foreign molecular components, PRRs activate the NF-κB, IRF3/IRF7, and inflammasome signaling pathways to trigger the production of type I interferons (IFNs) and pro-inflammatory cytokines, which are essential for host defense [47]. Type I IFNs are polypeptides that are secreted from infected cells, known for their roles in antivirus, anti-proliferation, and immunomodulation. IFNα and IFNβ are the most well-defined type I IFNs [39]. Most cell types synthesize IFNβ, while hematopoietic cells, particularly plasmacytoid dendritic cells (pDCs) predominantly produce IFNα. A single *IFNB* gene encodes IFNβ, whereas various IFNα isoforms are encoded by more than a dozen of distinct genes [103].

Secreted type I IFNs are capable of binding to their heterodimeric transmembrane receptors named as IFNα receptor (IFNAR), which consists of IFNAR1 and IFNAR2 subunits [68]. The canonical type I IFN-mediated innate immune responses rely on Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2)-mediated signaling cascade to phosphorylate signal transducer and activator of transcription 1 (STAT1) and STAT2 [51]. Activated STAT1 and STAT2 form dimers and translocate to the nucleus, which in turn interact with IFN regulatory factor 9 (IRF9) to constitute trimolecular complex IFN-stimulated gene factor (ISG3), thereby leading to the transcriptional activation of hundreds of interferon-stimulated genes (ISGs) [79, 103]. The expression of numerous ISGs establishes an antiviral state of infected cells by directly suppressing the viral proliferation and manipulating the immune signaling pathways. To sculpt immune responses that are appropriate for host defense and survival, type I IFN responses must be tightly controlled to initiate and terminate timely against viral infection.

Immune system has evolved a sophisticated mechanism to strictly regulate type I IFN production at multilayers, such as transcriptional activation and suppression, posttranscriptional regulation mediated by m^6A (N^6-methyladenosine) RNA modification, post-translational modifications (PTMs) including phosphorylation, ubiquitination, and other unconventional forms of PTMs [36, 60, 61, 89, 96]. Increasing attention has been paid to the indispensable roles of autophagy in regulation of the initiation, activation, and termination of type I IFN signaling and maintains the strength of type I IFN response during viral infection [14, 16]. Autophagy may serve as a primordial form of eukaryotic innate immune system to directly eliminate the invading pathogens [17]. Moreover, the principal functions of autophagy have evolved drastically and have been well integrated with innate and adaptive immunity at multilevels [78].

8.2 Autophagy is a Regulatory System of Eukaryotic Innate Immunity

Autophagy mediated by a number of autophagy-related (ATG) proteins, is a highly conserved lysosomal degradation system that delivers substrates to double-membrane vesicles termed as autophagosomes [55]. Autophagy is controlled

by mammalian target of rapamycin (mTOR) and AMP-activated protein kinase (AMPK), both function as upstream factors to influence autophagy through direct phosphorylation of ULK1 [31]. The ULK1 serine–threonine kinase complex, constituted by ULK1, FIP200, ATG13, and ATG101, plays an essential role in the initiation of autophagy through phosphorylating a list of downstream proteins involved in autophagy flux [15]. The Beclin-1 complex (involving Beclin-1, VPS34, and VPS15) binds to ATG14L or UVRAG to generate phosphatidylinositol 3-phosphate (PI3P) to act in autophagosome nucleation or endolysosomal and autophagolysosomal maturation. The vesicle containing ATG9A is the only transmembrane core ATG protein that provides the membrane for autophagosomes [19]. WIPI serves in early stage of the membrane extension at the PI3P production site with ATG2A or ATG2B [55]. The expansion and completion of the autophagosome membrane contains two ubiquitin-like protein coupling systems: ubiquitin (Ub)-like ATG12 conjugates with ATG5 and ATG16L1, and Ub-like LC3/GABARAP conjugates with phosphatidylethanolamine (PE) resided on the membrane [13]. LC3/GABARAP family proteins are attached to the autophagosome membrane after proteolysis by ATG4, and bind to cargo receptors such as p62, NDP52, or OPTN to participate in cargo recognition, which subsequently delivers the substrates to lysosomes for degradation [3, 26, 37].

Although autophagy is considered as a high-throughput degradation system, accumulating evidence has demonstrated that autophagy plays an important role in type I IFN responses [98]. Given that microorganisms invading and replication cause cell stress, autophagy process accompanies microbial infection [14]. However, since a major function of autophagy is to eliminate cytoplasmic components, the innate immune system potentiates autophagy to degrade and dispose of invading pathogens [17]. Furthermore, at the later phase of infection, autophagy collaborates with adaptive immunity through delivering virus-derived antigens for presentation to T lymphocytes [14, 16]. In particular, selective autophagy is specifically capable of clearing intracellular components, such as protein aggregates and damaged or unwanted organelles, and deficiency of these processes may cause abnormal type I IFN responses [98]. Meanwhile, numerous evidence suggests that microbial pathogens have evolved diversified strategies to either fight with or take advantage of autophagy to promote their proliferation [14]. The exact roles and detailed mechanisms of ATG proteins that govern autophagy during microbial infection remain largely a black box to be further clarified.

8.3 Autophagy Orchestrates Type I IFN Production

Type I IFNs are multifunctional cytokines that produced by diverse PRRs, DNA and RNA sensors, confer antiviral, antiproliferative, and immunomodulatory activities in cells [75]. Endosomal TLRs, including TLR3, TLR7, TLR8, and TLR9, sense viral RNA and unmethylated CpG DNA [42]. RLR family, consisting of RIG-I, melanoma

differentiation associated gene 5 (MDA5), and the laboratory of genetics and physiology 2 (LGP2), all harbor a central helicase domain and a carboxy-terminal domain, which are both required for RNA binding, while only RIG-I and MDA5 have a tandem caspase activation and recruitment domain (CARD) at the amino terminus to enable them to transfer the signal through mitochondrial antiviral signaling protein (MAVS) [12]. Several cytosolic DNA and RNA sensors such as cyclic GMP-AMP synthase (cGAS), IFNγ-inducible protein 16 (IFI16), DNA-dependent protein kinase (DNA-PK), and the probable ATP-dependent RNA helicases DDX41 and DDX60, recognize intracellular DNA or RNA and converge on stimulator of interferon genes (STING) to drive the production of type I IFNs [7]. Mounting evidence has revealed the participation of autophagy in orchestrating type I IFN responses. In the following section, the potential roles of autophagy in mediating the type I IFN signaling triggered by diverse PRRs will be discussed.

8.3.1 TLR Signaling

TLR3 recognizes double-stranded RNA (dsRNA), TLR7/TLR8 senses single-stranded RNA (ssRNA), and TLR9 recognizes unmethylated CpG DNA [100]. TLR13 (mice only) functions as a sensor for the bacterial 23S ribosomal RNA [42]. These endosome-resident receptors respond to pathogen-derived nucleic acids and function via two distinct signaling pathways: TLR3 activates TRIF while TLR7/TLR8, TLR9, and TLR13 activate MyD88. Both of the two adaptors induce the activation of transcription factor nuclear factor κ-light-chain enhancer of activated B cells (NF-κB), whereas IFN regulatory factor 3 (IRF3) is activated through the TRIF pathway and IRF7 through the MyD88 pathway [42, 62]. The phosphorylation of inhibitor IκB by the IκB kinase (IKK) complex is required for NF-κB activation. After phosphorylation, IκB undergoes poly-ubiquitination and subsequent proteasomal degradation, thus releasing NF-κB to the nucleus and turning on the transcription of downstream genes. IRF3 and IRF7 are phosphorylated by IKK-related kinases, TBK1/IKKε, which in turn form homodimers to translocate into the nucleus and form an enhanceosome complex together with NF-κB and other transcription factors to activate the transcription of type I IFN genes [12, 100].

In the recognition of viral RNA by TLR7, autophagy participates in transmission of the cytosolic viral replication intermediates to lysosomes and regulates the production of IFNα in pDCs [52]. The RNA of vesicular stomatitis virus (VSV) can be captured by autophagosomes and be delivered to endosomes, where to facilitate type I IFN signaling activation [24]. Upon human immunodeficiency virus type 1 (HIV-1) infection, pDCs synthesize IFNα via TLR7 signaling pathway in an autophagy-dependent manner [99]. In response to DNA-containing immune complexes (DNA-IC), IFNα production depends on LC3-associated phagocytosis (LAP). LAP contributes to TLR9 trafficking into a specialized IFN signaling compartment by a mechanism that involves ATG proteins, but not the conventional autophagic pre-

initiation complex, or adaptor protein-3 (AP-3) [34]. Under the activation of TLR9 by CpG DNA, LC3 and IKKα are recruited into endosomes to form the LC3-IKKα complex, which provokes IRF7-mediated type I IFN activation [32]. Collectively, these studies reveal that autophagy process plays an essential role in the initiation of TLR signaling pathway.

Meanwhile, autophagic degradation has also been reported to inhibit TLR-triggered type I IFN production. Enterovirus 71 (EV71) and coxsackievirus A16 (CA16) can suppress the TLR7-mediated type I IFN signaling pathway by promoting the degradation of endosome through autophagy [80]. Furthermore, selective autophagy is indispensable for the termination of TLR signaling. During *Salmonella typhimurium* infection, Tripartite motif-containing 32 (TRIM32) functions as a suppressor of TLR3/4-mediated type I IFN signaling by targeting TRIF for TAX1BP1-mediated selective autophagic degradation [94]. *ATG16L1* deficiency promotes the accumulation of TRIF and enhances downstream signaling in macrophages. Mice lacking *Atg16l1* in myeloid cells succumb to lipopolysaccharide-mediated sepsis but increase their elimination of intestinal *Salmonella typhimurium* in an interferon receptor-dependent manner. Human macrophages with the Crohn's disease-associated with ATG16L1 variant T300A, exhibit more production of IFNβ and IL-1β. An elevated IFN response gene signature is observed in patients with IBD who are resistant to treatment with an antibody to the cytokine TNF [74]. Following the treatment of poly(I:C), autophagy cargo receptor NDP52 prompts the inactivation of IRF3 by accelerating the autophagic degradation of adopter proteins TRIF and TRAF. Under normal conditions, ubiquitin-editing enzyme A20 restrains this autophagy process [38]. These findings indicate selective autophagy coordinates with innate immune system to shape the host defense. During viral infection, TLR activation tends to induce autophagy to improve IFN production, whereas the negative regulation of autophagy helps terminate TLR signaling.

8.3.2 RLR Signaling

Based on their unique features, RIG-I and MDA5 detect distinct viral RNA PAMPs. RIG-I exhibits a preference for ligands that are short (10–300 bp) dsRNA, which contains 5′-triphosphate ends with regions enriched in poly-U/UC or AU sequences (32–35 bp). By contrast, the precise natural RNA PAMP ligands of MDA5 remain poorly characterized. It is generally acknowledged that MDA5 binds internally to long dsRNA (greater than 1000–2000 bp) organized in higher ordered structures with no end specificity. PAMPs recognition induces conformational changes of RIG-I and MDA5 by releasing their N-terminal CARD. The exposed CARD is then ubiquitinated and interacts with MAVS CARD for signaling. MAVS recruits TBK1, which further phosphorylates and activates IRF3, resulting in IRF3 dimerization and transcription activation of type I IFN genes [95].

ATG5–ATG12 conjugation suppresses the production of type I IFN by binding to the CARD of RIG-I, which disrupts its association with MAVS and inhibits the RIG-I signaling [41]. Moreover, Beclin-1 plays a negative regulatory role in RIG-I-mediated type I IFN production by targeting the CARD of MAVS, hence blocking the RIG-I-MAVS interaction in an autophagy-independent manner [44]. Hepatitis C virus (HCV)-induced autophagy suppresses RIG-I signaling and type I IFN production, which also occurs during the Dengue virus (DENV) infection. Since HCV-induced autophagy up-regulates the ATG5 and Beclin-1 expressions [47, 48], which are negative regulators of type I IFN production. It can explain why autophagy induced by some species of viruses inhibits the host defenses. IRF3 is also a target of autophagy. Rubicon, an autophagy inhibitor, is involved in suppressing RLR-mediated type I IFN responses by inhibiting IRF3 dimerization via interacting with its IRF association domain [43]. Altogether, these findings indicate that ATG proteins function as regulators in RIG-I signaling.

Besides affecting type I IFN signaling by directly interacting with key immune factors, autophagy also plays an essential role in shutting down the type I IFN signaling activation by selective degradation. The leucine-rich repeat-containing protein 25 (LRRC25) binds with ISG15 (a product encoded by ISG) associated RIG-I, which facilitates the recognition of RIG-I by autophagy cargo receptor p62, thereby down-regulating type I IFN production via autophagic degradation of RIG-I [22]. Human parainfluenza virus type 3 (HPIV3) infection induces mitophagy to degrade MAVS and interfere with the RIG-I-mediated type I IFN signaling [21]. *ATG5* absence leads to the deficiency of autophagy process, thus resulting in the accumulation of dysfunctional mitochondria, MAVS, and mitochondrial ROS to augment RLR-mediated type I IFN signaling. Autophagy is required for the clearance of dysfunctional mitochondria to keep the RLR-mediated type I IFN production in balance [85]. IFN-induced Tetherin/BST2 recruits the E3 ubiquitin ligase MARCH8 to catalyze K27-linked ubiquitin chains on MAVS at lysine (K) 7, thus providing a recognition signal for the NDP52-directed autophagic degradation of MAVS and preventing the persistent activation of type I IFN signaling [40].

Atg16l deficiency in mice causes a resistance to intestinal bacterial pathogen *Citrobacter rodentium* infection and a phenotype with protection against chemical injury of the intestine in a type I IFN-dependent manner. Therefore, autophagy proteins prevent a spontaneous type I IFN activation to microorganism that is beneficial in the presence of infectious and noninfectious intestinal hazards [67]. Mitochondrial nucleotide-binding, leucine-rich repeats (NLR)-containing protein, NLRX1 attenuates type I IFN production but promotes autophagy upon viral infection. NLRX1-interacting partner, mitochondrial Tu translation elongation factor (TUFM) associates with ATG5-ATG12 and ATG16L1 to increase the activation of type I IFN and decrease autophagy [54]. Cytochrome c Oxidase (CcO) complex subunit COX5B specifically suppresses MAVS-mediated antiviral pathway by targeting MAVS. Mechanistically, MAVS activation induces the enhanced ROS production and COX5B expression, while COX5B negatively regulates MAVS signaling by inhibiting ROS production. COX5B coordinates with the autophagy process to tightly control MAVS aggregation, hence balancing the antiviral immune responses [97].

TRIM proteins are important regulators of both autophagy and antiviral immunity. Through a targeted RNAi screening assay the relevance of selected TRIM proteins in autophagy induced various viruses, TRIM23 is identified to mediate autophagy-induced restriction of multiple viruses in both RING E3 ligase and ADP ribosylation factor (ARF) GTPase activity-dependent manner. K27-linked auto-ubiquitination of the ARF domain is essential for the GTP hydrolysis activity of TRIM23 and activation of TBK1 by facilitating its dimerization and ability to phosphorylate the selective autophagy receptor p62. TRIM23 has dual E3 ubiquitin ligase and GTPase activities, which is a core component of the selective autophagic machinery [81].

8.3.3 cGAS/STING Signaling

The cGAS-STING signaling pathway is critical for sensing viral DNA and triggering type I IFN production [25]. The activity of cGAS is restrained by TTLL6-mediated mono-glutamylation modification. Cytosolic carboxypeptidase5 (CCP5) and CCP6 hydrolyze the mono-glutamylation and poly-glutamylation of cGAS, respectively, which subsequently restores cGAS activity [91]. Following viral DNA treatment, cGAS catalyzes the synthesis of cyclic guanosine monophosphate–adenosine monophosphate (cGAMP), the second messenger to phosphorylate and dimerize STING, thus recruiting TBK1 to activate IRF3-directed type I IFN gene expression [20]. Upon herpes simplex virus-1 (HSV-1) infection, Beclin-1 inhibits the DNA stimulation by targeting cGAS, suppressing the cGAMP synthesis and subsequent type I IFN production. Additionally, autophagic degradation of cytosolic pathogen DNA recognized by cGAS, hence indirectly attenuating the activation of cGAS-STING signaling [59]. ATG9A also negatively regulates dsDNA-mediated immune responses by suppressing the assembly of STING and TBK1 [73]. Furthermore, cGAMP leads to the dissociation of ULK1 from AMPK complex, and induces the phosphorylation of STING by ULK1 and subsequently degradation of STING, thereby inhibiting the production of type I IFNs [46].

Selective autophagic degradation of key immune factors also contributes to regulate the cGAS-STING signaling. cGAS can be attached with K48-linked ubiquitin chains and recognized by autophagy cargo receptor p62 for degradation, leading to the inhibition of cGAS-STING signaling [11]. Persistent activation of the cGAS-STING signaling pathway leads to the K63-linked ubiquitination and elimination of STING, thus mediating the selective autophagic degradation of active STING by p62 to avoid the excessive type I IFN production [71]. TRIM21, also functions as selective autophagy receptor to direct the autophagic degradation of IRF3 dimers and suppress cGAS-STING signaling pathway [45].

In summary, so far at least two major mechanisms are involved in the crosstalk between autophagy/autophagy-related proteins and type I IFN responses: the immune-regulatory role played by ATG proteins and the degradation of immune factors directed by selective autophagy. Autophagy strikes up an elegant and flexible system in shaping the regulatory network of PRR-mediated type I IFN signaling (Fig. 8.1).

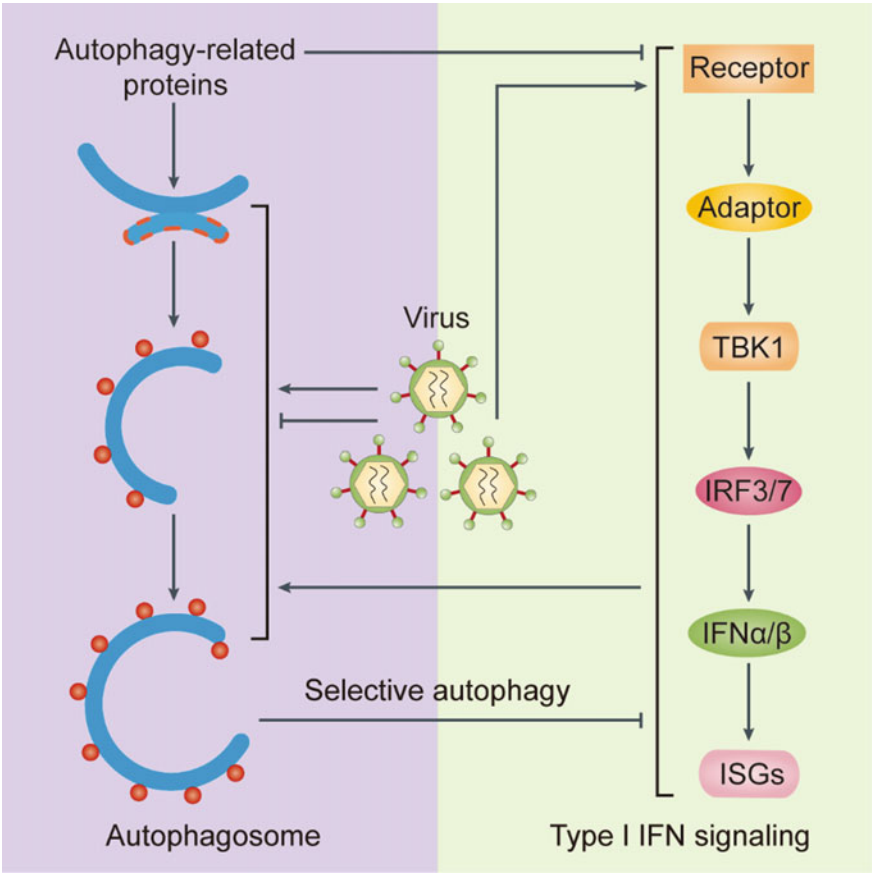


Fig. 8.1 Autophagy influences type I IFN responses. Autophagy-related proteins can directly interact with key immune factors in type I IFN signaling pathway, thus negatively regulating the production of type I IFNs. Moreover, viral infection can promote the recognition of innate immune factors by cargo receptors for selective autophagic degradation, which in turn shuts down the persistent activation of type I IFN responses

8.4 Autophagy Affects Type I IFN Responses

Once synthesized by the activation of PRR signaling, type I IFNs are released from the infected cell in a paracrine or autocrine manner, which in turn binds to IFNAR to stimulate downstream JAK-STAT pathway, thus activating the transcription of ISGs. The level of extracellular membrane IFNAR determines the activity of type I IFN responses. Diverse viruses have developed the strategies to mediate the degradation of IFN receptors to evade antiviral responses [90]. Currently, endocytosis and ubiquitination-mediated lysosomal degradation are considered the major pathways to control the IFNAR level [48, 49]. HCV infected Huh-7.5 cells when cultured

with free fatty acids (FFA), the intracellular lipid accumulation induces the endoplasmic reticulum (ER) stress response and down-regulates IFNAR1 level to shut down the JAK-STAT pathway [30]. HCV and FFA-induced autophagy promotes the expression of LAMP2A and its association between IFNAR1 to mediate the chaperone-mediated autophagic of IFNAR1 [50]. Moreover, HCV-induced ER stress and autophagy selectively degrade the IFNAR1 rather than type II (IFNγ) or type III (IFNλ) IFN receptors, which is crucial for the resistance of HCV against IFNα and ribavirin [8]. Altogether, these researches not only illustrate the interplay between chaperone-mediated autophagy and IFNAR expression but also indicate that type III IFN possesses a stronger antiviral activity than type I IFN in inhibiting HCV infection, and that the suppression of HCV-induced ER stress and chaperone-mediated autophagy is a potential strategy to reduce HCV resistance to type I IFNs.

8.5 Type I IFN Signaling Modulates Autophagy

Type I IFNs play an immune-regulatory role rather than a direct antiviral role in building up an antiviral state by the activation of numerous downstream ISG products. In addition to mediating the transcription of ISGs, IFNα/β also utilizes autophagy to combat against viral infection. IFNα/β induces the autophagic degradation of viral components and facilitates the delivery of PAMPs from the cytoplasm to TLR3-containing endosomes [18, 84]. Additionally, the antiviral mechanisms of several ISG products are associated with autophagy. Autophagy regulates type I IFN production and type I IFN signal transduction, and type I IFNs and ISG products eliminate viral particles by activation of autophagy as well, suggesting that there is a tight cross-regulation between autophagy and type I IFN signaling (Fig. 8.2).

8.5.1 Type I IFN Signaling Affects Autophagy

Autophagy has been implicated in innate immune responses against various intracellular pathogens. Recent studies have reported that autophagy can be triggered by pathogen-recognizing sensors, including TLRs and cGAS, to participate in type I IFN responses [53]. STING induces autophagy via a mechanism that is not dependent of the activation of TBK1 and IFN signaling. Through binding to cGAMP, STING translocates to the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and the Golgi in a COP-II complex and ARF GTPases dependent manner. STING-containing ERGIC functions as a membrane source for LC3 lipidation to promote autophagosome biogenesis. cGAMP mediated LC3 lipidation through a pathway that is dependent on WIPI2 and ATG5, but not ULK1 and VPS34-Beclin-1 kinase complexes. The cGAMP-induced autophagy is essential for the DNA and virus clearance in the cytosol. Intriguingly, STING from the sea anemone *Nematostella*

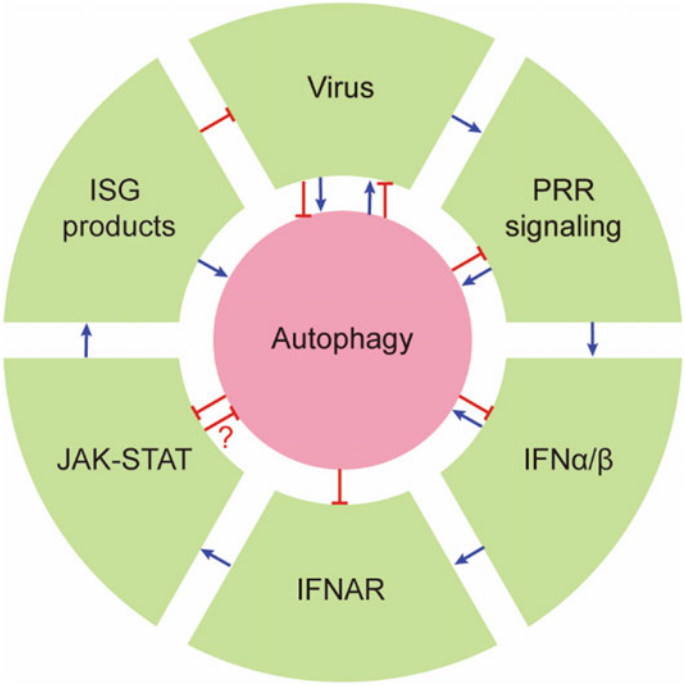


Fig. 8.2 The cross-regulation between autophagy and type I IFN signaling. Viral infection prompts the activation of autophagy, whereas some species of viruses suppress autophagy initiation. The primordial role of autophagy in antiviral immunity is to degrade the viral constituents, however, other viruses can subvert and exploit multiple steps of the autophagy process to evade type I IFN responses and promote their proliferation. Autophagy coordinates with type I IFN responses at multilayers, thus shaping the host defense against invading pathogens

vectensis activates autophagy but not IFN production in response to cGAMP stimulation, which suggests that induction of autophagy is a primordial function of the cGAS-STING signaling [29].

Moreover, STING harbors classic LC-3 interacting regions (LIRs) and mediates autophagy through its direct interaction with LC3. Upon stimulation, STING undergoes autophagic degradation immediately after TBK1 activation. The noncanonical autophagy induced by STING does not depend on ATG5, whereas other autophagy regulators such as ULK1, Beclin-1, ATG9, and p62 are dispensable. LIR mutants of STING abolished its interaction with LC3 and its activation of autophagy. Also, mutants that abolish STING dimerization and cGAMP binding diminished the STING-LC3 interaction and subsequent autophagy, suggesting that STING activation is indispensable for autophagy induction [63].

Transfection of poly(I:C) into the cytoplasm, or Sendai virus (SeV) infection, significantly induces autophagy in normal cells but not in *RIG-I*-depletion cells. Poly(I:C) transfection or SeV infection-induced autophagy in the cells lacking type I IFN signaling as the effect is not due to IFN production. The RIG-I-MAVS-TRAF6

signaling axis is crucial for RIG-I-induced autophagy. Upon RIG-I activation, Beclin-1 translocates to the mitochondria and interacts with TRAF6. Moreover, Beclin-1 undergoes K63-linked ubiquitination mediated by TRAF6 following RIG-I activation, thus triggering the initiation of autophagy. Since autophagy deficiency enhances type I IFN signaling, the induction of autophagy by the RIG-I pathway might also contribute to preventing excessive IFN productions through a negative-feedback loop [53].

8.5.2 Type I IFN Downstream Signaling Regulates Autophagy

Type I IFN signaling transduction includes the activation of JAK-STAT, phosphoinositide 3 kinase (PI3K)/AKT/mTOR, and mitogen-activated protein kinase (MAPK) pathways, which are critical for antiviral immunity by driving the expression of ISGs and provoking downstream events. These pathways are involved in autophagy process during starvation, inflammation, and antitumor immunity [4, 33, 75]. Type I IFN-induced immune responses may also be involved in virus-induced autophagy.

The JAK-STAT pathway is a canonical signaling pathway mediated by type I IFNs. Recent studies have indicated that type I IFN induces autophagy in multiple cancer cell lines and that several molecules of the JAK-STAT pathway are involved in this process [2, 57, 76, 102]. In human Burkitt lymphoma Daudi cells, type I IFN triggers autophagy in a STAT2-dependent manner [76]. IFNα induces autophagy in chronic myeloid leukemia cells and the activation of JAK1 and STAT1 facilitate the generation of Beclin-1, a critical ATG protein participates in VPS34 complex formation and recruits additional proteins, such as ATG14L, AMBRA1, UVRAG, Bcl-2, and Rubicon, to modulate the activity of VPS34 [102]. In MCF-7 breast cancer cells, IFNβ also initiates autophagy in an ATG7- and STAT1-dependent manner [2].

STAT1 also functions as a transcriptional suppressor of autophagy genes and autophagic activity. *STAT1* deficiency up-regulates the mRNA and protein levels of ULK1, as well as promotes autophagy flux. STAT1 bounds a putative regulatory sequence in the *ULK1* 5′-flanking region to function as a repressor of transcription activation of *ULK1*, thus negatively regulating autophagy flux [27].

The MAPK pathway is another important pathway activated by type I IFNs. JAK1 activation leads to the phosphorylation of VAV protein, which subsequently activates the downstream regulator Rac1 to phosphorylate and activate the MAPK-kinase kinase (MAPKKK). Therefore, MAPK kinase (MAPKK) is activated, thus resulting in the activation of MAPK [58]. MAPK is a number of highly conserved serine/threonine kinase family, including c-Jun N-terminal kinase 1/2/3 (JNK1/2/3), extracellular signal-related kinase 1/2 (ERK1/2), ERK3/4, ERK5/BMK1, ERK7/8, and p38 MAPK [101]. p38 MAPK activates the inflammatory responses by phosphorylating ULK1 and inhibiting autophagy in microglial cells [33]. JNK is involved in

triggering autophagy in cancer cells via up-regulating the expression of ATG5 and Beclin-1, and facilitating the dissociation of the Bcl-2/Beclin-1 complex [5, 56, 88]. Resveratrol (RSV) enhances the autophagic cell death of chronic myelogenous leukemia cells by elevating the p62 yields in a JNK-dependent manner [72]. Palmitate (PA) induces apoptosis in bone marrow mesenchymal stem cells by activating the JNK and p38 MAPK [64]. All together, these researches provide vital evidence for the crucial role of the MAPK pathway in inducing autophagy.

Type I IFNs also trigger the PI3K/AKT/mTOR pathway and mTORC1 servers as a direct negative regulator in autophagy initiation. Type I IFNs initiate autophagy via the PI3K/AKT/mTOR pathway in cancer cells. In human glioma cells, IFNβ-induced autophagy is dependent on the PI3K/AKT/mTOR and ERK1/2 signaling pathways [57]. Type I IFNs decrease the mTORC1 activity in human Burkitt lymphoma Daudi cells and further limit the PI3K/AKT/mTOR pathway, leading to the activation of autophagy [76].

The JAK-STAT, PI3K/AKT/mTOR, and MAPK signaling pathways are pivotal antiviral pathways. A great deal of studies have indicated that these pathways affect the initiation of autophagy in cancer and inflammation, and type I IFNs triggered autophagy in response to HCV has also been confirmed [18]. Key molecules of the type I IFN signaling pathway are also involved in virus-induced autophagy [70]. The roles of type I IFN and its downstream signaling pathways in autophagy activation upon viral infection is crucial. Recently, research on type I IFN-induced autophagy in cancer cells provides benefits for the study of antitumor [2, 102]. Therefore, it is important for exploiting new antiviral immune targets to further elucidate the interplay of the type I IFN responses and autophagy during viral infection.

8.6 ISG Products Participate in the Regulation of Autophagy

ISGs encode proteins that directly restrict viral infection and server as immune regulators. ISG products modulate autophagy and manipulate its crosstalk with antiviral immune responses. ISG products not only regulate virus-induced autophagy flux but also target key immune factor in type I IFN signaling for selective autophagic degradation, thereby influencing antiviral immunity.

IFN-induced and dsRNA-activated protein kinase (EIF2AK2/PKR) binds to Beclin-1 within the VPS34 complex, which initiates the formation of autophagosomes. PKR-mediated phosphorylation of the eukaryotic translation initiation factor 2 subunit 1 (EIF2S1/eIF2α) also induces autophagy [86, 87]. IFNβ-inducible protein SCOTIN transports HCV nonstructural 5A (NS5A) to autophagosomes for degradation to exert a vital role in repressing the HCV proliferation [44]. PML (also named as TRIM19) can be induced by IFNα/β/γ, which inhibits EV71-mediated autophagy to control viral replication [9, 10]. Tudor domain containing 7 (TDRD7) restrains the proliferation of paramyxovirus [83]. Additionally, TDRD7 inhibits the

paramyxovirus-triggered autophagy by suppressing the activity of AMPK, which is a positive factor upstream of autophagy initiation via activating ULK1 and suppressing mTORC1 [23, 35]. Ribonuclease L (RNase L), encoded by an ISG, cleaves viral RNA to produce small dsRNA, thus suppressing viral replication via activation of autophagy during early viral infections [6, 77]. RNase L initiates virus-induced autophagy by triggering the activation of JNK and PKR [1]. RNase L-induced autophagy can restrain the viral proliferation in early infection, however, in later stages, autophagy promotes the replication of the virus. Plenty researches support autophagy in promoting the replication of certain viruses, due to hijacking autophagy for their replication at the late stage of infection, which subverts the RNase L-induced antiviral autophagy.

ISG products manipulate selective autophagic degradation of immune factors in type I IFN signaling to regulate antiviral immune responses. TRIM14, induced by type I IFNs, can recruit USP14 to remove the K48-linked ubiquitin chains of cGAS, hence suppressing the p62-directed selective autophagic degradation of cGAS [11]. Ubiquitin-like modifier, ISG15 conjugates with target molecules, which is a process termed as ISGylation. Type I IFNs facilitate the ISGylation of Beclin-1 and inhibit the formation of VPS34 complex, therefore blocking autophagy flux [93]. ISG15 can also interact with histone deacetylase 6 (HDAC6) and p62 to mediate the degradation of ISG15-conjugated proteins through autophagy [69]. ISG15 is involved in the LRRC25-mediated degradation of RIG-I through p62-dependent selective autophagy [22]. Moreover, LRRC59 targets ISG15-associated RIG-I to inhibit the association between LRRC25 and RIG-I, thus disrupting the recognition of RIG-I by p62 [92]. These studies indicate that ISG15 works as a degradation signal for p62-mediated autophagy recognition of RIG-I.

TRIM5α, an IFN-inducible intercellular antiviral restriction factor, acts as autophagy receptor by recognizing viral proteins as cargoes, which is initially discovered as an antiviral restriction factor that recognizes the HIV capsid protein [28, 82]. TRIM5α initiates autophagy through recruitment of ULK1 and Beclin-1, then recognizes the HIV-1 capsid via the SPRY domain of TRIM5α, thus leading to premature capsid disassembly and autophagic degradation [15]. Another TRIM family member, TRIM22, is also up-regulated by viral infection. TRIM22 regulates virus-induced autophagy via the NF-κB/Beclin-1 pathway. TRIM22-augmented autophagy prevents intracellular virus to evade autophagic clearance, hence inhibiting the persistence of viral infection [65].

Tetherin (also referred to as BST2 or CD317) is an IFN-induced membrane protein that inhibits the release of diverse enveloped viral particles from infected cells [40]. Autophagy process and ATG proteins are hijacked by HIV-1 Vpu to circumvent Tetherin restriction of viral release. Tetherin and Vpu are presented in LC3-positive compartments. Vpu selectively interacts with LC3C and antagonizes Tetherin restriction. LC3C expression favors the removal of Tetherin from the HIV-1 budding site, and thus HIV-1 release in Tetherin-expressing cells. Moreover, ATG5 and Beclin-1, but not all the components of autophagy, act with LC3C to facilitate Vpu antagonism of Tetherin restriction. LAP contributes to Vpu counteraction of Tetherin restriction [66]. Tetherin not only functions as a cargo of autophagy but itself regulates the activation

of autophagy. Tetherin associates with the autophagy/mitophagy suppressor LRPPRC and suppresses LRPPRC from forming a complex with Beclin-1-Bcl-2, thus activating autophagy through releasing Beclin-1 to bind with VPS34 [104]. Moreover, Tetherin promotes the NDP52-mediated selective autophagic degradation of MAVS, therefore restraining the persistent type I IFN production [40].

8.7 Conclusion

Autophagy, a highly evolutionarily conserved process for the degradation and recycling of cytoplasmic constituents, has evolved comprehensive and multilevel mechanisms to regulate type I IFN responses. The primordial role of autophagy in innate immunity is to eliminate microbial infection through destructing the invading pathogens. Type I IFNs and IFN-stimulated gene products can harness autophagy to achieve the clearance of microbial infection, which strengthens the ability of immune system. Moreover, autophagy process integrates with the innate immune signaling pathway. ATG proteins directly govern type I IFN responses during viral infection, and selective autophagic degradation of immune factors and microbial constituents also contributes to the suppression of type I IFN signaling. Nevertheless, some invading microorganisms manipulate autophagy to elude host defense. The dominant function of autophagy in type I IFN responses remains as the suppression of type I IFN production, which represents that autophagy maintains the antimicrobial role and type I IFN signaling in a balance, thus avoiding harmful immunopathology. The accurate roles of ATG proteins and the detailed mechanisms underlying microbial infection-induced autophagy still remain elusive. Understanding the interplay between autophagy and type I IFN responses will help us identify novel diagnostic markers and develop strategies for clinical therapy of certain pathogen infections in future.

Acknowledgements This work was supported by the National Natural Science Foundation of China (31970700 and 31700760) and the Fundamental Research Funds for the Central Universities (18lgpy49).

References

1. Adnan M, Malathi SK (2012) RNase L induces autophagy via c-Jun N-terminal kinase and double-stranded RNA-dependent protein kinase signaling pathways. J Biol Chem 287:43651–43664
2. Ambjorn M, Ejlerskov P, Liu YW, Lees M, Jaattela M, Issazadeh-Navikas S (2013) IFNB1/interferon-β-induced autophagy in MCF-7 breast cancer cells counteracts its proapoptotic function. Autophagy 9:287–302

3. Anding AL, Baehrecke EH (2017) Cleaning house: selective autophagy of organelles. Dev Cell 41:10–22
4. Barutcu SA, Girnius N, Vernia S, Davis RJ (2018) Role of the MAPK/cJun NH2-terminal kinase signaling pathway in starvation-induced autophagy. Autophagy 14:1586–1595
5. Byun JY, Yoon CH, An S, Park IC, Kang CM, Kim MJ, Lee SJ (2009) The Rac1/MKK7/JNK pathway signals upregulation of Atg5 and subsequent autophagic cell death in response to oncogenic Ras. Carcinogenesis 30:1880–1888
6. Chakrabarti A, Ghosh PK, Banerjee S, Gaughan C, Silverman RH (2012) RNase L triggers autophagy in response to viral infections. J Virol 86:11311–11321
7. Chan YK, Gack MU (2016) Viral evasion of intracellular DNA and RNA sensing. Nat Rev Microbiol 14:360–373
8. Chandra PK, Bao LL, Song K, Aboulnasr FM, Baker DP, Shores N, Wimley WC, Liu SH, Hagedorn CH, Fuchs SY et al (2014) HCV infection selectively impairs type I but not type III IFN signaling. Am J Pathol 184:214–229
9. Chelbi-Alix MK, Pelicano L, Quignon F, Koken MHM, Venturini L, Stadler M, Pavlovic J, Degos L, De The H (1995) Induction of the PML protein by interferons in normal and APL cells. Leukemia (Basingstoke) 9:2027–2033
10. Chen DY, Feng CH, Tian XY, Zheng N, Wu ZW (2018) Promyelocytic leukemia restricts enterovirus 71 replication by inhibiting autophagy. Front Immunol 9:19
11. Chen MX, Meng QC, Qin YF, Liang PP, Tan P, He L, Zhou YB, Chen YJ, Huang JJ, Wang RF et al (2016) TRIM14 inhibits cGAS degradation mediated by selective autophagy receptor p62 to promote innate immune responses. Mol Cell 64:105–119
12. Chiang C, Gack MU (2017) Post-translational control of intracellular pathogen sensing pathways. Trends Immunol 38:39–52
13. Choi AMK, Ryter SW, Levine B (2013) Autophagy in human health and disease. N Engl J Med 368:651–662
14. Choi Y, Bowman JW, Jung JU (2018) Autophagy during viral infection—a double-edged sword. Nat Rev Microbiol 16:340–353
15. Clarke AJ, Simon AK (2019) Autophagy in the renewal, differentiation and homeostasis of immune cells. Nat Rev Immunol 19:170–183
16. Deretic V, Levine B (2018) Autophagy balances inflammation in innate immunity. Autophagy 14:243–251
17. Deretic V, Saitoh T, Akira S (2013) Autophagy in infection, inflammation and immunity. Nat Rev Immunol 13:722–737
18. Desai MM, Gong B, Chan TS, Davey RA, Soong L, Kolokoltsov AA, Sun JR (2011) Differential, type I interferon-mediated autophagic trafficking of hepatitis C virus proteins in mouse liver. Gastroenterology 141:674–685
19. Dikic I, Elazar Z (2018) Mechanism and medical implications of mammalian autophagy. Nat Rev Mol Cell Biol 19:349–364
20. Diner EJ, Burdette DL, Wilson SC, Monroe KM, Kellenberger CA, Hyodo M, Hayakawa Y, Hammond MC, Vance RE (2013) The innate immune DNA sensor cGAS produces a noncanonical cyclic dinucleotide that activates human STING. Cell Rep 3:1355–1361
21. Ding BB, Zhang LL, Li ZF, Zhong Y, Tang QP, Qin YL, Chen MZ (2017) The matrix protein of human parainfluenza virus type 3 induces mitophagy that suppresses interferon responses. Cell Host Microbe 21:538–547
22. Du Y, Duan TH, Feng YC, Liu QX, Lin M, Cui J, Wang RF (2018) LRRC25 inhibits type I IFN signaling by targeting ISG15-associated RIG-I for autophagic degradation. EMBO J 37:351–366
23. Egan DF, Shackelford DB, Mihaylova MM, Gelino S, Kohnz RA, Mair W, Vasquez DS, Joshi A, Gwinn DM, Taylor R et al (2011) Phosphorylation of ULK1 (hATG1) by AMP-activated protein kinase connects energy sensing to mitophagy. Science 331:456–461

24. Frenz T, Graalmann L, Detje CN, Doring M, Grabski E, Scheu S, Kalinke U (2014) Independent of plasmacytoid dendritic cell (pDC) infection, pDC triggered by virus-infected cells mount enhanced type I IFN responses of different composition as opposed to pDC stimulated with free virus. J Immunol 193:2496–2503
25. Gao DX, Wu JX, Wu YT, Du FH, Aroh C, Yan N, Sun LJ, Chen ZJJ (2013) Cyclic GMP-AMP synthase is an innate immune sensor of HIV and other retroviruses. Science 341:903–906
26. Gatica D, Lahiri V, Klionsky DJ (2018) Cargo recognition and degradation by selective autophagy. Nat Cell Biol 20:233–242
27. Goldberg AA, Nkengfac B, Sanchez AMJ, Moroz N, Qureshi ST, Koromilas AE, Wang S, Burelle Y, Hussain SN, Kristof AS (2017) Regulation of ULK1 expression and autophagy by STAT1. J Biol Chem 292:1899–1909
28. Grutter MG, Luban J (2012) TRIM5 structure, HIV-1 capsid recognition, and innate immune signaling. Curr Opin Virol 2:142–150
29. Gui X, Yang H, Li T, Tan XJ, Shi PQ, Li MH, Du FH, Chen ZJJ (2019) Autophagy induction via STING trafficking is a primordial function of the cGAS pathway. Nature 567:262–266
30. Gunduz F, Aboulnasr FM, Chandra PK, Hazari S, Poat B, Baker DP, Balart LA, Dash S (2012) Free fatty acids induce ER stress and block antiviral activity of interferon alpha against hepatitis C virus in cell culture. Virol J 9:12
31. Hansen M, Rubinsztein DC, Walker DW (2018) Autophagy as a promoter of longevity: insights from model organisms. Nat Rev Mol Cell Biol 19:579–593
32. Hayashi K, Taura M, Iwasaki A (2018) The interaction between IKKα and LC3 promotes type I interferon production through the TLR9-containing LAPosome. Sci Signal 11:10
33. He YL, She H, Zhang T, Xu HD, Cheng LH, Yepes M, Zhao YR, Mao ZX (2018) p38 MAPK inhibits autophagy and promotes microglial inflammatory responses by phosphorylating ULK1. J Cell Biol 217:315–328
34. Henault J, Martinez J, Riggs JM, Tian J, Mehta P, Clarke L, Sasai M, Latz E, Brinkmann MM, Iwasaki A et al (2012) Noncanonical autophagy is required for type I interferon secretion in response to DNA-immune complexes. Immunity 37:986–997
35. Hoyer-Hansen M, Bastholm L, Szyniarowski P, Campanella M, Szabadkai G, Farkas T, Bianchi K, Fehrenbacher N, Elling F, Rizzuto R et al (2007) Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-β, and Bcl-2. Mol Cell 25:193–205
36. Hu MM, Yang Q, Xie XQ, Liao CY, Lin H, Liu TT, Yin L, Shu HB (2016) Sumoylation promotes the stability of the DNA sensor cGAS and the adaptor STING to regulate the kinetics of response to DNA virus. Immunity 45:555–569
37. Huang TZ, Kim CK, Alvarez AA, Pangeni RP, Wan XC, Song X, Shi TP, Yang YY, Sastry N, Horbinski CM et al (2017) MST4 phosphorylation of ATG4B regulates autophagic activity, tumorigenicity, and radioresistance in glioblastoma. Cancer Cell 32:840–855
38. Inomata M, Niida S, Shibata K, Into T (2012) Regulation of Toll-like receptor signaling by NDP52-mediated selective autophagy is normally inactivated by A20. Cell Mol Life Sci 69:963–979
39. Ivashkiv LB, Donlin LT (2014) Regulation of type I interferon responses. Nat Rev Immunol 14:36–49
40. Jin SH, Tian S, Luo M, Xie WH, Liu T, Duan TH, Wu YX, Cui J (2017) Tetherin suppresses type I interferon signaling by targeting MAVS for NDP52-mediated selective autophagic degradation in human cells. Mol Cell 68:308–322
41. Jounai N, Takeshita F, Kobiyama K, Sawano A, Miyawaki A, Xin KQ, Ishii KJ, Kawaii T, Akira S, Suzuki K et al (2007) The Atg5–Atg12 conjugate associates with innate antiviral immune responses. Proc Natl Acad Sci USA 104:14050–14055
42. Kawai T, Akira S (2011) Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 34:637–650

43. Kim JH, Kim TH, Lee HC, Nikapitiya C, Uddina MB, Park ME, Pathinayake P, Lee ES, Chathuranga K, Herath TUB et al (2017) Rubicon modulates antiviral type I interferon (IFN) signaling by targeting IFN regulatory factor 3 dimerization. J Virol 91:14
44. Kim N, Kim MJ, Sung PS, Bae YC, Shin EC, Yoo JY (2016) Interferon-inducible protein SCOTIN interferes with HCV replication through the autolysosomal degradation of NS5A. Nat Commun 7:12
45. Kimura T, Jain A, Choi SW, Mandell MA, Schroder K, Johansen T, Deretic V (2015) TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. J Cell Biol 210:973–989
46. Konno H, Konno K, Barber GN (2013) Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. Cell 155:688–698
47. Kumar H, Kawai T, Akira S (2011) Pathogen recognition by the innate immune system. Int Rev Immunol 30:16–34
48. Kumar KGS, Barriere H, Carbone CJ, Liu JH, Swaminathan G, Xu P, Li Y, Baker DP, Peng JM, Lukacs GL et al (2007) Site-specific ubiquitination exposes a linear motif to promote interferon-alpha receptor endocytosis. J Cell Biol 179:935–950
49. Kumar KGS, Tang W, Ravindranath AK, Clark WA, Croze E, Fuchs SY (2003) SCFHOS ubiquitin ligase mediates the ligand-induced down-regulation of the interferon-alpha receptor. EMBO J 22:5480–5490
50. Kurt R, Chandra PK, Aboulnasr F, Panigrahi R, Ferraris P, Aydin Y, Reiss K, Wu T, Balart LA, Dash S (2015) Chaperone-mediated autophagy targets IFNAR1 for lysosomal degradation in free fatty acid treated HCV cell culture. PLoS One 10:21
51. Lazear HM, Schoggins JW, Diamond MS (2019) Shared and distinct functions of type I and type III interferons. Immunity 50:907–923
52. Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A (2007) Autophagy-dependent viral recognition by plasmacytoid dendritic cells. Science 315:1398–1401
53. Lee NR, Ban J, Lee NJ, Yi CM, Choi JY, Kim H, Lee JK, Seong J, Cho NH, Jung JU et al (2018) Activation of RIG-I-mediated antiviral signaling triggers autophagy through the MAVS-TRAF6-Beclin-1 signaling axis. Front Immunol 9:14
54. Lei Y, Wen HT, Yu YB, Taxman DJ, Zhang L, Widman DG, Swanson KV, Wen KW, Damania B, Moore CB et al (2012) The mitochondrial proteins NLRX1 and TUFM form a complex that regulates type I interferon and autophagy. Immunity 36:933–946
55. Levine B, Kroemer G (2019) Biological functions of autophagy genes: a disease perspective. Cell 176:11–42
56. Li DD, Wang L, Deng R, Tang J, Shen Y, Guo JF, Wang Y, Xia LP, Feng GK, Liu QQ et al (2009) The pivotal role of c-Jun NH2-terminal kinase-mediated Beclin 1 expression during anticancer agents-induced autophagy in cancer cells. Oncogene 28:886–898
57. Li YB, Zhu HY, Zeng X, Fan JJ, Qian XL, Wang SF, Wang ZY, Sun Y, Wang XD, Wang WW et al (2013) Suppression of autophagy enhanced growth inhibition and apoptosis of interferon-β in human glioma cells. Mol Neurobiol 47:1000–1010
58. Li YZ, Sassano A, Majchrzak B, Deb DK, Levy DE, Gaestel M, Nebreda AR, Fish EN, Platanias LC (2004) Role of p38α map kinase in type I interferon signaling. J Biol Chem 279:970–979
59. Liang QM, Seo GJ, Choi YJ, Kwak MJ, Ge JN, Rodgers MA, Shi MD, Leslie BJ, Hopfner KP, Ha T et al (2014) Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. Cell Host Microbe 15:228–238
60. Lin M, Zhao ZY, Yang ZF, Meng QC, Tan P, Xie WH, Qin YF, Wang RF, Cui J (2016) USP38 inhibits type I interferon signaling by editing TBK1 ubiquitination through NLRP4 signalosome. Mol Cell 64:267–281
61. Liu BY, Zhang M, Chu HL, Zhang HH, Wu HF, Song GH, Wang P, Zhao K, Hou JX, Wang X et al (2017) The ubiquitin E3 ligase TRIM31 promotes aggregation and activation of the signaling adaptor MAVS through Lys63-linked polyubiquitination. Nat Immunol 18:214–224

62. Liu J, Qian C, Cao XT (2016) Post-translational modification control of innate immunity. Immunity 45:15–30
63. Liu D, Wu H, Wang C, Li Y, Tian H, Siraj S, Sehgal SA, Wang X, Wang J, Shang Y et al (2019) STING directly activates autophagy to tune the innate immune response. Cell Death Differ 26:1735–1749
64. Liu YY, Wang N, Zhang SK, Liang QW (2018). Autophagy protects bone marrow mesenchymal stem cells from palmitate-induced apoptosis through the ROS-JNK/p38 MAPK signaling pathways. Mol Med Rep 18:1485–1494
65. Lou J, Wang YL, Zheng XM, Qiu WQ (2018) TRIM22 regulates macrophage autophagy and enhances Mycobacterium tuberculosis clearance by targeting the nuclear factor-multiplicity κB/beclin 1 pathway. J Cell Biochem 119:8971–8980
66. Madjo U, Leymarie O, Fremont S, Kuster A, Nehlich M, Gallois-Montbrun S, Janvier K, Berlioz-Torrent C (2016) LC3C contributes to Vpu-mediated antagonism of BST2/Tetherin restriction on HIV-1 release through a non-canonical autophagy pathway. Cell Reports 17:2221–2233
67. Martin PK, Marchiando A, Xu RL, Rudensky E, Yeung F, Schuster SL, Kernbauer E, Cadwell K (2018) Autophagy proteins suppress protective type I interferon signalling in response to the murine gut microbiota. Nat Microbiol 3:1131–1141
68. Mesev EV, LeDesma RA, Ploss A (2019) Decoding type I and III interferon signalling during viral infection. Nat Microbiol 4:914–924
69. Nakashima H, Nguyen T, Goins WF, Chiocca EA (2015) Interferon-stimulated gene 15 (ISG15) and ISG15-linked proteins can associate with members of the selective autophagic process, histone deacetylase 6 (HDAC6) and SQSTM1/p62. J Biol Chem 290:1485–1495
70. Orvedahl A, Sumpter R, Xiao GH, Ng A, Zou ZJ, Tang Y, Narimatsu M, Gilpin C, Sun QH, Roth M et al (2011) Image-based genome-wide siRNA screen identifies selective autophagy factors. Nature 480:113–117
71. Prabakaran T, Bodda C, Krapp C, Zhang BC, Christensen MH, Sun CL, Reinert L, Cai YJ, Jensen SB, Skouboe MK et al (2018) Attenuation of cGAS-STING signaling is mediated by a p62/SQSTM1-dependent autophagy pathway activated by TBK1. EMBO J 37:17
72. Puissant A, Robert G, Fenouille N, Luciano F, Cassuto JP, Raynaud S, Auberger P (2010) Resveratrol promotes autophagic cell death in chronic myelogenous leukemia cells via JNK-mediated p62/SQSTM1 expression and AMPK activation. Can Res 70:1042–1052
73. Saitoh T, Fujita N, Hayashi T, Takahara K, Satoh T, Lee H, Matsunaga K, Kageyama S, Omori H, Noda T et al (2009) Atg9a controls dsDNA-driven dynamic translocation of STING and the innate immune response. Proc Natl Acad Sci USA 106:20842–20846
74. Samie M, Lim JH, Verschueren E, Baughman JM, Peng I, Wong A, Kwon YS, Senbabaoglu Y, Hackney JA, Keir M et al (2018) Selective autophagy of the adaptor TRIF regulates innate inflammatory signaling. Nat Immunol 19:246–254
75. Schmeisser H, Bekisz J, Zoon KC (2014) New function of type I IFN: induction of autophagy. J Interferon Cytokine Res 34:71–78
76. Schmeisser H, Fey SB, Horowitz J, Fischer ER, Balinsky CA, Miyake K, Bekisz J, Snow AL, Zoon KC (2013) Type I interferons induce autophagy in certain human cancer cell lines. Autophagy 9:683–696
77. Siddiqui MA, Mukherjee S, Manivannan P, Malathi K (2015) RNase L cleavage products promote switch from autophagy to apoptosis by caspase-mediated cleavage of Beclin-1. Int J Mol Sci 16:17611–17636
78. Sil P, Muse G, Martinez J (2018) A ravenous defense: canonical and non-canonical autophagy in immunity. Curr Opin Immunol 50:21–31
79. Snell LM, McGaha TL, Brooks DG (2017) Type I interferon in chronic virus infection and cancer. Trends Immunol 38:542–557
80. Song J, Hu YJ, Li JQ, Zheng HW, Wang JJ, Guo L, Shi H, Liu LD (2018) Suppression of the toll-like receptor 7-dependent type I interferon production pathway by autophagy resulting from enterovirus 71 and coxsackievirus A16 infections facilitates their replication. Arch Virol 163:135–144

81. Sparrer KMJ, Gableske S, Zurenski MA, Parker ZM, Full F, Baumgart GJ, Kato J, Pacheco-Rodriguez G, Liang CY, Pornillos O et al (2017) TRIM23 mediates virus-induced autophagy via activation of TBK1. Nat Microbiol 2:1543–1557
82. Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J (2004) The cytoplasmic body component TRIM5 alpha restricts HIV-1 infection in old world monkeys. Nature 427:848–853
83. Subramanian G, Kuzmanovic T, Zhang Y, Peter CB, Veleeparambil M, Chakravarti R, Sen GC, Chattopadhyay S (2018) A new mechanism of interferon's antiviral action: induction of autophagy, essential for paramyxovirus replication, is inhibited by the interferon stimulated gene, TDRD7. PLoS Pathog 14:25
84. Sun JR, Desai MM, Soong L, Ou JHJ (2011) IFN α/β and autophagy: tug-of-war between HCV and the host. Autophagy 7:1394–1396
85. Tal MC, Sasai M, Lee HK, Yordy B, Shadel GS, Iwasaki A (2009) Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. Proc Natl Acad Sci USA 106:2770–2775
86. Talloczy Z, Jiang WX, Virgin HW, Leib DA, Scheuner D, Kaufman RJ, Eskelinen EL, Levine B (2002) Regulation of starvation- and virus-induced autophagy by the eIF2 alpha kinase signaling pathway. Proc Natl Acad Sci USA 99:190–195
87. Talloczy Z, Virgin HW, Levine B (2006) PKR-dependent autophagic degradation of herpes simplex virus type 1. Autophagy 2:24–29
88. Wei YJ, Sinha S, Levine B (2008) Dual role of JNK1-mediated phosphorylation of Bcl-2 in autophagy and apoptosis regulation. Autophagy 4:949–951
89. Winkler R, Gillis E, Lasman L, Safra M, Geula S, Soyris C, Nachshon A, Tai-Schmiedel J, Friedman N, Le-Trilling VTK et al (2019) m^6A modification controls the innate immune response to infection by targeting type I interferons. Nat Immunol 20:173–182
90. Xia C, Anderson P, Hahm B (2018) Viral dedication to vigorous destruction of interferon receptors. Virology 522:19–26
91. Xia P, Ye BQ, Wang S, Zhu XX, Du Y, Xiong Z, Tian Y, Fan ZS (2016) Glutamylation of the DNA sensor cGAS regulates its binding and synthase activity in antiviral immunity. Nat Immunol 17:369–378
92. Xian HF, Yang S, Jin SH, Zhang YX, Cui J (2019) LRRC59 modulates type I interferon signaling by restraining the SQSTM1/p62-mediated autophagic degradation of pattern recognition receptor DDX58/RIG-I. Autophagy 1–11
93. Xu DC, Zhang T, Xiao J, Zhu KZ, Wei R, Wu ZM, Meng HY, Li Y, Yuan JY (2015) Modification of BECN1 by ISG15 plays a crucial role in autophagy regulation by type I IFN/interferon. Autophagy 11:617–628
94. Yang Q, Liu TT, Lin H, Zhang M, Wei J, Luo WW, Hu YH, Zhong B, Hu MM, Shu HB (2017) TRIM32-TAX1BP1-dependent selective autophagic degradation of TRIF negatively regulates TLR3/4-mediated innate immune responses. PLoS Pathog 13:21
95. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T (2004) The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol 5:730–737
96. Zhang Y, Wang X, Zhang X, Wang J, Ma Y, Zhang L, Cao X (2019) RNA-binding protein YTHDF3 suppresses interferon-dependent antiviral responses by promoting FOXO3 translation. Proc Natl Acad Sci USA 116:976–981
97. Zhao YY, Sun XF, Nie XL, Sun LW, Tang TS, Chen DH, Sun QM (2012) COX5B regulates MAVS-mediated antiviral signaling through interaction with ATG5 and repressing ROS production. PLoS Pathog 8:16
98. Zhong ZY, Sanchez-Lopez E, Karin M (2016) Autophagy, inflammation, and immunity: a troika governing cancer and its treatment. Cell 166:288–298
99. Zhou DJ, Kang KH, Spector SA (2012) Production of interferon α by human immunodeficiency virus type 1 in human plasmacytoid dendritic cells is dependent on induction of autophagy. J Infect Dis 205:1258–1267

100. Zhou YL, He CX, Wang L, Ge BX (2017) Post-translational regulation of antiviral innate signaling. Eur J Immunol 47:1414–1426
101. Zhou YY, Li Y, Jiang WQ, Zhou LF (2015) MAPK/JNK signalling: a potential autophagy regulation pathway. Biosci Rep 35:10
102. Zhu S, Cao LZ, Yu Y, Yang LC, Yang MH, Liu K, Huang J, Kang R, Livesey KM, Tang DL (2013) Inhibiting autophagy potentiates the anticancer activity of IFN1@/IFN α in chronic myeloid leukemia cells. Autophagy 9:317–327
103. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G (2015) Type I interferons in anticancer immunity. Nat Rev Immunol 15:405–414
104. Zou J, Li WJ, Misra A, Yue F, Song K, Chen Q, Guo GH, Yi JL, Kimata JT, Liu LY (2015) The viral restriction factor Tetherin prevents leucine-rich pentatricopeptide repeat-containing protein (LRPPRC) from association with Beclin 1 and B-cell CLL/lymphoma 2 (Bcl-2) and enhances autophagy and mitophagy. J Biol Chem 290:7269–7279

Chapter 9
Selective Autophagy Regulates Innate Immunity Through Cargo Receptor Network

Yaoxing Wu and Jun Cui

Abstract Autophagy, an evolutionarily conserved cargo degradation process, is responsible to remove superfluous and unwanted cytoplasmic materials and maintain cellular homeostasis. Autophagy can be highly selective and target specific cargoes by utilizing multiple cargo receptors, which bind both ubiquitinated cargoes and autophagosomes. Mounting evidence has revealed the deep involvement of selective autophagy in innate immunity upon pathogen invasion, including eliminating microbial pathogens, initiating the anti-microbe responses, and inhibiting excessive immune responses. Given the importance of selective autophagy in innate immunity, how cargo receptors deliver pathogens and intracellular host constitutes to autophagosomes during infection remains to be elucidated. In this review, we summarize current evidence for the regulation of innate immunity by selective autophagy and try to elucidate the mechanisms employed by cargo receptor network in mediating diverse innate immune responses.

Keywords Selective autophagy · Cargo receptor · Ubiquitination · Immune response

9.1 Introduction

Macroautophagy, thereafter referred to as autophagy, is an evolutionarily conserved cargo degradation process, which maintains cellular homeostasis and provides energy substrates when facing various stress factors, including starvation, pathogenic infection, proteotoxic, and oxidative stress [41, 57]. Autophagy initiates with the sequestration of cellular substrates into a double-membrane structure termed as phagophore. The immature phagophore grows to envelop the engulfed contents and form an autophagosome. Autophagosome subsequently becomes its mature form by fusing

Y. Wu · J. Cui (✉)
MOE Key Laboratory of Gene Function and Regulation, State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou 510006, Guangdong, China
e-mail: cuij5@mail.sysu.edu.cn

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_9

with endosome or lysosome. After fusion, the inner membrane and the cytosolic contents undergoes degradation by lysosomal acid hydrolases and the resultant degradation products can be reused for nutrient recycling [8, 57].

Autophagy used to be regard as a nonselective bulk degradation system for amino acid and nutrients recycle [10, 35], however, accumulating evidence reveal that autophagy can be highly selective and target specific substrates for degradation [38]. Selectivity of autophagy is mediated by specialized autophagic adaptors, termed as cargo receptors [38], which physically bridge cellular cytosolic materials and autophagy components [90]. In selective autophagy, cargoes usually undergo ubiquitination and the ubiquitinated cargoes are recognized by cargo receptors for subsequent delivery to autophagosomes for degradation. Due to the diversity of the potential cargoes recognized by cargo receptors, selective autophagy is involved in various cellular processes, including balancing cellular homeostasis, regulating inflammation, removing invading pathogens, and eliminating damaged organelles [90]. Mounting evidence has suggested the involvement of selective autophagy during pathogenic microbial infection, including the elimination of microbial pathogens, generation of anti-pathogen factors and materials, microbial antigen presentation, initiation of anti-microbe responses, and inhibition of excessive immune responses [8, 82]. During the process of selective autophagy, a great deal of substrates, including invading pathogens, cytosolic microbial components, damaged organelles, and key molecules in type I interferon and inflammatory signaling pathways can be targeted by cargo receptors to autophagosomes [82]. Poly-ubiquitin chains serve as the most common signals for the recognition of cargoes destined for autophagy, while a part of cargoes are sensed by selective autophagic receptors through ubiquitin-independent mechanism, such as employing protein–protein association domains or utilizing ubiquitin-like, sugar- or lipid-based signals [35, 40]. However, the precise mechanisms employed by a limited amount of cargo receptors in targeting broad and diverse kinds of intracellular cargoes remain to be elucidated. Specifically, the question about how cargoes are tagged and how the modified cargoes are discriminated by cargo receptors when multiple potential targets exist in the cytosol during infection remains unexplored. One convincing explanation is that there are a number of host regulators, which serve as secondary autophagic receptors or co-receptors for the post-translational modification or protein–protein interaction of particular cargoes, which assists the recognition by cargo receptors. Diverse secondary receptors/co-receptors work synergistically to support a network for selective cargo receptors in targeting different kinds of substrates, which leads to modulate innate immune responses during pathogenic invasion. In this review, we summarize available evidence for selective autophagy in innate immunity and try to elucidate the mechanisms employed by selective autophagy in mediating diverse innate immune responses from three different levels: autophagic cargoes, cargo receptors, and recognition signals.

9.2 Selective Autophagy-Dependent Cargo Recognition and Degradation in Innate Immunity

9.2.1 Selective Autophagy Affects Microbial Pathogen Invasion

Invading pathogens are one of the specific targets for selective autophagic degradation. Autophagy machinery is used as a defensive system against microbial infection, which is referred to as xenophagy [11, 44]. Xenophagy targets and eliminates intracellular invading pathogens, including viruses, bacteria, and fungi, which serves as a critical part in innate immune responses [20]. Several pathogenic bacteria, including *Mycobacterium tuberculosis*, *Salmonella enterica*, and *Listeria monocytogenes* are shown to be involved in autophagic degradation [40]. One of the well-studied examples of autophagic degradation targeting invading bacteria is *Salmonella enterica* serovar Typhimurium (*S. typhimurium*). During invasion, *S. typhimurium* resides and replicates in the intracellular Salmonella-containing vacuoles (SCVs), but it can also leak out to the cytosol and be targeted by host ubiquitin conjugation system. The ubiquitinated *S. typhimurium* is subsequently recognized by autophagy cargo receptors and delivered to autophagy [31]. At least three different autophagy receptors, p62 [106], NDP52 [94], and OPTN [101] participate in the reorganization of ubiquitinated *S. typhimurium* by autophagosome in nonredundant manners. Individual silencing of these three cargo receptors is sufficient to enhance bacterial replication during infection in *Drosophila melanogaster*, *Caenorhabditis elegans*, mice, and human cell lines [42, 101]. Research from Vojo Deretic's group shows that p62 can also bring specific cytosolic proteins of *M. tuberculosis* to autolysosomes where they are processed from bacterial precursors into novel antimicrobial peptides, which reveals a different role of p62 in autophagic pathogen defense [73]. In addition to ubiquitin-dependent pathogen recognition, damaged vesicle can also be targeted by host protein galectin 8, which recruits NDP52-dependent autophagosome and promotes antibacterial autophagy [95].

During viral infection, selective autophagy also participates in the recognition and degradation of viral components and viral particles. Accumulating researches have shown the indispensable roles of selective autophagy during host innate defenses and tried to reveal the detail mechanism by autophagy in viral removal. Several studies reveal that autophagy can protect Sindbis virus (SINV)-mediated encephalitis [68]. p62 is shown to target SINV capsid protein and deliver viral capsid to autophagosome [68]. Host E3-ubiquin ligase, SMURF1, and host protein Fanconi anemia group C protein (FANCC) are necessary for the association and colocalization between p62 and SINV capsid protein [69, 92]. Selective autophagy can also restrict the infection of picornaviruses, such as poliovirus [8]. When the viral particle of poliovirus ruptures the endosomal membrane and releases its genome to cytosol, the exposure of β-galactosidases will be targeted by galectin 8, which results in the detection of damaged endosomes and autophagic degradation of viral RNA genome [89]. Hepatitis C

virus (HCV) has been demonstrated to induce autophagy for its own benefit, however, it has been demonstrated that host protein SHISA5 (also named SCOTIN) restricts HCV replication by associating with HCV nonstructural protein 5A (NS5A) and promoting its autophagic degradation [1, 36]. Histone deacetylase 6 (HDAC6) cooperates with HIV-1 restriction factor APOBEC3G and facilitates autophagic degradation of the virion infectivity factor Vif [53, 98]. p62 can selectively target the host trans-activator Tat, which is kidnapped by HIV for viral transcription, for autophagic degradation [78].

Collectively, selective autophagy not only directly promotes the autophagic degradation of invading pathogens, pathogens components, and host factors required for pathogens life cycle, but also facilitates microbial antigen presentation and the generation of antimicrobial peptides [8], and therefore initiating autophagy-mediated immune responses and defending against the invasion of multiple pathogens.

9.2.2 Selective Autophagy Regulates Inflammatory Cellular Process

During bacterial infection, several Toll-like receptors (TLRs) recognize pathogen-associated molecular patterns (PAMPs) and recruit their adaptor proteins, including MyD88 and IRAK family [34, 58]. Subsequently, IRAKs are activated to promote the oligomerization and ubiquitination of TRAF family proteins. Ubiquitinated TRAFs serve as a platform to recruit and activate TAK1 and IκB kinase (IKK) complex [67]. IKK complex then phosphorylates IκB and facilitates its degradation, which leads to the translocation of NF-κB to nucleus and subsequent production of pro-inflammatory cytokines [23]. Extensive studies have revealed the involvement of autophagy in regulating the activation of NF-κB in a selective manner. Early studies on p62 reveal its involvement in activation of NF-κB. Speckles of p62 can promote the oligomerization and ubiquitination of TRAF6 and the subsequent activation of NF-κB [59]. p62 can be induced by lipopolysaccharide (LPS) and promote the aggregation of MyD88. However, several recent studies show the negative regulatory roles of selective autophagy in NF-κB pathway [31]. Upon *S. typhimurium* infection, TAX1BP1 can target TRIF, a critical adaptor protein for TLR3/4, which results in its autophagic degradation and reduction of NF-κB signaling [103]. OPTN not only binds to RIPK1 and competes with NEMO, but also cooperates with CYLD to deubiquitinate NEMO, which leads to the inhibition of NF-κB activation [62, 108]. p62 can target activated IKKβ and lead to its degradation via selective autophagy, therefore reducing the activation of NF-κB [47].

Upon activation, several NOD-like receptors (NLRs) form large molecular platforms, called inflammasomes. After components assembly, inflammasomes can trigger caspase-1 to process the maturation and secretion of IL-1β and IL-18 [54]. Mature IL-1β and IL-18 can be sensed by different receptors and activate a series of inflammatory responses [13]. Multiple reports have revealed the involvement of

selective autophagy in suppression of inflammasome activation [12]. For instance, macrophage-specific deletion of p62 leads to the enhancement of damaged mitochondria and excessive inflammasome-related inflammation [107]. In addition to removing damaged mitochondria, p62 is also shown to directly regulate inflammasome [12]. During the activation of AIM2 inflammasome, ASC undergoes poly-ubiquitination and is subsequently targeted by p62, which results in the delivery of ASC to autophagosome and the inhibition of inflammasome [85]. Host E3 ubiquitin ligase, TRIM20 can target pro-caspase-1, NLRP1, and NLRP3, to promote their autophagic degradation [37]. TRIM11 serves as a receptor of AIM2 through its auto-poly-ubiquitination and facilities p62-dependent selective autophagic degradation of AIM2 [48].

In general, selective autophagy mainly serves as an anti-inflammatory factor and prevents excessive or overexuberant inflammation and inflammatory responses triggered by diverse agents of endogenous or infectious origin.

9.2.3 Selective Autophagy Regulates Antiviral Type I Interferon Responses

Upon the invasion of pathogenic microbe, RLRs, including RIG-I, MDA5, and LGP2 sense viral RNA in the cytosol, while a number of viral DNA sensors including DDX41, IFI16, and cGAS are responsible for recognizing intracellular viral DNA. Upon viral nucleic acid recognition, these sensors mediate the recruitment of their adaptor proteins, MAVS and STING. MAVS and STING then activate the kinase TBK1 to promote the phosphorylation of IRF3, which results in the transcriptional activation of type I interferon (IFN) signaling. The production of type I IFN can be released into the extracellular environment and induce a large spectrum of IFN-stimulated genes (ISGs) via the JAK-STAT pathway [80].

Recently, several studies have revealed the involvement of selective autophagy in regulating antiviral type I IFN responses. During RNA viral infection, p62 specifically targets to ISG15-associated RIG-I, therefore promoting the degradation of RIG-I in a selective manner [14]. In the resting state, cGAS undergoes K48-linked poly-ubiquitination at its lysine 414 site. Ubiquitinated cGAS is recognized by p62 and delivered to autophagosome for degradation [7]. Selective autophagy also participates in regulation of the adaptor proteins in IFN signaling pathway. NDP52 recognizes virus-triggered K27-linked ubiquitination on MAVS, and therefore leading to selective degradation of MAVS to suppress excessive activation of antiviral immune response [30]. During DNA viral infection, p62 can also target ubiquitinated STING and mediate its autophagic degradation in a TBK1-dependent manner. After the activation of cGAS-STING signaling, TBK1 is activated to phosphorylate p62, which stimulates the traffic of STING to autophagy-associated vesicles and degradation [74]. The transcription factor of IFN signal, IRF3 is also reported to go through selective degradation. TRIM21 can specifically target activated IRF3 and deliver the

IRF3 dimers to autophagosome, thus attenuating type I IFN signaling [37]. Taken together, selective autophagy functions at different levels of type I IFN responses by degrading receptors, adaptor proteins and transcription factors.

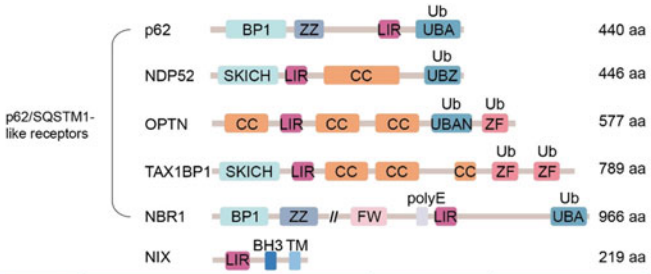
9.3 Selective Autophagy Mediated by Diverse Cargo Receptor Proteins During Infection

A simplified model to illustrate cargo receptor-mediated selective autophagy can be proposed: First, the cargoes need to recruit their molecular chaperones, such as E3 ubiquitin ligases, after receiving their degradation signals. Second, the autophagy cargoes get post-translational modification by their chaperones and the modified cargoes are recognized by diverse cargo receptors. The ubiquitin chains are the most common targets for the cargo receptors. Lastly, the substrates are trafficked to the autophagosomes for degradation by cargo receptors. During the selective autophagic degradation, autophagic cargo receptors function as a bridge to connect the autophagy substrates and the autophagosomes [90]. In this case, three domains of cargo receptors are always needed for their integrity—LC3-interacting region (LIR) domain (which is used to interact with the autophagosome decorated by LC3), dimerization domain and cargo recognizing domain (which is usually used to bind to the ubiquitin chains) [82]. Given the importance of cargo receptors and cargo receptors-mediated autophagic degradation during infection, we will discuss several key cargo receptors in the following sections.

9.3.1 p62

p62 is the first identified and studied mammalian autophagy cargo receptor. Since initially identified as a autophagy adaptor in 2005, p62 has been extensively studied about its binding partners, cellular localizations, domain structures as well as cellar functions [38]. p62 has multiple functional domains, including Phox and Bhem 1 (PB1) domain, a zinc finger domain, a TRAF6-binding (TB) motif, LIR domain, a KEAP-interacting region, and ubiquitin-associated (UBA) domain (Fig. 9.1) [79]. p62-mediated selective autophagy is reported in removal of invading pathogenic microbe [82]. Several bacterial species such as *S. typhimurium, Shigella flexneri* and *M. tuberculosis* can be selectively targeted by p62 and trafficked to nascent LC3-decorated membranes for degradation [18, 60, 106]. p62 is also reported to participate in removing the viral components during viral infection. For example, p62 targets SINV capsid and delivers capsid protein to the autophagosome for degradation [68].

In addition to mediating the elimination of invading pathogen, p62 is also involved in controlling pathogen-induced inflammation. Through interacting with TRAF6



Target Cargo	p62	NDP52	OPTN	TAX1BP1	other receptors
Bacteria or Bacterial components	Multiple bacterial species such as *S. typhimurium, S. flexneri and M. tuberculosis.*	Various pathogens including *Listeria, S.typhimurium, M. tuberculosis,* S. flexneri, etc. Galectin 8 signal on damaged *Salmonella*-containing vacuoles	Ubiquitinated *S. enterica* and *S. typhimurium*	Ubiquitinated *S. typhimurium*	NBR1 serves as a co-factor of p62 to targets bacteria to autophagosome.
Virus or Viral components	SINV capsid, host trans-activator Tat kidnapped by HIV.	Galectin 8 siganling binding to exposured β-galactosidases on endosomal membrane ruptured by poliovirus and polioviral RNA genome		TAX1BP1 is hijacked by MeV to promote the maturation of autophagosomes.	NBR1 targets the capsid proteins of CaMV in plant. SCOTIN targets HCV NS5A to autophagy. HDAC6 facilitates autophagic degradation of the HIV virion infectivity factor Vif. TRIM5α targets capsid protein of HIV to autophagosome
Acivactiors of Inflammatory Response	p62 promotes TRAF6 oligomerization. p62 transports IKKβ and p65 to autophagy. p62 targets AIM2 and ASC1 to autophagy.	NDP52 tatgets TLRs downstream adaptors to autophagy to reduce excessive NF-κB signaling	OPTN competes with NEMO for binding to RIPK1. OPTN associates with CYLD and deubiquitinases NEMO and RIPK1	TAX1BP1 assists A20, RNF11 and Itch to control NF-κB pathway. TAX1BP1 is recruited by TRIM32 to mediate the autophagic degradation of TRIF.	Tollip reduces TLR-sigaling by interacting with several TLRs and reducing autophosphorylation of IRAK1.
Key Molecules in Type I IFN Signaling	ISG15-decorated RIG-I and K48-linked ubiquitinated cGAS.	K27-linked ubiquitinated MAVS.	OPTN promotes the phosphorylation of TBK1 and IRF3 after stimulation by its TBK-1-binding domain.	TAX1BP1 translocates to mitochondria and promotes MAVS degradation. TAX1BP1 cooperates with A20 to remove K63-linked ubiquitination of TBK1 and IKKi.	NIX mediates the autophagic degradation of full-legnth MAVS through mitophagy.

Fig. 9.1 Domain structure of autophagy cargo receptors and their functions in innate immunity

using the TRAF6-binding motif, p62 is shown to participate in TRAF6 oligomerization and promote the K63-linked ubiquitination of NEMO [109]. Moreover, p62 can also capture A20, an NF-κB inhibitor, and sequester it to autophagosome, thus enhancing NF-κB signaling [32]. During the activation of NF-κB pathway, p62 is up-regulated and phosphorylated by activated TAK1 and TBK1, which creates a positive feedback loop [22, 46]. On the contrary, recent studies reveal that p62 is also involved in restricting excessive inflammatory responses by selectively promoting the autophagic degradation of key molecules in NF-κB pathway. F-box protein SKP2 can promote the interaction between p62 and IKKβ, which leads to the p62-dependent autophagic degradation of IKKβ [47]. LRRC25 interacts with the Rel homologous domain (RHD) of p65/RelA and promotes p65 recognition by p62, which results in the p62-dependent autophagic degradation of p65 [16, 105]. p62 also affects the assembly and the activation of inflammasomes, and therefore regulating inflammatory response. The macrophage-specific loss of p62 results in enhancement of damaged mitochondria and inflammasome-dependent production of IL-1β [107].

In addition to removing damaged mitochondria, p62 can also directly mediate the autophagic degradation of the components of inflammasomes. Upon the activation of AIM2 inflammasome, AIM2 molecular partner TRIM11 undergoes ubiquitination and is recognized by p62. Subsequently, p62 transports AIM2 and ASC to autophagosomes, which leads to the inhibition of inflammasomes activation [48, 85].

p62 is also reported to be involved in the regulation of viral-triggered type I IFN immune responses. During RNA viral infection, cytosol RNA sensor RIG-I binds to free ISG15, which is a recognition signal to recruit p62 and mediate the autophagic degradation of RIG-I with the help of LRRC25 [14]. Upon the activation of cGAS-STING signaling, STING is ubiquitinated and targeted by p62 to autophagosome [74]. Collectively, involvement of p62-mediated selective autophagy in host microbial defense affects various intracellular signaling pathways, which generates optimal anti-microorganism innate immune responses.

9.3.2 NDP52

NDP52, also known as CALCOCO2, was initially identified as another ubiquitin-associated and LC3-binding cargo receptor from Felix Randow's group in 2009 (Fig. 9.1). NDP52 was shown to be associated with ubiquitin-labeled *S. typhimurium* and restricted the replication of this pathogen [94]. Subsequent studies reveal that NDP52 can target various pathogens including *Shigella*, *Listeria*, *M. tuberculosis* and *Streptococcus pyogenes* to autophagosomes for their selective degradation [52, 60, 82, 100], which confirms the indispensable involvement of NDP52 in xenophagy. Although xenophagy is reported to be mediated by several different cargo receptors, it seems that the recruitment of different adaptors is independent of each other and the mechanism in targeting diverse microbes is different. For instance, p62 and NDP52 can deliver *Shigella* to autophagosomes dependently of actin-septin, while they can target *Listeria* to autophagosomes in an actin-septin-independent manner [60]. Galectins is another recognition signal for NDP52. NDP52 targets Galectin 8 and promotes autophagic degradation of *Salmonella* [95]. Several host proteins are also shown to facilitate the NDP52-dependent xenophagy. For instance, Parkin, the E3 ligase of NDP52, recruits NDP52 to *M. tuberculosis* to autophagosomes for degradation in a TBK1 phosphorylation-dependent manner [25]. GFP-bound Rab35 accumulates on the bacteria-containing endosomes and recruits NDP52 to promote xenophagy of Group A *Streptococcus* (GAS) [56].

NDP52 also participates in reducing inflammation and IFN signaling. NDP52 is capable of reducing excessive NF-κB signaling by targeting TLRs downstream adaptors for autophagic degradation [15, 96]. V248A mutants of NDP52, which affects its binding capacity of ubiquitinated cargo, results in high level of NF-κB activity, thereby causing more inflammatory responses in Crohn disease (CD) patients [15, 96]. NDP52 is also involved in the regulation of virus-triggered induction of IFN signaling. Upon viral infection, MAVS undergoes K27-linked ubiquitination by MARCH8. Ubiquitinated MAVS is subsequently targeted by NDP52 and goes through autophagic degradation, thus restricting excessive activation of type I IFN signaling [30].

9.3.3 OPTN

Another cargo receptor involved in xenophagy is optineurin (OPTN). Dikic group demonstrates the role of OPTN in restricting the growth of *S. enterica* during infection [101]. During this process, TBK1, which serves as a key factor in OPTN-mediated xenophagy and mitophagy, phosphorylates OPTN at S177, S473, and S513, which promotes recruitment of OPTN to ubiquitinated *S. enterica* and damaged mitochondria and enhances the association of OPTN and LC3 [76, 101]. Besides OPTN, p62, and NDP52 are also shown to be recruited to *S. typhimurium* and restrict bacterial replication in a nonredundant manner [94, 106]. One of the possible reasons is that their UBD domains can recruit diverse E3 ligases to mediate different types of ubiquitin chains of *S. typhimurium* [81].

OPTN also modulates inflammation by negatively regulating NF-κB pathway. The C-terminal of OPTN contains UBD, which shows striking homology to the UBD of NEMO [108]. UBDs facilitate the binding of OPTN and NEMO to K63- and linear (M1-linked) ubiquitin chains, but not the K48-linked ubiquitin chains [19]. Due to the similar UBDs, OPTN competes with NEMO for binding to RIPK1 upon TNFα stimulation, and thus dampening downstream activation of NF-κB signaling [91, 108]. Structural studies reveal that the capability of OPTN for linear ubiquitination binding is also critical for suppression of NF-κB activation [64]. OPTN can also associate with CYLD and promote the deubiquitination of NEMO and RIPK1, which further inhibits NF-κB activation [62]. Moreover, OPTN also binds to interleukin-1 (IL-1) receptor-associated kinase 1 (IRAK-1) and prevents the ubiquitination of TRAF6, thereby inhibiting the IL-1β-triggered NF-κB signaling [93]. On the contrary, in vivo studies show that OPTN mutants or OPTN deficiency does not affect NF-κB activation upon TNF or TLR stimulation, which proposes a controversial role of OPTN in NF-κB pathway [61, 87].

N-terminal of OPTN contains a TBK-1-binding domain, which indicates the potential involvement of OPTN in type I IFN pathway [55, 86]. OPTN mutants lacking UBD or TBK1-binding region or OPTN deficiency leads to the diminished phosphorylation of TBK1 and IRF3 and decreased secretion of IFN productions upon LPS and poly(I:C) treatment [19, 55, 61]. Subsequent studies show that OPTN mutants in UBD (D477 N) results in the reduced phosphorylation of TBK1 upon LPS or poly(I:C) treatment, which indicates the capability of binding to ubiquitin chains of OPTN is required for the activation of OPTN-TBK1 complex and the downstream induction of IFN signaling [2, 86].

9.3.4 TAX1BP1

The NDP52 close homolog TAX1BP1 was also shown in 2015 to associate with ubiquitinated *S. typhimurium* and participated in the bacterial removal through xenophagy [97]. In addition, TAX1BP1 is also reported to affect the viral replication [82]. Both

TAX1BP1 and NDP52 are required for the replication of Measles Virus (MeV). Although the physical interaction between cargo receptor proteins and MeV proteins is reported, the mechanism by how MeV utilizes TAX1BP1 and NDP52 to promote its replication remains unknown [71].

Several studies reveal the multiple roles of TAX1BP1 in regulating inflammation and IFN response upon infection [82]. TAX1BP1 can assist several negative regulators, including A20, RNF11, and Itch to control NF-κB pathway and inflammation upon LPS and TNF stimulation [83, 84]. TAX1BP1 is recruited by TRIM32 to mediate the autophagic degradation of TRIF, an important adaptor for TLR3/4, and inhibits TLR3/4-mediated activation of NF-κB and IFN signaling [103]. TAX1BP1 can also translocate to mitochondria and promote the degradation of MAVS, thereby inhibiting virus-triggered apoptosis [9]. TAX1BP1 cooperates with A20 to remove the K63-linked ubiquitination of TBK1 and IKKi, thus blocking virus-mediated activation of IRF3 [70]. Collectively, TAX1BP1 associates with A20, and assists A20 to restrict excessive IFN production and inflammation.

9.3.5 Involvements of Other Cargo Receptors During Infection

Besides the well-known cargo receptors described above, p62-like receptors were reported to mediate the innate immune response during infection. p62 homolog NBR1 is believed to interact with p62 via their PB1 domains and functions as a cofactor of p62 (Fig. 9.1) [39, 43]. In some cases, NBR1 can function independently and mediate viral infection. For instance, NBR1 targets the capsid proteins of cauliflower mosaic virus (CaMV) in plant and promotes their autophagic degradation in a selective way, which restricts the establishment of CaMV infection [21]. However, the independent involvement of NBR1 in mammalian during infection needs to be elaborated. Tollip is the mammalian homolog of Cue, the first identified aggrephagy receptors in yeasts [38]. Tollip was reported to have dual functions in regulating TLR-mediated immune responses [6]. On one hand, Tollip interacts with IL-1R, TLR2, and TLR4 and reduces the TLR-mediated signaling [4]. On the other hand, Tollip binds to IRAK1 and inhibits the autophosphorylation of IRAK1 [5, 45]. Interestingly, following TLR stimulation, activated and auotphosphorylated IRAK1 and IRAK4 can promote the phosphorylation of Tollip, which leads to its dissociation with IRAK1 and initiation of downstream signaling [5]. Mitochondrial protein NIX was the first identified mammalian cargo receptor for ubiquitin- and Parkin-independent mitophagy [38, 66]. NIX can mediate mitophagy by directly connecting to ATG8 family protein on autophagosome [33, 66]. NIX-mediated selective autophagy is also involved in regulating innate immune response [49, 75]. For example, full-length MAVS tends to aggregate in spontaneous condition and goes through NIX-mediated mitophagic degradation. Interestingly, N-terminally truncated forms of MAVS can stabilize full-length MAVS by inhibiting NIX-mediated mitophagy,

thereby inducing the downstream expression of type I IFN [75]. In addition, NIX can also promote the activation of NF-κB signaling in gliomas, however, the detailed mechanism requires further investigation [49].

9.4 Recognition Signals for Selective Autophagy in Innate Immunity

Ubiquitin, a small and highly conserved protein with 76 amino acids, can repeatedly be attached to the internal lysine residues of itself to form chains with various topologies and functions [29]. Modification of a substrate with diverse topological ubiquitin chains results in different cellular fates, including activation, proteasomal degradation, lysosomal degradation, and autophagic degradation, etc. [40]. During the formation of autophagosome, ubiquitin chains on the surface of cargoes provide the most common signals that can be targeted by diverse cargo receptors and lead to the autophagosome formation [40]. Besides, the ubiquitin-like modification is also involved in cargo recognition and degradation. Collectively, based on the type of cargo receptor and the cargo recognition system, selective autophagy can be divided into ubiquitin-dependent, ubiquitin-like-dependent, and ubiquitin-independent pathways [35].

9.4.1 Ubiquitin-Dependent Signals for Selective Autophagy

A majority of recent studies have revealed the fundamental roles of ubiquitination in selective autophagy [35, 40, 77]. During autophagy, autophagic cargoes are modified with ubiquitin and subsequently targeted by the ubiquitin-binding domain of the cargo receptors to transport them to autophagosome [28]. Upon pathogenic microbial invasion, ubiquitin-dependent signal serves as an indispensable role in innate immune system [26, 35]. Upon infection, it is an effective way to remove the cytosolic microbes by detecting the ubiquitinated microbes and mediating their degradation through autophagosome. Several E3 ubiquitin ligases were reported to combat microbial infection by mediating xenophagy of pathogens and recruiting autophagic adaptor proteins, including RNF166 on *S. enterica*, Smurf1, and Parkin on *M. tuberculosis* and Smurf1 on SINV [17, 24, 52, 69]. Several other E3 ligases, including LRSAM1, LUBAC, and ARIH1 are reported to directly attach ubiquitin chains to intracellular pathogens [26, 35]. LRSAM1 senses *Salmonella*, *Listeria*, autophagy-susceptible *Shigella*, and *Escherichia coli* through its LRR domain and promotes their K6- and K27-linked ubiquitination through RING domain [27]. ARIH1 and LUBAC recognize *Salmonella* and catalyze K48- and M1-linked ubiquitin chains on the outer surface of *Salmonella*, respectively [65, 72]. Upon *Salmonella* infection, LRSAM1, LUBAC, and ARIH1 work synergistically to target *Salmonella* for

ubiquitination [72]. Once *Salmonella* is ubiquitinated by these host E3 ligases, the pathogens and their ubiquitin chains are targeted by diverse cargo receptors including p62, NDP52, TAX1BP1, and OPTN to the autophagosome [35, 60, 94, 97, 101, 106]. In addition, M1- and K63-linked ubiquitin chains presented on cytosolic *Salmonella* also serves as a signaling platform to trigger the activation of NF-κB pathway, thereby promoting the secretion of pro-inflammatory cytokines and restricting the bacterial proliferation [65, 99].

In addition to directly mediating autophagic degradation of pathogens, ubiquitin modification system also participates in regulating microbe-triggered innate immune response in an autophagy-dependent manner. Cytosol DNA sensor cGAS undergoes K48-linked ubiquitination and can be recognized by p62 for autophagic degradation [7], and thus restricting the basal level of IFN induction. Upon DNA viral infection, TRIM11 binds to AIM2 and undergoes auto-ubiquitination. Cargo receptor p62 targets ubiquitinated TRIM11 and delivers AIM2-TRIM11 complex to autophagosome, thereby inhibiting AIM2 inflammasome [48]. During RNA viral infection, virus-triggered K27-linked ubiquitination of MAVS mediated by Tetherin (also known as BST2) and MARCH8 can be targeted by NDP52 for autophagic degradation, thus avoiding the excessive activation of IFN signaling [30]. Taken together, tight control of ubiquitin signal is indispensable for proper activation of host innate immune response and effective elimination of invaded intracellular pathogenic microbes [26].

9.4.2 Ubiquitin-like Signals

Ubiquitin-like (UBL) proteins FAT10 and ISG15 are induced by type I IFN, which indicates the potential involvement of these UBLs during innate immune responses [35]. In fact, FAT10 is shown to decorate cytosolic *S. Typhimurium*, which promotes the subsequent recruitment of p62 and NDP52. Although *FAT10* depletion or overexpression in cells can barely affect the bacterial replication, *Fat10* deficiency in mice leads to a higher susceptibility to *S. Typhimurium*, which suggests the involvement of FAT-10 in autophagic defense against pathogens [88]. ISG15 binds to p62 and HDAC6 and promotes the autophagic degradation of aggregated proteins [63]. Absence of intracellular ISG15 leads to the enhancement and amplification of IFN response, which is possibly due to the decrease of USP18, the negative regulator of IFN signaling [3, 104]. Recent studies reveal that LRRC25 specifically binds to ISG15-associated RIG-I and recruits p62, which leads to the autophagic degradation of RIG-I and the reduction of RIG-I-mediated type I IFN signaling [14].

9.4.3 *Ubiquitin-Independent Selective Autophagy*

In addition to ubiquitin- and UBL-dependent selective autophagy, cytosolic cargo proteins can be delivered to autophagy independent of ubiquitin. Two major mechanisms are involved in controlling selective autophagy independent of ubiquitin during pathogen microbial invasion [35].

First of all, several cargo receptors have been identified, which can directly bind their substrates to autophagy under certain circumstances without the participation of the ubiquitin system [35]. During HIV infection, IFN-inducible restriction factor TRIM5α serves as a selective autophagy receptor which targets capsid protein of HIV to autophagosome and facilitates the assembly of autophagy-activating complexes [50, 51]. Host endoplasmic reticulum (ER) transmembrane protein SCOTIN can associate with hepatitis C virus (HCV) nonstructural protein 5A (NS5A) and promote its autophagic degradation of NS5A without affecting the overall flow of autophagy [8, 36]. NIX can target full-length MAVS which aggregates spontaneously to selective autophagy, and thus avoiding spontaneous induction and accidental activation of IFN signaling [75].

Another mode of ubiquitin-independent autophagy involves sensing of diverse signals exposed to targets. Cytosol-exposed glycans is considered as the recognition signaling for antibacterial autophagy for galectin 8, a cytosolic lectin. By binding host glycans exposed to damaged *Salmonella*-containing vacuoles, galectin 8 recruits other autophagy factors such as NDP52 and delivers bacteria and damaged lysosomes for autophagic degradation [95].

Collectively, specialized autophagy receptors can regulate ubiquitin-dependent or ubiquitin-independent selective autophagy by sensing diverse recognition signals (Fig. 9.2), which suggests precise regulation of autophagy during innate immune responses.

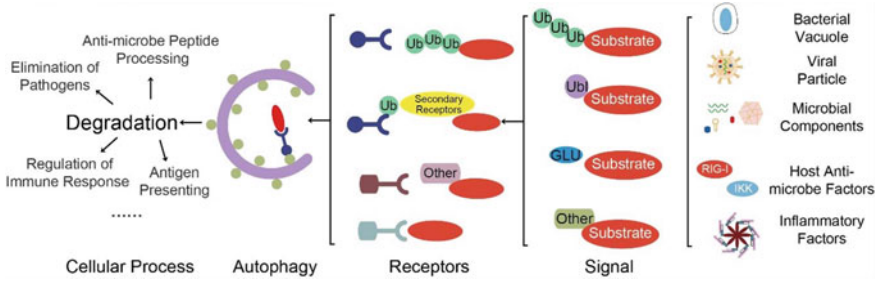


Fig. 9.2 Schematic overview of the participation of selective autophagy during pathogenic microbial invasion. Upon infection, various intracellular contents, including pathogens, host anti-microbe, and inflammatory factors become the targets for selective autophagy (Right). These autophagic cargoes will be decorated by diverse modification signal and targeted by specific cargo receptors to autophagosome (Middle). As a result, selective autophagy upon infection can lead to multiple cellular process, including removing the pathogens and avoiding excess immune responses (Left)

9.5 Regulation of Innate Immune Signaling by Manipulating Cargo Receptor Network

Accumulating evidence has revealed the involvement of selective autophagy as well as its cargo receptors in recognizing the degradation signals and mediating the degradation of a variety of substrates [35, 82]. However, the mechanisms about how diverse substrates are modified, and how specific cargo receptors target the particular substrates in the presence of multiple potential targets in the cytosol, are the major questions existing at the moment. To address these questions, we propose a secondary autophagic receptor/co-receptor models employed cargo receptors to assist their recognition of multitudinous cargoes. The secondary receptors/co-receptors are kinds of host proteins which mediate the modifications of the autophagic cargoes and promote the recognition of specific cargo receptors. Upon stimulation, the cargoes are first recruited to the secondary receptors/co-receptors, such as E3 ligases, and go through the post-translational modification with the assistance of the secondary receptors. As the secondary receptors/co-receptors generally lack LC3 interacting regions, which lead to their incapability to transport the cargoes to autophagosome. Instead, secondary receptors/co-receptors and the modification on the cargoes recruit specific cargo receptors, which results in the delivery of cargoes to autophagosome and their degradation (Fig. 9.3). The participation of various secondary receptors/co-receptors leads to the precision cargo recognition during selective autophagy and the coordination between them generates the cargo receptor network in manipulating diverse cellular processes.

During the invasion of pathogens, manipulation of cargo receptor network by secondary receptors/co-receptors also contribute to the regulation of innate immune response. The most common example is the manipulation of cargoes' ubiquitination by E3 ligases. Various E3 ligases, such as LRSAM1, LUBAC, MARCH8, and ARIH1 are shown to mediate the ubiquitin chains of the cargoes and promote the recognition of the cargo receptors [35]. On the contrary, host factors also reverse the ubiquitination process to block selective autophagy, therefore enhancing the innate immune signaling. For instance, cGAS undergoes K48-linked ubiquitination which is targeted by p62 to autophagy in the resting state. Upon HSV-1 infection, TRIM14 is up-regulated as an ISG product and recruits USP14 to cGAS for deubiquitination, therefore inhibiting the p62-mediated degradation of cGAS and enhancing anti-HSV-1 immune response [7].

Besides regulating the ubiquitin chains on the substrates, secondary receptors also mediate innate immune responses through auto-ubiquitination. Upon DNA virus infection, TRIM11 binds to AIM2 and undergoes auto-poly-ubiquitination, which is targeted by p62. TRIM11 acts as a secondary receptor to deliver AIM2 to autophagy for degradation in a p62-dependent manner, thereby inhibiting the activation of AIM2 inflammasome [48].

In addition to the ubiquitin signals, secondary receptors/co-receptors also assist cargo receptors to recognize the UBL signals. LRRC25 functions as a secondary receptor in recognizing ISG15-associated RIG-I following with the recruitment of

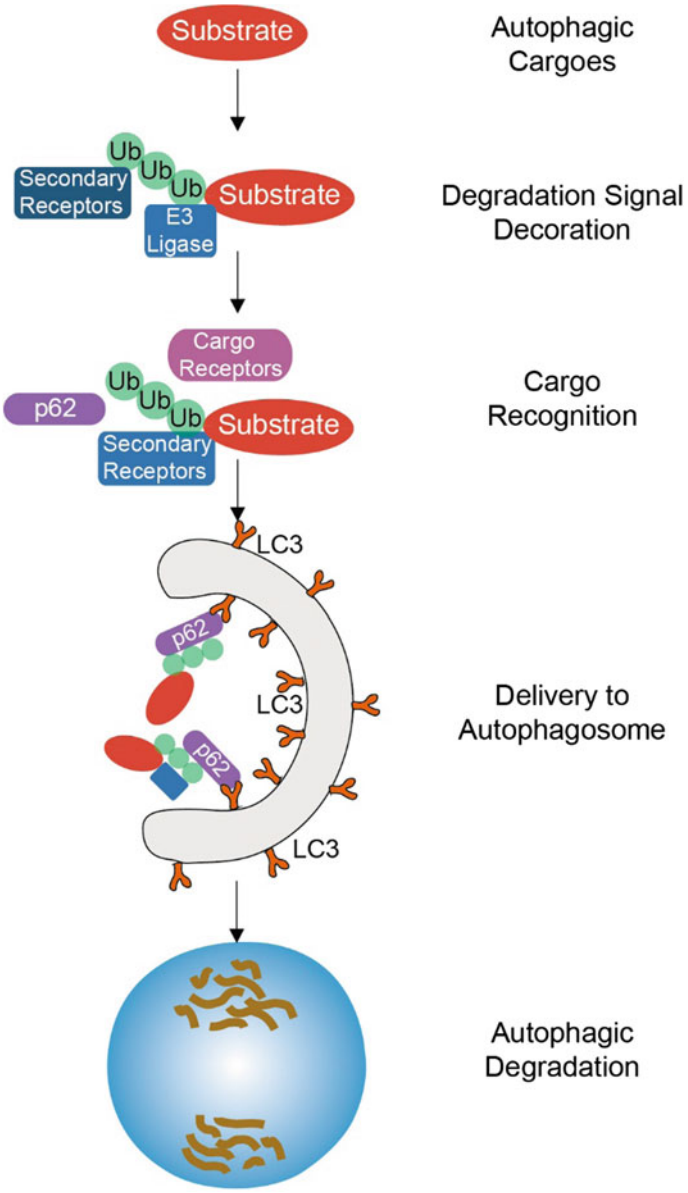


Fig. 9.3 Schematic overview of cargo receptor network. During activation, autophagic cargoes first recruit secondary receptors/co-receptors and undergoes post-translational modification. Subsequently, the secondary receptors/co-receptors assist cargo receptors to recognize the substrates and the degradation signals. Cargo receptors then deliver the substrates to autophagosomes for degradation

p62, which leads to the autophagic degradation of RIG-I and inhibition of excessive type I IFN response [14]. Interestingly, LRRC59, the homolog of LRRC25, competitively binds to RIG-I and blocks the association between RIG-I and LRRC25, thereby leading to the stronger antiviral immune responses [102].

Collectively, various secondary receptors/co-receptors help specific cargo receptors in recognizing particular substrates, which support a cargo receptor network to provide the precise control and cargo selection of selective autophagy. Manipulation of cargo receptor network contributes to the diverse cellular processes, including the restriction of innate immune signaling.

9.6 Conclusion and Perspective

As a major catabolic pathway in mammals, autophagy monitors and controls the invasion and replication of cytosolic pathogenic microbes by direct degrading pathogens or pathogenic components, regulating IFN and inflammatory responses and promoting antigen presentation. Utilizing a set of specialized cargo receptors, autophagy can be selective, and target specific substrates, including aggregated proteins, damaged organelles, and invading pathogens. During the microbial infection, broad and diverse mechanism is employed by selective autophagy in mediating innate immunity. With the examples discussed in the above section, the mechanism used by cargo receptors can be divided into several clusters (Fig. 9.2): (i) host factors, such as E3 ubiquitin ligases or antibacterial restriction factors, promote the ubiquitination of the cargoes under certain circumstances and the ubiquitinated substrates are targeted by cargo receptors to the autophagy; (ii) several secondary receptor/co-receptor proteins bridge the substrates and their specific cargo receptors, thereby leading to the selective autophagic degradation of the cargo proteins; (iii) several modifications of the cargoes, such as glycans, can be directly sensed by cargo receptors to autophagosome. For instance, cytosol-exposed glycans from ruptured *Salmonella* vacuoles can be targeted by NDP52 to xenophagy; (iv) substrates can be directly recognized by specialized receptor proteins in a ubiquitin-independent manner. Selective autophagy of host and pathogenic cargoes leads to diverse cellular functional outcomes during microbial infection, including elimination of microbial pathogens, inhibition of excessive immune response, and even transition of different cellular processes. However, the exact functions of selective autophagy and the detailed mechanisms that govern autophagy during pathogenic infection are still being elucidated. More autophagic cargoes, cargo receptors, and recognition signals involved in selective autophagy during infection are still required further investigation in future.

Acknowledgements This work was supported by the National Natural Science Foundation of China (31870862, 31700760, and 31800751), Science and Technology Planning Project of Guangzhou, China (201804010385), and the Fundamental Research Funds for the Central Universities (18lgpy53).

References

1. Ait-Goughoulte M, Kanda T, Meyer K, Ryerse JS, Ray RB, Ray R (2008) Hepatitis C virus genotype 1a growth and induction of autophagy. J Virol 82:2241–2249
2. Bakshi S, Taylor J, Strickson S, McCartney T, Cohen P (2017) Identification of TBK1 complexes required for the phosphorylation of IRF3 and the production of interferon beta. Biochem J 474:1163–1174
3. Bogunovic D, Byun M, Durfee LA, Abhyankar A, Sanal O, Mansouri D, Salem S, Radovanovic I, Grant AV, Adimi P et al (2012) Mycobacterial disease and impaired IFN-gamma immunity in humans with inherited ISG15 deficiency. Science 337:1684–1688
4. Bulut Y, Faure E, Thomas L, Equils O, Arditi M (2001) Cooperation of Toll-like receptor 2 and 6 for cellular activation by soluble tuberculosis factor and Borrelia burgdorferi outer surface protein A lipoprotein: role of Toll-interacting protein and IL-1 receptor signaling molecules in Toll-like receptor 2 signaling. J Immunol 167:987–994
5. Burns K, Clatworthy J, Martin L, Martinon F, Plumpton C, Maschera B, Lewis A, Ray K, Tschopp J, Volpe F (2000) Tollip, a new component of the IL-1RI pathway, links IRAK to the IL-1 receptor. Nat Cell Biol 2:346–351
6. Capelluto DG (2012) Tollip: a multitasking protein in innate immunity and protein trafficking. Microbes Infect 14:140–147
7. Chen M, Meng Q, Qin Y, Liang P, Tan P, He L, Zhou Y, Chen Y, Huang J, Wang RF et al (2016) TRIM14 Inhibits cGAS degradation mediated by selective autophagy receptor p62 to promote innate immune responses. Mol Cell 64:105–119
8. Choi Y, Bowman JW, Jung JU (2018) Autophagy during viral infection—a double-edged sword. Nat Rev Microbiol
9. Choi YB, Shembade N, Parvatiyar K, Balachandran S, Harhaj EW (2017) TAX1BP1 restrains virus-induced apoptosis by facilitating itch-mediated degradation of the mitochondrial adaptor MAVS. Mol Cell Biol 37
10. Deng Z, Purtell K, Lachance V, Wold MS, Chen S, Yue Z (2017) Autophagy receptors and neurodegenerative diseases. Trends Cell Biol 27:491–504
11. Deretic V (2010) Autophagy in infection. Curr Opin Cell Biol 22:252–262
12. Deretic V, Levine B (2018) Autophagy balances inflammation in innate immunity. Autophagy 14:243–251
13. Dinarello CA (2009) Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 27:519–550
14. Du Y, Duan T, Feng Y, Liu Q, Lin M, Cui J, Wang RF (2018) LRRC25 inhibits type I IFN signaling by targeting ISG15-associated RIG-I for autophagic degradation. EMBO J 37:351–366
15. Ellinghaus D, Zhang H, Zeissig S, Lipinski S, Till A, Jiang T, Stade B, Bromberg Y, Ellinghaus E, Keller A et al (2013) Association between variants of PRDM1 and NDP52 and Crohn's disease, based on exome sequencing and functional studies. Gastroenterology 145:339–347
16. Feng Y, Duan T, Du Y, Jin S, Wang M, Cui J, Wang RF (2017) LRRC25 functions as an Inhibitor of NF-κB signaling pathway by promoting p65/RelA for autophagic degradation. Sci Rep 7:13448
17. Franco LH, Nair VR, Scharn CR, Xavier RJ, Torrealba JR, Shiloh MU, Levine B (2017) The ubiquitin ligase Smurf1 functions in selective autophagy of mycobacterium tuberculosis and anti-tuberculous host defense. Cell Host Microbe 21:59–72
18. Franco LH, Nair VR, Scharn CR, Xavier RJ, Torrealba JR, Shiloh MU, Levine B (2017) The ubiquitin ligase Smurf1 functions in selective autophagy of mycobacterium tuberculosis and anti-tuberculous host defense. Cell Host Microbe 22:421–423
19. Gleason CE, Ordureau A, Gourlay R, Arthur JS, Cohen P (2011) Polyubiquitin binding to optineurin is required for optimal activation of TANK-binding kinase 1 and production of interferon beta. J Biol Chem 286:35663–35674
20. Gomes LC, Dikic I (2014) Autophagy in antimicrobial immunity. Mol Cell 54:224–233

21. Hafren A, Macia JL, Love AJ, Milner JJ, Drucker M, Hofius D (2017) Selective autophagy limits cauliflower mosaic virus infection by NBR1-mediated targeting of viral capsid protein and particles. Proc Natl Acad Sci USA 114:E2026–E2035
22. Hashimoto K, Simmons AN, Kajino-Sakamoto R, Tsuji Y, Ninomiya-Tsuji J (2016) TAK1 regulates the Nrf2 antioxidant system through modulating p62/SQSTM1. Antioxid Redox Sig 25:953–964
23. Hayden MS, Ghosh S (2008) Shared principles in NF-kappaB signaling. Cell 132:344–362
24. Heath RJ, Goel G, Baxt LA, Rush JS, Mohanan V, Paulus GLC, Jani V, Lassen KG, Xavier RJ (2016) RNF166 determines recruitment of adaptor proteins during antibacterial autophagy. Cell Rep 17:2183–2194
25. Heo JM, Ordureau A, Paulo JA, Rinehart J, Harper JW (2015) The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDP52 recruitment and TBK1 activation to promote mitophagy. Mol Cell 60:7–20
26. Herhaus L, Dikic I (2018) Regulation of Salmonella-host cell interactions via the ubiquitin system. Int J Med Microbiol 308:176–184
27. Huett A, Heath RJ, Begun J, Sassi SO, Baxt LA, Vyas JM, Goldberg MB, Xavier RJ (2012) The LRR and RING domain protein LRSAM1 is an E3 ligase crucial for ubiquitin-dependent autophagy of intracellular Salmonella Typhimurium. Cell Host Microbe 12:778–790
28. Husnjak K, Dikic I (2012) Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. Annu Rev Biochem 81:291–322
29. Ikeda F, Dikic I (2008) Atypical ubiquitin chains: new molecular signals. 'Protein Modifications: Beyond the Usual Suspects' review series. EMBO Rep 9:536–542
30. Jin S, Tian S, Luo M, Xie W, Liu T, Duan T, Wu Y, Cui J (2017) Tetherin suppresses type I interferon signaling by targeting MAVS for NDP52-mediated selective autophagic degradation in human cells. Mol Cell 68(308–322):e304
31. Johansen T, Lamark T (2011) Selective autophagy mediated by autophagic adapter proteins. Autophagy 7:279–296
32. Kanayama M, Inoue M, Danzaki K, Hammer G, He YW, Shinohara ML (2015) Autophagy enhances NFκB activity in specific tissue macrophages by sequestering A20 to boost antifungal immunity. Nat Commun 6:5779
33. Kanki T (2010) Nix, a receptor protein for mitophagy in mammals. Autophagy 6:433–435
34. Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11:373–384
35. Khaminets A, Behl C, Dikic I (2016) Ubiquitin-dependent and independent signals in selective autophagy. Trends Cell Biol 26:6–16
36. Kim N, Kim MJ, Sung PS, Bae YC, Shin EC, Yoo JY (2016) Interferon-inducible protein SCOTIN interferes with HCV replication through the autolysosomal degradation of NS5A. Nat Commun 7:10631
37. Kimura T, Jain A, Choi SW, Mandell MA, Schroder K, Johansen T, Deretic V (2015) TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. J Cell Biol 210:973–989
38. Kirkin V (2019) History of the selective autophagy research: how did it begin and where does it stand today? J Mol Biol 1–25
39. Kirkin V, Lamark T, Sou YS, Bjorkoy G, Nunn JL, Bruun JA, Shvets E, McEwan DG, Clausen TH, Wild P et al (2009) A role for NBR1 in autophagosomal degradation of ubiquitinated substrates. Mol Cell 33:505–516
40. Kirkin V, McEwan DG, Novak I, Dikic I (2009) A role for ubiquitin in selective autophagy. Mol Cell 34:259–269
41. Klionsky DJ (2007) Autophagy: from phenomenology to molecular understanding in less than a decade. Nat Rev Mol Cell Biol 8:931–937
42. Kohler LJ, Roy CR (2017) Autophagic targeting and avoidance in intracellular bacterial infections. Curr Opin Microbiol 35:36–41
43. Lamark T, Perander M, Outzen H, Kristiansen K, Overvatn A, Michaelsen E, Bjorkoy G, Johansen T (2003) Interaction codes within the family of mammalian Phox and Bem1p domain-containing proteins. J Biol Chem 278:34568–34581

44. Levine B (2005) Eating oneself and uninvited guests: autophagy-related pathways in cellular defense. Cell 120:159–162
45. Li T, Hu J, Li L (2004) Characterization of Tollip protein upon Lipopolysaccharide challenge. Mol Immunol 41:85–92
46. Ling J, Kang Y, Zhao R, Xia Q, Lee DF, Chang Z, Li J, Peng B, Fleming JB, Wang H et al (2012) KrasG12D-induced IKK2/ β/NF-κB activation by IL-1α and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. Cancer Cell 21:105–120
47. Liu K, Zhang L, Zhao Q, Zhao Z, Zhi F, Qin Y, Cui J (2018) SKP2 attenuates NF-κB signaling by mediating IKKβ degradation through autophagy. J Mol Cell Biol 10:205–215
48. Liu T, Tang Q, Liu K, Xie W, Liu X, Wang H, Wang RF, Cui J (2016) TRIM11 suppresses AIM2 inflammasome by degrading AIM2 via p62-dependent selective autophagy. Cell Rep 16:1988–2002
49. Lu Y, Wang L, He M, Huang W, Li H, Wang Y, Kong J, Qi S, Ouyang J, Qiu X (2012) Nix protein positively regulates NF-kappaB activation in gliomas. PLoS One 7:e44559
50. Mandell MA, Jain A, Arko-Mensah J, Chauhan S, Kimura T, Dinkins C, Silvestri G, Munch J, Kirchhoff F, Simonsen A et al (2014) TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition. Dev Cell 30:394–409
51. Mandell MA, Kimura T, Jain A, Johansen T, Deretic V (2014) TRIM proteins regulate autophagy: TRIM5 is a selective autophagy receptor mediating HIV-1 restriction. Autophagy 10:2387–2388
52. Manzanillo PS, Ayres JS, Watson RO, Collins AC, Souza G, Rae CS, Schneider DS, Nakamura K, Shiloh MU, Cox JS (2013) The ubiquitin ligase parkin mediates resistance to intracellular pathogens. Nature 501:512–516
53. Marin M, Rose KM, Kozak SL, Kabat D (2003) HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat Med 9:1398–1403
54. Martinon F, Mayor A, Tschopp J (2009) The inflammasomes: guardians of the body. Ann Rev Immunol 27:229–265
55. Meena NP, Zhu G, Mittelstadt PR, Giardino Torchia ML, Pourcelot M, Arnoult D, Ashwell JD, Munitic I (2016) The TBK1-binding domain of optineurin promotes type I interferon responses. FEBS Lett 590:1498–1508
56. Minowa-Nozawa A, Nozawa T, Okamoto-Furuta K, Kohda H, Nakagawa I (2017) Rab35 GTPase recruits NDP52 to autophagy targets. EMBO J 36:2790–2807
57. Mizushima N, Yoshimori T, Ohsumi Y (2011) The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol 27:107–132
58. Moresco EM, LaVine D, Beutler B (2011) Toll-like receptors. Curr Biol 21:R488–R493
59. Moscat J, Diaz-Meco MT (2009) p62 at the crossroads of autophagy, apoptosis, and cancer. Cell 137:1001–1004
60. Mostowy S, Sancho-Shimizu V, Hamon MA, Simeone R, Brosch R, Johansen T, Cossart P (2011) p62 and NDP52 proteins target intracytosolic Shigella and Listeria to different autophagy pathways. J Biol Chem 286:26987–26995
61. Munitic I, Giardino Torchia ML, Meena NP, Zhu G, Li CC, Ashwell JD (2013) Optineurin insufficiency impairs IRF3 but not NF-κB activation in immune cells. J Immunol 191:6231–6240
62. Nagabhushana A, Bansal M, Swarup G (2011) Optineurin is required for CYLD-dependent inhibition of TNFα-induced NF-κB activation. PLoS One 6:e17477
63. Nakashima H, Nguyen T, Goins WF, Chiocca EA (2015) Interferon-stimulated gene 15 (ISG15) and ISG15-linked proteins can associate with members of the selective autophagic process, histone deacetylase 6 (HDAC6) and SQSTM1/p62. J Biol Chem 290:1485–1495
64. Nakazawa S, Oikawa D, Ishii R, Ayaki T, Takahashi H, Takeda H, Ishitani R, Kamei K, Takeyoshi I, Kawakami H et al (2016) Linear ubiquitination is involved in the pathogenesis of optineurin-associated amyotrophic lateral sclerosis. Nat Commun 7:12547
65. Noad J, von der Malsburg A, Pathe C, Michel MA, Komander D, Randow F (2017) LUBAC-synthesized linear ubiquitin chains restrict cytosol-invading bacteria by activating autophagy and NF-κB. Nat Microbiol 2:17063

66. Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, Rogov V, Lohr F, Popovic D, Occhipinti A et al (2010) Nix is a selective autophagy receptor for mitochondrial clearance. EMBO Rep 11:45–51
67. Oeckinghaus A, Hayden MS, Ghosh S (2011) Crosstalk in NF-κB signaling pathways. Nat Immunol 12:695–708
68. Orvedahl A, MacPherson S, Sumpter R Jr, Talloczy Z, Zou Z, Levine B (2010) Autophagy protects against Sindbis virus infection of the central nervous system. Cell Host Microbe 7:115–127
69. Orvedahl A, Sumpter R Jr, Xiao G, Ng A, Zou Z, Tang Y, Narimatsu M, Gilpin C, Sun Q, Roth M et al (2011) Image-based genome-wide siRNA screen identifies selective autophagy factors. Nature 480:113–117
70. Parvatiyar K, Barber GN, Harhaj EW (2010) TAX1BP1 and A20 inhibit antiviral signaling by targeting TBK1-IKKi kinases. J Biol Chem 285:14999–15009
71. Petkova DS, Verlhac P, Rozieres A, Baguet J, Claviere M, Kretz-Remy C, Mahieux R, Viret C, Faure M (2017) Distinct contributions of autophagy receptors in measles virus replication. Viruses 9
72. Polajnar M, Dietz MS, Heilemann M, Behrends C (2017) Expanding the host cell ubiquitylation machinery targeting cytosolic Salmonella. EMBO Rep 18:1572–1585
73. Ponpuak M, Davis AS, Roberts EA, Delgado MA, Dinkins C, Zhao Z, Virgin HW 4th, Kyei GB, Johansen T, Vergne I et al (2010) Delivery of cytosolic components by autophagic adaptor protein p62 endows autophagosomes with unique antimicrobial properties. Immunity 32, 329–341
74. Prabakaran T, Bodda C, Krapp C, Zhang BC, Christensen MH, Sun C, Reinert L, Cai Y, Jensen SB, Skouboe MK et al (2018) Attenuation of cGAS-STING signaling is mediated by a p62/SQSTM1-dependent autophagy pathway activated by TBK1. EMBO J 37
75. Qi N, Shi Y, Zhang R, Zhu W, Yuan B, Li X, Wang C, Zhang X, Hou F (2017) Multiple truncated isoforms of MAVS prevent its spontaneous aggregation in antiviral innate immune signalling. Nat Commun 8:15676
76. Richter B, Sliter DA, Herhaus L, Stolz A, Wang C, Beli P, Zaffagnini G, Wild P, Martens S, Wagner SA et al (2016) Phosphorylation of OPTN by TBK1 enhances its binding to Ub chains and promotes selective autophagy of damaged mitochondria. Proc Natl Acad Sci USA 113:4039–4044
77. Rogov V, Dotsch V, Johansen T, Kirkin V (2014) Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. Mol Cell 53:167–178
78. Sagnier S, Daussy CF, Borel S, Robert-Hebmann V, Faure M, Blanchet FP, Beaumelle B, Biard-Piechaczyk M, Espert L (2015) Autophagy restricts HIV-1 infection by selectively degrading Tat in $CD4^+$ T lymphocytes. J Virol 89:615–625
79. Sanchez-Martin P, Saito T, Komatsu M (2019) p62/SQSTM1: 'Jack of all trades' in health and cancer. FEBS J 286:8–23
80. Schneider WM, Chevillotte MD, Rice CM (2014) Interferon-stimulated genes: a complex web of host defenses. Annu Rev Immunol 32:513–545
81. Shaid S, Brandts CH, Serve H, Dikic I (2013) Ubiquitination and selective autophagy. Cell Death Differ 20:21–30
82. Sharma V, Verma S, Seranova E, Sarkar S, Kumar D (2018) Selective autophagy and xenophagy in infection and disease. Front Cell Dev Biol 6:147
83. Shembade N, Harhaj NS, Liebl DJ, Harhaj EW (2007) Essential role for TAX1BP1 in the termination of TNF-α-, IL-1- and LPS-mediated NF-κB and JNK signaling. EMBO J 26:3910–3922
84. Shembade N, Parvatiyar K, Harhaj NS, Harhaj EW (2009) The ubiquitin-editing enzyme A20 requires RNF11 to downregulate NF-κB signalling. EMBO J 28:513–522
85. Shi CS, Shenderov K, Huang NN, Kabat J, Abu-Asab M, Fitzgerald KA, Sher A, Kehrl JH (2012) Activation of autophagy by inflammatory signals limits IL-1β production by targeting ubiquitinated inflammasomes for destruction. Nat Immunol 13:255–263

86. Slowicka K, van Loo G (2018) Optineurin functions for optimal immunity. Front Immunol 9:769
87. Slowicka K, Vereecke L, Mc Guire C, Sze M, Maelfait J, Kolpe A, Saelens X, Beyaert R, van Loo G (2016) Optineurin deficiency in mice is associated with increased sensitivity to Salmonella but does not affect proinflammatory NF-κB signaling. Eur J Immunol 46:971–980
88. Spinnenhirn V, Farhan H, Basler M, Aichem A, Canaan A, Groettrup M (2014) The ubiquitin-like modifier FAT10 decorates autophagy-targeted Salmonella and contributes to Salmonella resistance in mice. J Cell Sci 127:4883–4893
89. Staring J, von Castelmur E, Blomen VA, van den Hengel LG, Brockmann M, Baggen J, Thibaut HJ, Nieuwenhuis J, Janssen H, van Kuppeveld FJ et al (2017) PLA2G16 represents a switch between entry and clearance of Picornaviridae. Nature 541:412–416
90. Stolz A, Ernst A, Dikic I (2014) Cargo recognition and trafficking in selective autophagy. Nat Cell Biol 16:495–501
91. Sudhakar C, Nagabhushana A, Jain N, Swarup G (2009) NF-κB mediates tumor necrosis factor α-induced expression of optineurin, a negative regulator of NF-κB. PLoS One 4:e5114
92. Sumpter R Jr, Sirasanagandla S, Fernandez AF, Wei Y, Dong X, Franco L, Zou Z, Marchal C, Lee MY, Clapp DW et al (2016) Fanconi Anemia Proteins function in mitophagy and immunity. Cell 165:867–881
93. Tanishima M, Takashima S, Honda A, Yasuda D, Tanikawa T, Ishii S, MaruYama T (2017) Identification of optineurin as an interleukin-1 receptor-associated kinase 1-binding protein and its role in regulation of MyD88-dependent signaling. J Biol Chem 292:17250–17257
94. Thurston TL, Ryzhakov G, Bloor S, von Muhlinen N, Randow F (2009) The TBK1 adaptor and autophagy receptor NDP52 restricts the proliferation of ubiquitin-coated bacteria. Nat Immunol 10:1215–1221
95. Thurston TL, Wandel MP, von Muhlinen N, Foeglein A, Randow F (2012) Galectin 8 targets damaged vesicles for autophagy to defend cells against bacterial invasion. Nature 482:414–418
96. Till A, Lipinski S, Ellinghaus D, Mayr G, Subramani S, Rosenstiel P, Franke A (2013) Autophagy receptor CALCOCO2/NDP52 takes center stage in Crohn disease. Autophagy 9:1256–1257
97. Tumbarello DA, Manna PT, Allen M, Bycroft M, Arden SD, Kendrick-Jones J, Buss F (2015) The autophagy receptor TAX1BP1 and the molecular motor Myosin VI are required for clearance of Salmonella Typhimurium by autophagy. PLoS Pathog 11:e1005174
98. Valera MS, de Armas-Rillo L, Barroso-Gonzalez J, Ziglio S, Batisse J, Dubois N, Marrero-Hernandez S, Borel S, Garcia-Exposito L, Biard-Piechaczyk M et al (2015) The HDAC6/APOBEC3G complex regulates HIV-1 infectiveness by inducing Vif autophagic degradation. Retrovirology 12:53
99. van Wijk SJL, Fricke F, Herhaus L, Gupta J, Hotte K, Pampaloni F, Grumati P, Kaulich M, Sou YS, Komatsu M et al (2017) Linear ubiquitination of cytosolic Salmonella Typhimurium activates NF-κB and restricts bacterial proliferation. Nat Microbiol 2:17066
100. von Muhlinen N, Thurston T, Ryzhakov G, Bloor S, Randow F (2010) NDP52, a novel autophagy receptor for ubiquitin-decorated cytosolic bacteria. Autophagy 6:288–289
101. Wild P, Farhan H, McEwan DG, Wagner S, Rogov VV, Brady NR, Richter B, Korac J, Waidmann O, Choudhary C et al (2011) Phosphorylation of the autophagy receptor optineurin restricts Salmonella growth. Science 333:228–233
102. Xian H, Yang S, Jin S, Zhang Y, Cui J (2019) LRRC59 modulates type I interferon signaling by restraining the SQSTM1/p62-mediated autophagic degradation of pattern recognition receptor DDX58/RIG-I. Autophagy 1–11
103. Yang Q, Liu TT, Lin H, Zhang M, Wei J, Luo WW, Hu YH, Zhong B, Hu MM, Shu HB (2017) TRIM32-TAX1BP1-dependent selective autophagic degradation of TRIF negatively regulates TLR3/4-mediated innate immune responses. PLoS Pathog 13:e1006600
104. Zhang X, Bogunovic D, Payelle-Brogard B, Francois-Newton V, Speer SD, Yuan C, Volpi S, Li Z, Sanal O, Mansouri D et al (2015) Human intracellular ISG15 prevents interferon-α/β over-amplification and auto-inflammation. Nature 517:89–93

105. Zhang X, Zhang MC, Wang CT (2018) Loss of LRRC25 accelerates pathological cardiac hypertrophy through promoting fibrosis and inflammation regulated by TGF-beta1. Biochem Biophys Res Commun 506:137–144
106. Zheng YT, Shahnazari S, Brech A, Lamark T, Johansen T, Brumell JH (2009) The adaptor protein p62/SQSTM1 targets invading bacteria to the autophagy pathway. J Immunol 183:5909–5916
107. Zhong Z, Umemura A, Sanchez-Lopez E, Liang S, Shalapour S, Wong J, He F, Boassa D, Perkins G, Ali SR et al (2016) NF-κB restricts inflammasome activation via elimination of damaged Mitochondria. Cell 164:896–910
108. Zhu G, Wu CJ, Zhao Y, Ashwell JD (2007) Optineurin negatively regulates TNFα- induced NF-κB activation by competing with NEMO for ubiquitinated RIP. Curr Biol 17:1438–1443
109. Zotti T, Scudiero I, Settembre P, Ferravante A, Mazzone P, D'Andrea L, Reale C, Vito P, Stilo R (2014) TRAF6-mediated ubiquitination of NEMO requires p62/sequestosome-1. Mol Immunol 58:27–31

Chapter 10
Autophagy and Immune-Related Diseases

Peng Tan, Youqiong Ye, Jingrong Mao and Lian He

Abstract Autophagy is an intracellular degradation and recycling machinery by which cellular materials are delivered to the lysosome for disposal. Beyond lysosomal degradation, autophagy genes play additional roles in secretion and membrane-trafficking pathways. Ranging from cell-intrinsic and cell-type-specific regulation of innate inflammatory signaling pathways to intercellular cross talk through secretion of soluble factors (e.g., shaping the MHC immunopeptidome for T cell response, etc.), autophagy exerts multiple functions in driving inflammation and modulating the pathological progression of immune-related disorders such as neurodegenerative diseases, inflammatory bowel diseases, autoimmunity, and metabolic diseases. Notably, owing to the complexity of autophagy process involving multiple proteins and stepwise assembly of protein complexes, noncanonical forms of autophagy or autophagic proteins, which function beyond autophagy, are equally important in the maintenance of cellular homeostasis and pathogenesis. This chapter summarizes the most up-to-date findings of autophagy proteins in the regulation of immune-related diseases. Understanding of the autophagy machinery offers therapeutic strategies for treating inflammatory diseases.

Keywords Autophagy · Autoimmune · Neurodegeneration · Inflammatory bowel disease · Metabolic disease · Aging

P. Tan (✉) · L. He
Institute of Biosciences and Technology, College of Medicine, Texas A&M University, Houston, TX 77030, USA
e-mail: tpengsysu@gmail.com

Y. Ye (✉)
Department of Biochemistry and Molecular Biology, University of Texas Health Science Center at Houston McGovern Medical School, Houston, TX 77030, USA
e-mail: youqiong.ye@uth.tmc.edu

J. Mao
Xiangya Hospital, Central South University, Changsha 410008, People's Republic of China

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_10

10.1 Introduction

Autophagy, in general cytoprotective, is critical for resolving the internal and environmental challenges such as oxidative stress, nutrient starvation, accumulation of damaged proteins, and organelles. In selective autophagy, the proteins or organelle substrates either contain an LC3-interacting (LIR) motif that directly binds LC3 on the forming phagophore or labeled with a ubiquitin signal which then binds to adaptor proteins (e.g., p62) to bridge them with LC3-conjugated phagophore membrane are determined as cargoes to be degraded in autophagosome (TRIM-mediated autophagy, ER-phagy, mitophagy, micronuclei, lipophagy, xenophagy, etc.). Besides, autophagy-related (ATG) genes contribute to the secretion of inflammatory cytokines, granules, or antimicrobial peptides through exocytosis in intestinal Paneth cell and the release of pathogenic protein-containing exosomes [37].

Autophagy is closely related to immune function and inflammation. It orchestrates innate and adaptive immune responses and shapes T cell immunity through the delivery of peptides to major histocompatibility complex (MHC) class II molecule [48, 59]. Intrinsic or environmental perturbation or dysfunction of autophagy results in accomplice of cellular homeostasis and induction of disease-prone inflammation [76]. For instance, malfunction of ATG genes are considered to be responsible for the immune cell death and failure to generate immune memory [9, 31, 53, 71], changes of cell state or cell fate during viral infection and in the tumor microenvironment [12, 43], differentiation defects [46], ER stress [20], impaired antigen presentation [18], metabolic reprogramming [61], failure to clear pathogen or dying cells (e.g., through LC3-associated phagocytosis) [26], and accumulation of damaged mitochondria contributed to inflammation-induced multiple aging-associated pathologies [19].

Genome-wide association studies (GWAS) and experimental models have linked risk alleles and anomalous expression in ATG genes with tissue-destructive inflammation and the pathogenesis of several autoimmune and inflammatory disorders [21, 30, 39, 42, 54, 76, 77] including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), inflammatory bowel disease (IBD), neurodegenerative disease, metabolic disease, pancreatitis, aging, and cancer. Therefore, autophagy is an attractive therapeutic target to modulate the effector and memory functions of immune cells as well as to treat patients with immune disorders.

In this chapter, we summarize and discuss this network of communication between autophagy and immune-related diseases, but with less focus on inflammation-induced cancer that is recently reviewed [76]. We also discuss herein molecular mechanisms regulating phenotypic and functional profile through immune-modulating programs (microbiome, metabolism, and cell–cell interplay), and present recent single-cell technology for interrogating complex human disease and mapping risk variants to cell types and pathways.

10.2 Autophagy in CNS Autoimmunity

Multiple sclerosis (MS), is a common axonal demyelination and neurodegeneration led by dysfunction and apoptosis of oligodendrocytes (OLs) in central nervous system (CNS), correlates with the involvement of autoreactive $CD4^+$ T cells that invade across the compromised blood–brain barrier, reactivated and accumulated through myelin antigen presentation by dendric cells (DCs) within the CNS [14]. Experimental autoimmune encephalomyelitis (EAE) is a model for MS, where expanded $CD4^+$ T cells in CNS are largely specific for myelin oligodendrocyte glycoprotein (MOG) immunogenic peptide 35–55 ($MOG_{35–55}$). Induction of pathogenic $CD4^+$- and IL-17-producing $\gamma\delta^+$ T cells in EAE triggers the immune suppression by clonally expanded regulatory $CD8^+$ T cells [55].

Autophagy is essential for the maintenance of T cell survival and development as well as the functional integrity under activating environment. *Becn1*-deletion in $CD4^+$ T cells (CD4-Cre) are prone to cell death (less susceptible in Th17 cells) with elevated expression of apoptotic proteins such as caspase-8 upon TCR stimulation, without affecting the $CD4^+CD8^+$ thymocytes development (similarly found in CD4-Cre *Atg7*-deficient mice, whereas thymic iNKT cell development is blocked and mature iNKT cells are absent in secondary lymphoid organs) [31, 56]. Mice with the *Becn1*-deficiency in $CD4^+$ T cells, therefore, fail to mount autoimmune responses in EAE [31]. Autoimmune disease often exhibits loss of immunosuppressive Treg cells. In regulatory T (Treg) cells, autophagy is highly activated. *Atg5* or *Atg7* is essential in maintaining Foxp3 expression of Treg cells and immune homeostasis and supporting the survival fitness of mature Treg cells that are activated by peripheral environmental cues as exemplified in *Foxp3*-Cre conditional knockout mice. Autophagy deficiency in Treg cells upregulates PI3K-PDK1-mTORC1 signaling and c-Myc-mediated glycolytic metabolism (increase in cell size, CD71 and CD98 expression, suppressed Foxp3, Foxo3, and Bach2), leading to defective Treg function [70].

Absence of *Atg5* in conventional DC attenuates the myelin peptide presentation following phagocytosis of damaged and dying OLs. *Atg7* or *Atg16l* deficiency in DC also ameliorates the onset and severity of EAE by reducing the priming of $CD4^+$ T cells. In vivo treatment of CNS-penetrating Na^+ and K^+ pump inhibitor, cardiac glycoside neriifolin that also targets Beclin-1 and phagocytosis or autophagy (e.g., LAP), or autophagy–lysosomal fusion inhibitor chloroquine (CQ), reduces EAE severity [2, 5, 30]. Consistent with the importance of autophagy found in the DC-mediated EAE, the cytotoxic T-lymphocyte-associated protein 4 (CTLA4) on $Foxp3^+$ Treg cells suppresses DC-mediated EAE response by suppressing autophagy machinery in DCs. Binding of CTLA4 to DC promotes the activation of PI3K/Akt/mTOR pathway and subsequent FoxO1 phosphorylation and cytoplasm retention. As the transcription target of FoxO1, the transcript of autophagy component microtubule-associated protein 1 light chain 3β (Lc3b) is diminished and autophagosome-mediated antigen processing is abolished. Treating human DCs with Abatacept (CTLA4-Ig, marketed as Orencia), an antibody containing the fusion of Fc region of IgG1 and the extracellular domain of CTLA4, suppresses LC3B expression and autophagosome formation [2].

10.3 Autophagy in Neurodegeneration

Autophagy in neuron plays physiologically vital role in the clearance of aggregation-prone forms of misfolded proteins characteristic of the major late-onset neurodegenerative diseases such as Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Parkinson's disease (PD) (e.g., Tau, β-amyloid, Huntingtin, α-Synuclein, prions) [50]. Suppression of basal autophagy in mice deficient for *Atg5* in neuronal cells (Nestin-Cre) develops axonal swelling and deficits in motor function as phenotypes of neurodegeneration [24].

AD, for example, clinically distinguished by memory loss and cognitive dysfunctions, is characterized by aggregation and accumulation of extracellular β-amyloid (Aβ) plaques and intracellular tau protein neurofibrillary tangles. Aβ peptide is formed by sequential cleavage of a larger Aβ precursor protein (APP) through β- and γ-secretases. Aβ comprises a set of 40–43-residue polypeptides and is progressively built up in the brains of AD, leading to synaptic dysfunction, inflammation, and neuronal cell death. Soluble Aβ dimers mediate the glutamatergic neuronal firing and blockade of the reuptake of synaptically released glutamate by astrocytes and neurons, leading to cycles of neuronal hyperactivation (a key feature of early stages of AD and before the plaque formation) [78]. Autophagy mediates the secretion of Aβ and Aβ plaque load. Autophagy deficiency leads to intracellular accumulation of neurotoxic Aβ and neurodegeneration can be exacerbated by Aβ amyloidosis in a mouse model where *Atg7* was conditionally deleted (CamKII-Cre) in forebrain excitatory neurons [49]. Besides neuronal autophagy, inflammation is increasingly recognized as a critical factor driving the progression of neurodegenerative diseases. Dysfunction of neuronal immune cells drives the disease-prone inflammation (e.g., inflammasome-induced apoptosis-associated speck-like protein containing a CARD (ASC) specks in Aβ aggregation) [68, 69] and accumulation of Aβ plaques and tau aggregates including astrocytes, microglia, perivascular macrophages, invaded peripheral macrophages [10]. Multiple variants associated with an increased risk of developing AD are in genes related to immune functions including triggering receptor expressed on myeloid cells-2 (*TREM2*), *CR1*, *CD33*, *ABCA7*, *EPHA1* [77]. Arginine-to-histidine hypomorphic variants at position 47 (R47H) or 62 (R62H) of TREM2 in human as well as TREM2 deficiency in mouse increase the risk for AD by impeding the accumulation of microglia around Aβ plaques and phagocytosis of Aβ plaques. In AD mouse model 5xFAD (Four familial Alzheimer disease), which mimics AD pathology due to the expression of AD-associated mutant APP (K670N/M671L, I716V, V717I) and presenilin 1 (PS1; M146L, L286V) under neural-specific elements of the mouse Thy1 promoter, TREM2-deficiency in microglia or microglia with TREM2 R47H alleles display markedly increased autophagy, impaired microglia fitness, and capacity to clear Aβ plaques, through inhibiting mTOR activation and anabolic metabolism. While studies demonstrate a beneficial role of autophagy in AD neurons, autophagy induced by TREM2-deficiency in microglia is detrimental to the microglia function in AD [67]. Also, loss of plaque-associated microglia (with TREM2 R47H) facilitates the formation of tau aggregates [38].

Parkinson disease (PD) is a neurodegenerative disorder characterized by the loss of dopamine-containing neurons (DNs). The underlying mechanisms regarding the loss of DNs include mitochondrial dysfunction, impairment of autophagic degradation machinery, and neuroinflammatory processes associated with glial cell activation. Mutation in the lysosomal enzyme glucocerebrosidase (encoded by GBA1; E326K, T369M, N370S, L444P) has emerged as the risk factor associated with PD. Decreased glucocerebrosidase activity, which metabolizes glucosylceramide to ceramide and glucose, is associated with the autophagy–lysosomal pathway and mitochondrial dysfunction as well as α-synuclein accumulation [3, 60]. Several other risk genes in pathways of secretory autophagy and mitophagy, such as TMEM230, PINK1 (PARK6), Parkin (PARK2), SMPD1, ATP13A2 (PARK9), LRRK2, SNCA, and MAPT, have also been linked to PD [47]. Absence of T cells in *Rag1*$^{-/-}$ and *Tcrb*$^{-/-}$ mice attenuate the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic cell death. This T cell-mediated dopaminergic toxicity requires the expression of FasL but not IFN-γ, since a reduction of MPTP-induced dopaminergic cell death can be seen in mice in *Rag1*$^{-/-}$ mice reconstituted with FasL-deficient but not with IFN-γ-deficient splenocytes [6]. MSDC-0160 targeting the protein complex for transporting pyruvate into the mitochondria, mitochondrial pyruvate carrier (MPC), and downstream mTOR pathway, improves locomotor behavior and survival of nigral dopaminergic neurons, normalizes autophagy flux, and reduces neuroinflammation in glial fibrillary acidic protein (GFAP), ionized calcium-binding adapter molecule 1 (Iba-1), and inducible nitric oxide synthase (iNOS)) in MPTP-treated mice and in the Engrailed1 (En$^{+/-}$) mouse model of PD [17].

10.4 Autophagy in Inflammatory Bowel Disease

Inflammatory bowel diseases (IBD) are complex diseases affected by genetic, immunological, and microbial factors of the gastrointestinal tract, including Crohn's disease (CD) and ulcerative colitis (UC). Autophagy has been demonstrated critical in maintaining the intestinal immune homeostasis, epithelial barrier, and antimicrobial function of Paneth cells [4, 33]. The evidence for the implication of autophagy in the pathogenesis of IBD came from GWAS, which revealed polymorphisms in the autophagy-related genes as susceptibility factors for CD. ATGL16L1 mutation (T300A) is the major risk allele for CD, enhancing its susceptibility to caspase cleavage. T300A knock-in, intestinal organoid deletion, or conditional knockout mice shows loss of Paneth cells and enhanced enterocyte TNF-α-induced necroptosis through IL-22-cGAS-STING, induce ER stress and an ER-stress sensor inositol requiring enzyme 1 α (IRE1α)-mediated barrier-protective IgA response, aberrant Th2 cell expansion and inflammation in response to microbiota antigens, and decreased Foxp3^{+} Treg cells in IBD [1, 20, 29, 44]. In addition to the function in the intestinal immune modulation, ATG16L1, ATG5, and ATG12 are also required for plasma membrane repair in response to bacterial pore-forming toxins independent of autophagy function. *ATG16L1* deficiency or cells harboring the ATG16L1

T300A variant causes lysosomal accumulation of cholesterol and ultimately defective membrane repair, linking IBD to plasma membrane integrity [64]. In *Salmonella Typhimurium*-infected Paneth cells, antimicrobial protein lysozyme is excluded from lysosomal degradation, contained in an $LC3^+$ vesicles, and released into the extracellular space via the $Rab8\alpha^+$ secretory autophagy [52]. Crypts from *S. Typhimurium*-infected *Atg16L1*^{T300A} transgenic mice do not produce lysozyme-filled $LC3^+$ vesicles and show reduced bacterial killing. ER stress induced by bacteria infection triggers the secretory autophagy through protein kinase RNA-like endoplasmic reticulum kinase (PERK)-elongation initiation factor 2α (eIF2α) pathway, which requires IL-22 produced the type 3 innate lymphoid cells (ILC3) upon interaction with DC (TLR/MyD88-mediated recognition of bacteria patterns) [52]. Immunity-related GTPase M (IRGM) plays a critical role in the formation and activation of autophagy initiation complex by interacting with ULK1 and Beclin-1 and the association with pattern recognition receptors including NOD2 and ATG16L1, all of which are CD risk variants, regulating the antimicrobial autophagy response [8, 62]. Moreover, IRGM appears to have a protective function in anti-inflammation by exerting suppression on the NACHT, LRR, and PYD domains containing protein 3 (NLRP3) inflammasome-mediated gut inflammation through selective autophagy degradation of NLRP3 and ASC and protects cells from pyroptosis in DSS-induced colitis [45, 51]. G protein-coupled receptor 65 (GPR65) maintains the lysosomal pH and function through H^+-sensing. IBD-associated GPR65 I231L missense variant downregulates GPR65 signaling and impairs the intracellular bacterial clearance. Mice lacking Gpr65 increases the susceptibly to *Citrobacter rodentium*-induced colitis [34]. Other IBD-associated and autophagy-related risk factors include myotubularin-related protein 3 (*MTMR3*), leucine-rich repeat kinase 2 (*LRRK2*), protein tyrosine phosphatase non-receptor type 2 (*PTPN2*), and SMAD Specific E3 Ubiquitin Protein Ligase 1 (*SMURF1*).

Autophagy in intestinal epithelial cells (IECs) has a role in the control of microbiota composition. Impaired autophagy alters the fecal microbiota [35, 66, 73]. IBD-associated risk variants in healthy individuals (NOD2, IRGM, ATG16L1, CARD9, and FUT2) are associated with bacterial handling, a decrease of the acetate-to-butyrate converter, *Roseburia spp*, to produce short-chain fatty acid butyrate essential for maintaining intestinal epithelial cell homeostasis [13, 27]. *Atg7*-deficiency (ΔIEC) enriches *Clostridium leptum*, *Eubacterium cylindroides*, and *Bacteroides fragilis*. Similarly, *Atg5*-deficient mice (ΔIEC) exhibit an increased number of pro-inflammatory bacteria, Pasteurellaceae, *Candidatus Athromitus*, and segmented filamentous bacteria (SFB), which are able to induce pathogenic T cell differentiation and a reduction of *Lachnospiraceae* and *Ruminococcaceae* families and *Akkermansia muciniphila*. Together, these data highlighted how autophagy in host cells regulates intestinal microbial communities and limits the expansion of pathobionts and how the pathobionts have adapted to subvert this process to evade host cells.

Efforts have been made to explore the molecular events in the host–microbiome cross talk by which any defect in autophagy could lead to disrupted intestinal epithelial function, gut dysbiosis, defect in antimicrobial peptide secretion by Paneth cells, ER stress in enterocytes, and aberrant immune responses to pathogenic

bacteria, which are hallmarks of IBD pathogenesis. Recently, single-cell genomics have inferred cell-type-specific functions for risk variants (pathways in metabolism, autophagy, inflammation, epithelial barrier integrity, and cell–cell interactions) that links to the progression of IBD (including the identification of $BEST4^+$ enterocytes, microfold-like cells and $IL13RA2^+IL11^+$ inflammatory fibroblasts, and $CD8^+IL17^+$ cells) [22, 63]. Multi-omics of the gut microbiome and host–microbe interactions propose the imbalanced microbiome composition, altered metabolites, differential gene expression in inflamed ileum and rectum biopsies, and strain-specific gut inflammation in IBD [7, 16, 40, 57, 58].

10.5 Autophagy in Inflammation-Related Metabolic Diseases

Accumulating evidence suggests the importance of autophagy in metabolic diseases including obesity, diabetes, nonalcoholic fatty liver disease (NAFLD), or nonalcoholic steatohepatitis (NASH). Fatty acids are up-taken by liver cells and converted into triglycerides in lipid droplets. Autophagy contributes to the breakdown of lipids through colocalization with the lysosome-associated membrane protein type 1 (LAMP1). Hepatocyte-specific deletion of *Atg7* (Albumin-Cre) leads to hepatic lipid accumulation [61]. In obese mouse (ob/ob) and high-fat diet (HFD) models, obesity induces downregulation of autophagy genes such as *Atg7* through calpain 2. β cell-specific deletion of *Atg7* (RIP-Cre) impairs glucose tolerance and β cell functions [28]. Suppression of autophagy shows defective insulin sensitivity and ER stress that could be reversed by reconstitution of *Atg7* expression [72]. Conversely, Rubicon, a Beclin-1-interacting negative regulator for autophagosome–lysosome fusion, is upregulated in NAFLD patients. Hepatocyte-specific knockout of Rubicon boosts autophagy and displays improvement of liver steatosis and attenuation of ER stress [65]. A Beclin-1 homolog protein Beclin-2 is involved in two distinct degradation pathways: the autophagy pathway and the G protein-coupled receptor (GPCR)-associated sorting protein 1 (GASP1)-dependent, DADLE ([D-Ala2, D-Leu5]-Enkephalin) agonist-induced endolysosomal degradation of GPCRs including DOR, CB1R, and a nonrecycling mutant form of the β-adrenergic (β-Ala) receptor. Loss of Beclin-2 in mice showed impaired autophagy and increased CB1R-induced food intake, leading to diabetes and insulin resistance in HFD condition [25]. The transcription factor EB (TFEB) is an essential component of lysosomal biogenesis and autophagy flux. TFEB translocates into the myonuclei in muscle during exercise controlling the glucose metabolism [41]. Thus, dietary and exercise lifestyle or pharmacological modulation of autophagy could be beneficial in the management of metabolic diseases [75].

10.6 Autophagy in Organismal Aging and Lifespan

The target of rapamycin (TOR), a suppressor of autophagy, is activated through phosphoinositide 3-kinase (PI3K) and the protein kinase AKT upon binding of insulin and insulin-like growth factors (IGF) to the cell surface insulin receptor. On the contrary, adenosine monophosphate-activated protein kinase (AMPK), which is activated upon caloric restriction (CR) and energy depletion, is a stress-responsive inhibitor of TOR that activates autophagy. Prolonged TOR activation is associated with age-related pathologies such as declined cardiac performance, skeletal muscle degeneration, mitochondrial dysfunction, and brain atrophy. CR delays age-associated pathologies in primate species possibly through the TOR suppression and induction of autophagy [11].

Sestrins is induced upon TOR-induced ROS in a feedback manner. Loss of Sestrins prompts aging pathologies that could be phenocopied by inhibition of autophagy in *Drosophila* [36], hinting an inhibitory role of autophagy in aging and longevity. Age-related decline in autophagy function (including selective autophagy) could lead to accumulation of functionally disabled organelles and aging of tissues [23]. Restoration of autophagy could have an antiaging function in maintaining functional metabolism in viral organs [74]. Indeed, mice with constitutively active autophagy due to a Phe121Ala mutation in Beclin-1 ($Becn1^{F121A/F121A}$) abolishing Beclin-1 and BCL2 interaction, significantly extend the lifespan and delays age-related pathologies such as fibrosis and spontaneous cancer development in organs [15]. $Becn1^{F121A/F121A}$ transgenic mice prove the aging phenotypes observed in klotho deficiency [32], indicating Beclin-1 autophagy could be a potential mechanism underlying the antiaging mechanism of klotho and other longevity-related signal pathways. It will be attractive to explore the mechanisms of autophagy during aging and pharmacological interventions that induce autophagy with possible effects on longevity and lifespan extension in humans.

10.7 Conclusions and Perspectives

Up to now, many studies have been performed to explore the cell-type-specific function of ATG genes in the regulation of diverse biological pathways including those in the immune responses and tissue homeostasis in health and disease conditions. Mutations in genes required for autophagy pathways have been implicated in the pathogenesis of immune-related diseases and cancer. Although these genes were first discovered in the classical autophagy pathways, those findings have inferred new functions related to noncanonical autophagy or autophagy-independent mechanisms. Further research is needed to define a cell-intrinsic autophagy network and the function of autophagy in the cell–cell interplay. More specific ways and disease context-dependent regimens are needed in terms of targeting autophagy.

References

1. Aden K, Tran F, Ito G, Sheibani-Tezerji R, Lipinski S, Kuiper JW, Tschurtschenthaler M, Saveljeva S, Bhattacharyya J, Hasler R et al (2018) ATG16L1 orchestrates interleukin-22 signaling in the intestinal epithelium via cGAS-STING. J Exp Med 215:2868–2886
2. Alissafi T, Banos A, Boon L, Sparwasser T, Ghigo A, Wing K, Vassilopoulos D, Boumpas D, Chavakis T, Cadwell K et al (2017) Tregs restrain dendritic cell autophagy to ameliorate autoimmunity. J Clin Invest 127:2789–2804
3. Beavan MS, Schapira AH (2013) Glucocerebrosidase mutations and the pathogenesis of Parkinson disease. Ann Med 45:511–521
4. Bel S, Pendse M, Wang Y, Li Y, Ruhn KA, Hassell B, Leal T, Winter SE, Xavier RJ, Hooper LV (2017) Paneth cells secrete lysozyme via secretory autophagy during bacterial infection of the intestine. Science 357:1047–1052
5. Bhattacharya A, Parillon X, Zeng S, Han S, Eissa NT (2014) Deficiency of autophagy in dendritic cells protects against experimental autoimmune encephalomyelitis. J Biol Chem 289:26525–26532
6. Brochard V, Combadiere B, Prigent A, Laouar Y, Perrin A, Beray-Berthat V, Bonduelle O, Alvarez-Fischer D, Callebert J, Launay JM et al (2009) Infiltration of $CD4^+$ lymphocytes into the brain contributes to neurodegeneration in a mouse model of Parkinson disease. J Clin Invest 119:182–192
7. Brown EM, Kenny DJ, Xavier RJ (2019) Gut microbiota regulation of T cells during inflammation and autoimmunity. Annu Rev Immunol 37:599–624
8. Chauhan S, Mandell MA, Deretic V (2015) IRGM governs the core autophagy machinery to conduct antimicrobial defense. Mol Cell 58:507–521
9. Chen M, Hong MJ, Sun H, Wang L, Shi X, Gilbert BE, Corry DB, Kheradmand F, Wang J (2014) Essential role for autophagy in the maintenance of immunological memory against influenza infection. Nat Med 20:503–510
10. Chitnis T, Weiner HL (2017) CNS inflammation and neurodegeneration. J Clin Invest 127:3577–3587
11. Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW et al (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325:201–204
12. Cunha LD, Yang M, Carter R, Guy C, Harris L, Crawford JC, Quarato G, Boada-Romero E, Kalkavan H, Johnson MDL et al (2018) LC3-associated phagocytosis in myeloid cells promotes tumor immune tolerance. Cell 175(429–441):e416
13. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, Bultman SJ (2011) The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab 13:517–526
14. Falcao AM, van Bruggen D, Marques S, Meijer M, Jakel S, Agirre E, Samudyata, Floriddia EM, Vanichkina DP, Ffrench-Constant C et al (2018) Disease-specific oligodendrocyte lineage cells arise in multiple sclerosis. Nat Med 24:1837–1844
15. Fernandez AF, Sebti S, Wei Y, Zou Z, Shi M, McMillan KL, He C, Ting T, Liu Y, Chiang WC et al (2018) Disruption of the beclin 1-BCL2 autophagy regulatory complex promotes longevity in mice. Nature 558:136–140
16. Franzosa EA, Sirota-Madi A, Avila-Pacheco J, Fornelos N, Haiser HJ, Reinker S, Vatanen T, Hall AB, Mallick H, McIver LJ et al (2019) Gut microbiome structure and metabolic activity in inflammatory bowel disease. Nat Microbiol 4:293–305
17. Ghosh A, Tyson T, George S, Hildebrandt EN, Steiner JA, Madaj Z, Schulz E, Machiela E, McDonald WG, Escobar Galvis ML et al (2016) Mitochondrial pyruvate carrier regulates autophagy, inflammation, and neurodegeneration in experimental models of Parkinson's disease. Sci Transl Med 8:368ra174
18. Graham DB, Luo C, O'Connell DJ, Lefkovith A, Brown EM, Yassour M, Varma M, Abelin JG, Conway KL, Jasso GJ et al (2018) Antigen discovery and specification of immunodominance hierarchies for MHCII-restricted epitopes. Nat Med 24:1762–1772

19. Green DR, Galluzzi L, Kroemer G (2011) Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. Science 333:1109–1112
20. Grootjans J, Krupka N, Hosomi S, Matute JD, Hanley T, Saveljeva S, Gensollen T, Heijmans J, Li H, Limenitakis JP et al (2019) Epithelial endoplasmic reticulum stress orchestrates a protective IgA response. Science 363:993–998
21. Gukovsky I, Li N, Todoric J, Gukovskaya A, Karin M (2013) Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. Gastroenterology 144(1199–1209):e1194
22. Haber AL, Biton M, Rogel N, Herbst RH, Shekhar K, Smillie C, Burgin G, Delorey TM, Howitt MR, Katz Y et al (2017) A single-cell survey of the small intestinal epithelium. Nature 551:333–339
23. Hansen M, Rubinsztein DC, Walker DW (2018) Autophagy as a promoter of longevity: insights from model organisms. Nat Rev Mol Cell Biol 19:579–593
24. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H et al (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 441:885–889
25. He C, Wei Y, Sun K, Li B, Dong X, Zou Z, Liu Y, Kinch LN, Khan S, Sinha S et al (2013) Beclin 2 functions in autophagy, degradation of G protein-coupled receptors, and metabolism. Cell 154:1085–1099
26. Heckmann BL, Boada-Romero E, Cunha LD, Magne J, Green DR (2017) LC3-associated phagocytosis and inflammation. J Mol Biol 429:3561–3576
27. Imhann F, Vich Vila A, Bonder MJ, Fu J, Gevers D, Visschedijk MC, Spekhorst LM, Alberts R, Franke L, van Dullemen HM et al (2018) Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. Gut 67:108–119
28. Jung HS, Chung KW, Won Kim J, Kim J, Komatsu M, Tanaka K, Nguyen YH, Kang TM, Yoon KH, Kim JW et al (2008) Loss of autophagy diminishes pancreatic beta cell mass and function with resultant hyperglycemia. Cell Metab 8:318–324
29. Kabat AM, Harrison OJ, Riffelmacher T, Moghaddam AE, Pearson CF, Laing A, Abeler-Dorner L, Forman SP, Grencis RK, Sattentau Q et al (2016) The autophagy gene Atg16l1 differentially regulates Treg and TH2 cells to control intestinal inflammation. Elife 5:e12444
30. Keller CW, Sina C, Kotur MB, Ramelli G, Mundt S, Quast I, Ligeon LA, Weber P, Becher B, Munz C et al (2017) ATG-dependent phagocytosis in dendritic cells drives myelin-specific CD4(+) T cell pathogenicity during CNS inflammation. Proc Natl Acad Sci USA 114:E11228–E11237
31. Kovacs JR, Li C, Yang Q, Li G, Garcia IG, Ju S, Roodman DG, Windle JJ, Zhang X, Lu B (2012) Autophagy promotes T-cell survival through degradation of proteins of the cell death machinery. Cell Death Differ 19:144–152
32. Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsugi T, Ohyama Y, Kurabayashi M, Kaname T, Kume E et al (1997) Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390:45–51
33. Larabi A, Barnich N, Nguyen HTT (2019) New insights into the interplay between autophagy, gut microbiota and inflammatory responses in IBD. Autophagy 1–14
34. Lassen KG, McKenzie CI, Mari M, Murano T, Begun J, Baxt LA, Goel G, Villablanca EJ, Kuo SY, Huang H et al (2016) Genetic coding variant in GPR65 alters lysosomal pH and links lysosomal dysfunction with colitis risk. Immunity 44:1392–1405
35. Lavoie S, Conway KL, Lassen KG, Jijon HB, Pan H, Chun E, Michaud M, Lang JK, Gallini Comeau CA, Dreyfuss JM et al (2019) The Crohn's disease polymorphism, ATG16L1 T300A, alters the gut microbiota and enhances the local Th1/Th17 response. Elife 8
36. Lee JH, Budanov AV, Park EJ, Birse R, Kim TE, Perkins GA, Ocorr K, Ellisman MH, Bodmer R, Bier E et al (2010) Sestrin as a feedback inhibitor of TOR that prevents age-related pathologies. Science 327:1223–1228

37. Levine B, Kroemer G (2019) Biological functions of autophagy genes: a disease perspective. Cell 176:11–42
38. Leyns CEG, Gratuze M, Narasimhan S, Jain N, Koscal LJ, Jiang H, Manis M, Colonna M, Lee VMY, Ulrich JD et al (2019) TREM2 function impedes tau seeding in neuritic plaques. Nat Neurosci 22:1217–1222
39. Lin NY, Beyer C, Giessl A, Kireva T, Scholtysek C, Uderhardt S, Munoz LE, Dees C, Distler A, Wirtz S et al (2013) Autophagy regulates TNFα-mediated joint destruction in experimental arthritis. Ann Rheum Dis 72:761–768
40. Lloyd-Price J, Arze C, Ananthakrishnan AN, Schirmer M, Avila-Pacheco J, Poon TW, Andrews E, Ajami NJ, Bonham KS, Brislawn CJ et al (2019) Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. Nature 569:655–662
41. Mansueto G, Armani A, Viscomi C, D'Orsi L, De Cegli R, Polishchuk EV, Lamperti C, Di Meo I, Romanello V, Marchet S et al (2017) Transcription factor EB controls metabolic flexibility during exercise. Cell Metab 25:182–196
42. Martinez J, Cunha LD, Park S, Yang M, Lu Q, Orchard R, Li QZ, Yan M, Janke L, Guy C et al (2016) Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. Nature 533:115–119
43. Martinez-Martin N, Maldonado P, Gasparrini F, Frederico B, Aggarwal S, Gaya M, Tsui C, Burbage M, Keppler SJ, Montaner B et al (2017) A switch from canonical to noncanonical autophagy shapes B cell responses. Science 355:641–647
44. Matsuzawa-Ishimoto Y, Shono Y, Gomez LE, Hubbard-Lucey VM, Cammer M, Neil J, Dewan MZ, Lieberman SR, Lazrak A, Marinis JM et al (2017) Autophagy protein ATG16L1 prevents necroptosis in the intestinal epithelium. J Exp Med 214:3687–3705
45. Mehto S, Jena KK, Nath P, Chauhan S, Kolapalli SP, Das SK, Sahoo PK, Jain A, Taylor GA, Chauhan S (2019) The Crohn's disease risk factor IRGM limits NLRP3 inflammasome activation by impeding its assembly and by mediating its selective autophagy. Mol Cell 73(429–445):e427
46. Mizushima N, Levine B (2010) Autophagy in mammalian development and differentiation. Nat Cell Biol 12:823–830
47. Moors TE, Hoozemans JJ, Ingrassia A, Beccari T, Parnetti L, Chartier-Harlin MC, van de Berg WD (2017) Therapeutic potential of autophagy-enhancing agents in Parkinson's disease. Mol Neurodegener 12:11
48. Munz C (2016) Autophagy proteins in antigen processing for presentation on MHC molecules. Immunol Rev 272:17–27
49. Nilsson P, Loganathan K, Sekiguchi M, Matsuba Y, Hui K, Tsubuki S, Tanaka M, Iwata N, Saito T, Saido TC (2013) Abeta secretion and plaque formation depend on autophagy. Cell Rep 5:61–69
50. Nixon RA (2013) The role of autophagy in neurodegenerative disease. Nat Med 19:983–997
51. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, Roberts RG, Nimmo ER, Cummings FR, Soars D et al (2007) Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet 39:830–832
52. Ponpuak M, Mandell MA, Kimura T, Chauhan S, Cleyrat C, Deretic V (2015) Secretory autophagy. Curr Opin Cell Biol 35:106–116
53. Pua HH, Dzhagalov I, Chuck M, Mizushima N, He YW (2007) A critical role for the autophagy gene Atg5 in T cell survival and proliferation. J Exp Med 204:25–31
54. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW et al (2007) Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 39:596–604
55. Saligrama N, Zhao F, Sikora MJ, Serratelli WS, Fernandes RA, Louis DM, Yao W, Ji X, Idoyaga J, Mahajan VB et al (2019) Opposing T cell responses in experimental autoimmune encephalomyelitis. Nature 572:481–487

56. Salio M, Puleston DJ, Mathan TS, Shepherd D, Stranks AJ, Adamopoulou E, Veerapen N, Besra GS, Hollander GA, Simon AK et al (2014) Essential role for autophagy during invariant NKT cell development. Proc Natl Acad Sci USA 111:E5678–E5687
57. Schirmer M, Garner A, Vlamakis H, Xavier RJ (2019) Microbial genes and pathways in inflammatory bowel disease. Nat Rev Microbiol 17:497–511
58. Schirmer M, Smeekens SP, Vlamakis H, Jaeger M, Oosting M, Franzosa EA, Horst RT, Jansen T, Jacobs L, Bonder MJ et al (2016) Linking the human gut microbiome to inflammatory cytokine production capacity. Cell 167:1897
59. Schmid D, Pypaert M, Munz C (2007) Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. Immunity 26:79–92
60. Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, Bar-Shira A, Berg D, Bras J, Brice A et al (2009) Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med 361:1651–1661
61. Singh R, Kaushik S, Wang Y, Xiang Y, Novak I, Komatsu M, Tanaka K, Cuervo AM, Czaja MJ (2009) Autophagy regulates lipid metabolism. Nature 458:1131–1135
62. Singh SB, Davis AS, Taylor GA, Deretic V (2006) Human IRGM induces autophagy to eliminate intracellular mycobacteria. Science 313:1438–1441
63. Smillie CS, Biton M, Ordovas-Montanes J, Sullivan KM, Burgin G, Graham DB, Herbst RH, Rogel N, Slyper M, Waldman J et al (2019) Intra- and inter-cellular rewiring of the human colon during ulcerative colitis. Cell 178(714–730):e722
64. Tan JMJ, Mellouk N, Osborne SE, Ammendolia DA, Dyer DN, Li R, Brunen D, van Rijn JM, Huang J, Czuczman MA et al (2018) An ATG16L1-dependent pathway promotes plasma membrane repair and limits Listeria monocytogenes cell-to-cell spread. Nat Microbiol 3:1472–1485
65. Tanaka S, Hikita H, Tatsumi T, Sakamori R, Nozaki Y, Sakane S, Shiode Y, Nakabori T, Saito Y, Hiramatsu N et al (2016) Rubicon inhibits autophagy and accelerates hepatocyte apoptosis and lipid accumulation in nonalcoholic fatty liver disease in mice. Hepatology 64:1994–2014
66. Tsuboi K, Nishitani M, Takakura A, Imai Y, Komatsu M, Kawashima H (2015) Autophagy protects against colitis by the maintenance of normal gut microflora and secretion of mucus. J Biol Chem 290:20511–20526
67. Ulland TK, Song WM, Huang SC, Ulrich JD, Sergushichev A, Beatty WL, Loboda AA, Zhou Y, Cairns NJ, Kambal A et al (2017) TREM2 maintains microglial metabolic fitness in Alzheimer's disease. Cell 170(649–663):e613
68. Venegas C, Kumar S, Franklin BS, Dierkes T, Brinkschulte R, Tejera D, Vieira-Saecker A, Schwartz S, Santarelli F, Kummer MP et al (2017) Microglia-derived ASC specks cross-seed amyloid-beta in Alzheimer's disease. Nature 552:355–361
69. Voet S, Srinivasan S, Lamkanfi M, van Loo G (2019) Inflammasomes in neuroinflammatory and neurodegenerative diseases. EMBO Mol Med 11
70. Wei J, Long L, Yang K, Guy C, Shrestha S, Chen Z, Wu C, Vogel P, Neale G, Green DR et al (2016) Autophagy enforces functional integrity of regulatory T cells by coupling environmental cues and metabolic homeostasis. Nat Immunol 17:277–285
71. Xu X, Araki K, Li S, Han JH, Ye L, Tan WG, Konieczny BT, Bruinsma MW, Martinez J, Pearce EL et al (2014) Autophagy is essential for effector CD8(+) T cell survival and memory formation. Nat Immunol 15:1152–1161
72. Yang L, Li P, Fu S, Calay ES, Hotamisligil GS (2010) Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab 11:467–478
73. Yang L, Liu C, Zhao W, He C, Ding J, Dai R, Xu K, Xiao L, Luo L, Liu S et al (2018). Impaired autophagy in intestinal epithelial cells alters gut microbiota and host immune responses. Appl Environ Microbiol 84
74. Zhang C, Cuervo AM (2008) Restoration of chaperone-mediated autophagy in aging liver improves cellular maintenance and hepatic function. Nat Med 14:959–965
75. Zhang Y, Sowers JR, Ren J (2018) Targeting autophagy in obesity: from pathophysiology to management. Nat Rev Endocrinol 14:356–376

76. Zhong Z, Sanchez-Lopez E, Karin M (2016) Autophagy, inflammation, and immunity: a troika governing cancer and its treatment. Cell 166:288–298
77. Zhou Y, Ulland TK, Colonna M (2018) TREM2-dependent effects on microglia in Alzheimer's disease. Front Aging Neurosci 10:202
78. Zott B, Simon MM, Hong W, Unger F, Chen-Engerer HJ, Frosch MP, Sakmann B, Walsh DM, Konnerth A (2019) A vicious cycle of beta amyloid-dependent neuronal hyperactivation. Science 365:559–565

Chapter 11
Targeting Autophagy with Small-Molecule Modulators in Immune-Related Diseases

Lan Zhang and Bo Liu

Abstract Autophagy, a highly conserved and multistep lysosomal degradation process, plays a pivotal role in maintaining cellular and physiological homeostasis. Of note, autophagy controls intracellular homeostasis and cell responses to stresses by regulating the self-renewal, maturation, and survival of immune cells. And dysregulation of autophagy in immune cells may contribute to the inflammatory disorders and defect in immune responses against invasive pathogens. Accumulating evidence have indicated that dysregulated autophagy participates in the pathology of immune-related diseases. Therefore, targeting autophagy might represent a promising therapeutic strategy for treatment of immune-related diseases. In this chapter, we focus on discussing the link between autophagy and pathogenesis of immune-related diseases, as well as the dysregulation of autophagy-related signaling pathways, in different diseases. Moreover, we highlight the therapeutic potential of currently used small-molecule modulators of autophagy for treatment of immune-related diseases and illustrate the mechanisms of these small-molecule modulators.

Keywords Autophagy · Small-molecule modulators · Immune-related diseases · Targeted therapy · Therapeutic potential

11.1 Introduction

Autophagy is a highly conserved cellular self-digestion process by which cellular components are targeted for degradation via lysosomes. Autophagy in mammalian cells can be categorized into three main ways: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA) [37, 52, 64], of which, the most intensively

L. Zhang
School of Life Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China

B. Liu (✉)
State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University, and Collaborative Innovation Center for Biotherapy, Chengdu 610041, China
e-mail: liubo2400@163.com

J. Cui (ed.), *Autophagy Regulation of Innate Immunity*, Advances in Experimental Medicine and Biology 1209, https://doi.org/10.1007/978-981-15-0606-2_11

studied is macroautophagy (hereafter referred to as autophagy). Autophagy is the major regulated catabolic mechanism that involves the delivery of cytoplasmic cargo sequestered inside double-membrane vesicles to the lysosome and is highly regulated by a number of autophagy-related genes (ATGs) [77]. In general, autophagy plays a Janus role and is implicated in certain human diseases [73, 90]. Moderate autophagy is regarded as a cytoprotective mechanism. It governs the degradation of damaged, denatured, aging, and loss-of-function cells, organelles, and biomacromolecules such as denatured proteins and nucleic acids, which provide raw materials for cell regeneration and reparation. Also, autophagy can resist the invasion of pathogens and protect cells from detrimental cellular components [45, 79].

The immune system is responsible for the surveillance and defense against the invasiveness of different exogenous pathogens and the maintenance of internal homeostasis by maintaining proper immune tolerance and regulation. In fact, immune surveillance and defense are the main immune responses. Based on the types of exogenous antigens, immune responses can exert antiviral, antibacterial, and antitumor functions depending on the diverse immunocytes that synthetically construct the immune defense. Elements, such as the inappropriate exposure to self-antigens, dysregulation of immune responses, and stimulation of cross antigens, may launch autoimmunity and promote the generation of certain immune diseases [6, 59]. Of note, autophagy not only acts as an adaptive supplier of nutrients, to maintain cellular energy metabolism under stress conditions such as nutrients and growth factor deprivation, but also serves as a functional regulator to maintain cellular integrity and homeostasis [50]. Accumulating evidence has recently revealed that autophagic pathways and autophagy-related proteins involved in this process play important roles in regulating infections, inflammation, and immunity. Autophagy can promote self-tolerance, an essential component of immune functions. While abnormal self-tolerance may promote immune-related diseases including Crohn's disease (CD), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis (MS) [50, 60, 71].

In this chapter, we discuss the role of autophagy and the dysregulation of autophagy-related signaling pathways in immune-related diseases, and how small-molecule modulators of autophagy used in the treatment of immune-related diseases have already been shown to affect autophagy induction as part of their mode of action.

11.2 Dysregulation of Autophagy in Immune-Related Diseases

It is well-known that autophagy plays a pivotal role in maintaining intracellular homeostasis and cell responses to stresses by controlling the self-renewal, maturation, and survival of immune cells, including T cells and B cells [4, 18]. In addition, autophagy is also required for the maturation of monocytes into macrophages, which

is closely associated the intracellular processing of inflammatory cytokines [95]. However, accumulating evidence has indicated that dysregulated autophagy often contributes significantly to the pathology of immune-related diseases, including CD, SLE, RA, and MS. Consequently, we discuss the dysregulation of autophagy and their relationship in each immune disease (Table 11.1).

11.2.1 Crohn's Disease

Crohn's disease (CD) and ulcerative colitis (UC) are the two different chronic conditions of inflammatory bowel disease (IBD), mainly characterized by inflammatory response and immune disorders in gastrointestinal tract [5]. Extensive studies have suggested that the etiology of CD is implicated with environmental and genetic factors that result in dysfunction of the epithelial barrier and deregulation of the mucosal immune response to gut microbiota [10]. Over the past decade, several susceptibility genes have been identified and linked the pathology of CD to autophagy by genome-wide association studies (GWAS). For instance, *autophagy-related 16-like 1* (*ATG16L1*) and *immunity-related GTPase family M* (*IRGM)*, two autophagy-related genes, have been identified as genetic risk factors for CD in consideration of their polymorphisms [24, 54, 63, 70]. In spite such genes are associated with the pathogenesis of CD, the function of autophagy in chronic inflammation and susceptibility to CD remains unclear. Studies have shown the genetic variations of *ATG16L*1 and *IRGM* can lead to defect in autophagy, causing prolonged survival and defective clearance of invasive bacteria [26, 40]. For example, the CD-associated ATG16L1 (T300A) variant exhibited defective ability of autophagy-mediated intracellular clearance of enteral pathogen *Salmonella Typhimurium* in intestinal epithelial cells [40]. Consequently, Cadwell et al. showed that ATG16L1 may be implicated in the pathogenesis of CD via a distinct mechanism. In a mouse model with hypomorphic ATG16L1 expression ($ATG16L1^{HM}$), Paneth cells exhibited markable granule abnormalities with impaired granule exocytosis pathway. Further transcriptional analysis revealed that the ATG16L1-deficient Paneth cells could raise the expression of genes associated with peroxisome proliferator-activated receptor (PPAR) pathways, adipocytokine signaling, and lipid metabolism. More importantly, CD patients with homozygous for the ATG16L1 risk allele showed similar granule abnormalities of Paneth cell to those observed in mice [9]. Another study found that stimulation of lipopolysaccharide (LPS) resulted in ATG16L1-deficient macrophages to produce high levels of IL-1β and IL-18. Deficiency of ATG16L1 in mice hematopoietic cells also leads to high susceptibility with dextran sulfate sodium (DSS)-induced acute colitis, which is often used as an experimental colitis model to investigate the pathology of CD. Moreover, treatment with anti-IL-1β and IL-18 antibodies could ameliorate the mortality and loss of body weight in DSS-treated ATG16L1-deficient mice, suggesting the important role of ATG16L1 in immune responses against intestinal inflammation [72]. More recently, Murthy et al. revealed that the amino acids 296–299 of ATG16L1 contain a caspase cleavage motif and that the

Table 11.1 The dysregulation of autophagy associated with pathogenesis of immune diseases

Disease	Autophagy-related gene and protein	Mechanism associated with pathogenesis of immune diseases	Cell type or animal model	References
Crohn's disease	ATG16L1	ATG16L1 (T300A) variant exhibits defective autophagy-mediated clearance of pathogen	Intestinal epithelial cells	[40]
		Hypomorphic ATG16L1 expression exhibits granule abnormalities with impaired exocytosis pathway	Paneth cells and ATG16L1^{HM} mouse model	[9]
		ATG16L1 deficiency produces high levels of IL-1β and IL-18	LPS-stimulated macrophages	[72]
	IRGM	IRGM deficiency decreases the clearance of bacteria with impaired autophagy induction	Intestinal epithelial cells and macrophages	[43, 44, 54]
	NOD2	NOD2 variants impair MDP-induced activation of autophagy, NF-κB, and MAPK signaling pathways	Epithelial cells, macrophages, and dendritic cells	[28]
	ATG4B	ATG4B deficiency exhibits an increase of pro-inflammatory cytokines in response to infectious bacteria	Paneth cells and ATG4B-deficient mice	[8]

(continued)

Table 11.1 (continued)

Disease	Autophagy-related gene and protein	Mechanism associated with pathogenesis of immune diseases	Cell type or animal model	References
Systemic lupus erythematosus	mTOR	IL-21 block differentiation and function of Treg cell through suppression of autophagy via activating mTOR	Treg cell	[34]
	ATG5	Knockout of ATG5 eliminates increased serum level of inflammatory cytokine and autoantibody	TLR7 transgenic mouse model of SLE	[82]
		SNP of ATG5 (rs573775) increases production of IL-10	–	[49]
	LRRK2	LRRK2 deficiency attenuates autoantibody production	Pristane-induced mice model of SLE	[94]
	LAP	LAP deficiency increases levels of pro-inflammatory cytokines and autoantibodies	LAP-deficient mice	[51]
Rheumatoid arthritis	ALFY, p62	Decreased expression of ALFY facilitates cell death of RA-FLS	FLS	[35]
	ATG7	Knockout of ATG7 reduces numbers of osteoclasts, alleviates TNF-α-induced bone erosion, proteoglycan loss, and chondrocyte death	Osteoclasts	[47]
	FIP200	Deletion of FIP200 leads to osteopenia in mice through reduced level of autophagy	Osteoblasts	[48]

(continued)

Table 11.1 (continued)

Disease	Autophagy-related gene and protein	Mechanism associated with pathogenesis of immune diseases	Cell type or animal model	References
Multiple sclerosis	ATG5, Parkin	Activates autophagy/mitophagy and increases TNF-α production	–	[2, 65]
	ATG5	Increases TNF production	EAE mice model	[66]
	Beclin-1	Knockdown of Beclin-1 increases PTGS2 expression through activation of the ROS-MAPK1/3 pathway	EAE mice model	[15]
	ATG7	Inhibits autophagy by conditional knockout of ATG7 to suppress survival of autoreactive $CD4^+$ T cells	Dendritic cells and EAE mice model	[7]
	ATG16L2	Inhibits autophagy	–	[91]

ATG16L1 (T300A) variant significantly enhances the sensitization to caspase-3-mediated degradation of ATG16L1, which lead to the impaired autophagy in CD [57].

IRGM, as a member of p47 immunity-related guanosine triphosphatase family, is induced by IFN-γ and activates autophagy as a mechanism to eliminate the invasive pathogens. Genetic studies have revealed that there are two polymorphisms of IRGM including a "silent" SNP within the coding region (c.313C > T) and a 20 kb deletion upstream of the IRGM gene, which are highly associated with CD risk [54, 63]. Previous studies have revealed the knockdown of *IRGM* resulted in defective autophagy and decreased internalized bacteria within autophagosomes in *Salmonella Typhimurium* infected HeLa cells, whereas overexpression of IRGM reversed this phenomenon [54]. Also, the depletion of *IRGM* increased the intracellular replication of adherent-invasive *Escherichia coli* (AIEC) in human intestinal epithelial cells and macrophages, which was caused by impaired autophagy induction [43, 44].

Nucleotide oligomerization domain 2 (NOD2), the first locus identified as a risk factor for CD, is a member of the NOD-like receptors (NLR) family, also acts as an intracellular sensor of pathogen/microbe-associated molecular patterns (P/MAMP), which play important roles in innate immunity. Until now, three mutations in NOD2 have been found to be strongly associated with CD onset, including two amino acid substitutions (R702W and G908R) as well as a frameshift mutation (L1007fsinsC) that results in a truncated NOD2 protein [31, 62]. Thereafter, studies showed that muramyl dipeptide (MDP)-mediated NOD2 activation could induce autophagy in dendritic cells. ATG16L1 can be recruited to plasma membrane at the bacterial entry site by NOD2, thus initiating autophagosome formation to parcel and clear the invading pathogens. These studies were also the first to link the function of NOD proteins with autophagy induction [13, 78]. Furthermore, a recent study demonstrated that MDP stimulation of epithelial cells, macrophages, and dendritic cells activated autophagy and the nuclear factor κB (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways, as well as increased the killing of *Salmonella*. The expression of ATG16L1 and NOD2 seem to be required in these responses, which were impaired by CD-associated variants of NOD2. Interestingly, the ATG16L1 (T300A) variant only blocked the antibacterial pathway in epithelial cell lines and not in primary macrophages or dendritic cells, indicating that is altered in a cell- and function-specific manner by CD-associated mutations [28].

With the exception of *ATG16L1*, *IRGM*, and *NOD2* genes, another study has reported a significant association of CD susceptibility with an intronic polymorphism in the autophagy gene ULK1 [25]. In addition, the Paneth cell of *ATG4B*$^{-/-}$ mice was found to display granule abnormalities and increased susceptibility to DSS-induced colitis. Meanwhile, the ATG4B-deficient mice also exhibit markable increase of pro-inflammatory cytokines in response to infectious bacteria, which are alleviated by antibiotic treatments or bone marrow transplantation from wild-type mice. These findings indicate that ATG4B may serve as a novel protective target in inflammatory colitis, especially for the treatment of CD [8].

11.2.2 Systemic Lupus Erythematosus

The systemic lupus erythematosus (SLE) is a chronic autoimmune disease, which is characterized by dysregulation of B cells, T cells, and mature plasma cells, as well as increased production of autoantibodies, resulting in systemic inflammation and multiple organ damage [39, 83, 84]. A large number of studies have shown that autophagy plays an important role in the pathogenesis and disease progression of SLE. For instance, the hyperactivation of T cells can enhance the cytokine and autoantigen associated signaling, which play a crucial role in the pathogenesis of SLE, and also lead to dysfunction of B cells [67]. Intriguingly, the T cells of SLE patients displayed notable dysregulated autophagy, accompanied by growing numbers of autophagic vesicle and reduced autophagic flux. Meanwhile, the $CD4^+$ T cells from SLE patients also exhibit increased accumulation of LC3-II compared to its levels in $CD4^+$ T cells from healthy donors, indicating that autophagic flux may be disrupted in T cells of SLE patients [1, 23]. Recently, IL-21, a pro-inflammatory cytokine, has been reported to block differentiation and function of regulatory T cells (Treg) in patients with SLE, through suppression of autophagy via activating mTORC1 and mTORC2 [34].

Autophagy is dysregulated in both T-cell and B-cell of patients with SLE, but it seems more significantly abnormal in B cells. For example, suppressor of T-cell receptor signaling 1 (STS-1), a protein tyrosine phosphatase, was overexpressed in B cells from patients with SLE, as well as in mouse model (MRL/lpr mice), thereby upregulating IFN-α-induced autophagy in B cells via activating the JAK1-STAT1 signaling pathway [17]. The researchers also found that B-cell autophagy was activated in the early stages of disease in SLE mouse model, as well as in patients with SLE, which is required for the differentiation of B cells into plasma cells [12]. Additionally, the expression of autophagy-related genes such as *BECN1*, *LC3*, and *p62* have been reported to be simultaneously increased in peripheral blood mononuclear cells (PBMCs) of SLE patients, indicating autophagosomes formation might be activated and their degradation were blocked in SLE [85]. Furthermore, in a toll-like receptor 7 (TLR7) transgenic mouse model of SLE, B cell-specific abrogation of autophagy by knockout of *ATG5* can eliminate the increased serum level of inflammatory cytokine and production of autoantibody, as identified as the hallmarks of SLE [82]. Orosomucoid-like 3 (ORMDL3), as a key endoplasmic reticulum stress (ERS)/unfolded protein response (UPR) inducer, was shown to be upregulated both in PBMCs from patients with SLE and in the spleen of lupus mice. ORMDL3 facilitated the survival of splenic B cells via suppressing apoptosis and promoting autophagy via the ATF6-Beclin-1 autophagy pathway, suggesting that it may play a pivotal role in B cell differentiation and survival in the pathogenesis of SLE [14]. Interestingly, genetic studies have also revealed the *ATG5* gene is associated with increased susceptibility for developing SLE. For example, a single-nucleotide polymorphism (SNP) of ATG5 (rs573775) was found to increase the risk of SLE and the production of IL-10, which is closely related to functional IL-10 genotype [49]. In addition, other

studies also identified several ATG5 SNPs in Chinese populations as genetic risk factor for development of SLE [96, 101]. Besides *ATG5* gene, a more recent study has reported the polymorphism of autophagy-related *LRRK2* gene (rs2638272) showing important correlation with susceptibility to SLE in northern Han Chinese population [97]. More recently, it has been found that *LRRK2* expression is upregulated in B cells from SLE patients and has significant association with disease severity. Moreover, *LRRK2* deficiency largely attenuates the pathological symptoms and autoantibody production in pristane-induced mice model of SLE [94].

LC3-associated phagocytosis (LAP), a non-canonical form of autophagy, has been found to conduce to the engulfment and clearance of dying cells and pathogens via an endocytic and lysosomal delivery process. In LAP-deficient mice, dying cells can be engulfed but not efficiently degraded, leading to increased levels of pro-inflammatory cytokines and autoantibodies, which might accelerate the development of SLE-like disease. On the contrary, in mice deficient of *FIP200* or *ULK1*, dying cells are engulfed, macrophages produce IL-10 but not inflammatory cytokines, and no lupus-like disease is observed [51]. Therefore, defects in LAP, rather than canonical autophagy, may contribute to the pathogenesis of SLE.

11.2.3 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory autoimmune disease characterized by cartilage degradation, synovial hyperplasia, and fibroblast-like synoviocytes (FLS) infiltration, resulting in the progression of bone and joint damage. Extensive studies have demonstrated several mechanisms contributing to the survival of synovial fibroblasts in RA, including upregulation of antiapoptotic factors and downregulation of proapoptotic factors, as well as enhanced autophagy [86, 87]. For example, a study shows that the apoptosis ratio of RA-FLS has a negative correlation to the expression levels autophagy markers such as Beclin-1 and LC3 [87]. Additionally, autophagy is significantly increased in $CD4^+$ T cells from RA patients, and the increased apoptosis resistance is also observed in $CD4^+$ T cells from RA patients, which is reversed by autophagy inhibition. Moreover, autophagy inhibition reduces both arthritis incidence and disease severity in an arthritis mouse model, indicating that enhanced autophagy may contribute to RA pathogenesis [80]. However, autophagy activation on the survival of RA-FLS is controversial. A study has been reported that decreased expression of Autophagy-linked FYVE protein (ALFY) and the aggregation of p62-positive polyubiquitinated protein facilitate cell death of RA-FLS under ERS, indicating that autophagy plays a promotive role in cell death of FLS under ERS. Whereas, inhibition of proteasomal activity of RA-FLS increases LC3-II protein levels and prolongs their survival, suggesting that increased autophagy can also promote RA-associated synovitis [35]. In addition, researchers have found that autophagy-related proteins are elevated in the synovial tissues from patients with active RA, which is strongly associated with the serum levels of several RA activity-related markers and correlated with disease severity [102]. Moreover,

IL-17 and IL-17-producing T helper (Th17) cells have been found to impair and show resistance to apoptosis in RA-FLS through induction of mitochondrial dysfunction and autophagy [36].

Interestingly, autophagy seems to play different roles in bone homeostasis of RA. For example, a study has been reported that autophagy is activated by TNF-α in osteoclasts of patients with RA, as indicated by increased expression of Beclin-1 and ATG7. And abrogation of autophagy by knockout of *ATG7* results in reduced numbers of osteoclasts, as well as alleviates TNF-α-induced bone erosion, proteoglycan loss, and chondrocyte death, indicating that autophagy has a vital role in TNF-induced osteoclast differentiation and osteoclast-mediated bone resorption [47]. Conversely, osteoblast-specific deletion of *FIP200* leads to osteopenia in mice through reduced level of autophagy, suggesting autophagy plays a positive role in osteoblast nodule formation and differentiation [48].

11.2.4 Multiple Sclerosis

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) with characteristics including inflammatory disorder, demyelination, and neurodegeneration, which is mainly caused by autoreactive T cells that attack the myelin sheath in the CNS [46]. Autophagy is closely associated with autoimmune diseases such as MS. However, the mechanism of autophagy in the pathogenesis of MS is still elusive. Previous studies have found that the expression of ATG5 is elevated in experimental autoimmune encephalomyelitis (EAE) mice model of MS, as well as in blood and brain tissues from MS patients [2]. Another study also found that both ATG5 and Parkin expression levels are increased in blood and cerebrospinal fluid (CSF) of patients with MS, indicating that autophagy/mitophagy play an important role in the pathology of MS. In addition, TNF-α concentration in CSF has positive correlations with both ATG5 and Parkin levels, suggesting that activation of autophagy/mitophagy is closely associated with inflammatory stimuli in MS [65]. Distinct from abovementioned studies, a recent study has been reported that the expression level of ATG5 is increased in T cells rather than B cells of MS patients compared with control group. However, this process is not associated with autophagy induction. Additionally, there is a positive correlation between *ATG5* and *TNF* in mRNA levels, suggesting that autophagy-independent increase of ATG5 expression might contribute to the proinflammatory capacity of T cells in MS patients [66]. Similar to ATG5, suppression of autophagy by knockdown of *BECN1* obviously enhances the therapeutic effects of mesenchymal stem cells (MSCs) in EAE, which is mainly dependent on inhibition of autoreactive $CD4^+$ T cells [15]. Moreover, inhibition of autophagy by conditional knockout of *ATG7* in dendritic cells (DCs), the most potent antigen-presenting cells (APCs) in the immune system, also has a protective effect in the EAE mice model of MS [7]. Conversely, ATG16L2, as a component of ATG5–ATG12–ATG16 complex, has been shown to decrease in blood samples of MS patients compared with healthy control, indicating the complicated role of autophagy in pathogenesis of MS [33, 91].

Thus, further study needs to be conducted to illustrate the interrelation of autophagy and MS, according to different cell types and different onset stages of MS.

11.3 Small-Molecule Modulators of Autophagy in Immune-Related Diseases

With the progress of unraveling the relationship between autophagy and pathogenesis of immune diseases, a large number of small-molecule modulators that regulate components of the autophagic pathway have been discovered in recent years (Table 11.2). These agents often directly or indirectly link to the autophagy response with the modulation of immunoregulatory signaling pathways. Next, we discuss the currently used small-molecule modulators of autophagy in immune-related diseases and illustrate the mechanisms of these agents in this section.

11.3.1 Small-Molecule Modulators of Autophagy for Treatment of CD

In recent years, a number of autophagy-modulating drugs have been identified as potential therapeutic agents for the treatment of CD. For instance, sirolimus (also known as rapamycin), which binds FKBP12 and inhibits the mTORC1 complex, has been used in clinical trials for the treatment of CD. Sirolimus seems to be effective as rescue therapy with severe refractory CD by modulating autophagy to improve symptoms and healing in both adult and child patients [53, 58]. CD-associated genetic variants that regulate bacterial recognition (NOD2, IRGM) and autophagy (ATG16L1) are involved with reduced clearance of intracellular bacteria. Recently, BRD5631, a small-molecule enhancer of autophagy, has been reported to enhance autophagy through an mTOR-independent pathway to clear invasive bacteria and suppress IL-1β production in cells harboring the CD-associated allele of ATG16L1 (T300A) [41]. Furthermore, celastrol, a triterpene extracted from the root bark of the Chinese medicinal plant *Tripterygium wilfordii*, has been found to ameliorate DSS-induced colon injury and inflammation in mouse model of UC, with the mechanism of inhibition of NF-κB signaling, modulation of oxidative stress, inflammatory cytokines, and intestinal homeostasis [75]. A recent study has indicated that celastrol could ameliorate the inflammation of IL-10 deficient mice, a mouse model of CD with the induction of autophagy by suppressing the PI3K/AKT/mTOR signaling pathway [99]. Additionally, another study showed that docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid, could attenuate experimental chronic colitis in *IL-10*$^{-/-}$ mice by triggering autophagy via inhibition of the mTOR pathway, as demonstrated by suppression

Table 11.2 Small-molecule modulators of autophagy in immune diseases

Disease	Modulator	Autophagy-related mechanism	Cell/Animal model	Clinical trial	References
Crohn's disease	Sirolimus	Induces autophagy by inhibiting mTORC1 activity	–	Phase IV	[53, 58]
	BRD5631	Induces autophagy via an mTOR-independent pathway	HeLa cells harboring the CD-associated allele of ATG16L1 (T300A)	–	[41]
	Celastrol	Induces autophagy by suppressing the PI3K/Akt/mTOR signaling pathway	IL-10 deficient mice	–	[99]
	Docosahexaenoic acid (DHA)	Induces autophagy via inhibition of the mTOR pathway	IL-10 deficient mice	–	[98]
	Chlorpromazine	Induces autophagy via dose-dependent increase of LC3	Monocyte-derived macrophages (MDM)	–	[74]
	AMA0825	Induces autophagy via ROCK inhibition	DSS-induced colitis mice model	–	[27]
	Azathioprine	Induces autophagy via mTORC1 signaling and stimulation of PERK	THP-1-derived macrophages	–	[29]
Systemic lupus erythematosus	Sirolimus	Induces autophagy by inhibiting mTORC1 activity	(NZBxNZW)F1 mice	Phase I/II	[42, 69]

(continued)

Table 11.2 (continued)

Disease	Modulator	Autophagy-related mechanism	Cell/Animal model	Clinical trial	References
	Hydroxychloroquine	Inhibits autophagy by blocking fusion of autophagosomes with lysosomes	–	Phase II	[68]
	Pioglitazone	Activates PPARγ, and indirectly modulates mTOR activity and activates autophagy	MRL/lpr mice model of SLE	Phase I/II	[3, 81]
Rheumatoid arthritis	Hydroxychloroquine	Inhibits autophagy by blocking fusion of autophagosomes with lysosomes	–	Phase IV	[56, 61]
	Resveratrol	Suppresses oxidative stress and increases mitochondrial reactive oxygen species (mtROS) production by inhibiting autophagy	Adjuvant-arthritis (AA) rats model	–	[93]
	Metformin	Induces autophagy via AMPK-mediated ULK1 activation	Macrophages, KRN/I-A^{g7} (K/BxN) mice	–	[32, 89]
	Astragalus polysaccharides	Induces autophagy by inhibiting PI3K/AKT/mTOR pathway	IL-1β-stimulated RSC-364 cells, RA-FLS	–	[55]

(continued)

Table 11.2 (continued)

Disease	Modulator	Autophagy-related mechanism	Cell/Animal model	Clinical trial	References
	Artesunate	Induces autophagy by inhibiting PI3K/AKT/mTOR pathway	Chondrocytes of rats with RA	–	[20]
	Triptolide	Inhibits autophagy by decreasing LC3 expression	Neutrophil, adjuvant-arthritis (AA) rats model	–	[30]
Multiple sclerosis	Sirolimus	Induces autophagy by inhibiting mTOR activity	EAE mice model	–	[16, 19, 21, 88]
	Clioquinol	Induces autophagy by elevated expression of Beclin-1 and LC3	MOG-induced EAE mice model		[11]
	1,25-dihydroxyvitamin D3	Induces autophagy by elevated expression of Beclin-1	EAE mice model		[100]
	Hydroxychloroquine	Inhibits autophagy by blocking fusion of autophagosomes with lysosomes	Human microglia and EAE mice model	–	[38]

of colonic pro-inflammatory cytokines and reduction of inflammatory cells infiltration [98]. An FDA-approved drug, chlorpromazine, also used as autophagy inducer, has been identified to overcome bacterial killing defect in Niemann–Pick disease type C1 (NPC1), whereas mutations in NPC1 predispose to early-onset IBD with Crohn's disease phenotype and granuloma formation [74]. Obstruction of intestinal fibrosis is a common complication of CD. Recent studies have demonstrated that AMA0825, a small-molecule inhibitor of Rho kinases (ROCKs), could prevent and reverse intestinal fibrosis by diminishing MRTF and p38 MAPK activation and increasing autophagy in fibroblasts, which would be utilized as a promising add-on therapy for CD [27]. Furthermore, the immunomodulator azathioprine, widely used as IBD drugs, has been reported to induce autophagy via mechanisms involving modulation of mTORC1 and stimulation of the UPR sensor PERK, leading to enhancement of clearance of intracellular AIEC and repression of AIEC-induced production of TNF-α [29].

11.3.2 Small-Molecule Modulators of Autophagy for Treatment of SLE

In recent years, autophagy has been regarded as a therapeutic target in SLE treatment. Sirolimus, as an mTOR inhibitor, was originally approved immunosuppressive agent for organ transplantation. In a previous study, sirolimus treatment has been shown to reduce disease activity and requirement for prednisone therapy in patients with active SLE, compared with standard treatment [22]. Sirolimus has been also demonstrated to be effective for treatment of lupus mice, which is characterized by reduced levels of autoantibodies, proteinuria, and prolonged survival in mice [69]. More recently, a phase I/II clinical study has been reported that sirolimus treatment shows obvious progressive improvement of disease activity in patients with active SLE, which is correlated to correction of pro-inflammatory T-cell lineage specification [42]. Additionally, a recent study has revealed that the activities of mTORC1 and mTORC2 in SLE Treg cells were increased, whereas the expression of GATA-3 and CTLA-4 as well as the level of autophagy were reduced. Nevertheless, IL-21 contributed to the defects of autophagy and dysfunction of Treg cells in patients with SLE. Interestingly, treatment with rapamycin enhanced transforming growth factor β (TGF-β) production and induced autophagy via blockade of mTORC1 and mTORC2, meanwhile restored the expression of GATA-3 and CTLA-4 and corrected Treg cell function [34]. Considering the important role of autophagy in immune-related diseases and the fact that autophagy is usually dysfunctional in SLE, hydroxychloroquine, as an autophagy inhibitor to prevent autophagosome–lysosome fusion, has been studied in clinical trials and regarded to be an effective therapy for SLE. However, the use of hydroxychloroquine could also cause safety issue such as retinopathy that needs to be frequently monitored on such therapies [68]. Pioglitazone was approved by FDA for treatment of T2DM because it increases cell sensitivity to insulin. As an

agonist of PPARγ, pioglitazone can indirectly modulate mTOR activity and activate autophagy, thus it has also been applied in clinical trial studies in patients with SLE and exhibited some beneficial effects [3, 81].

11.3.3 Small-Molecule Modulators of Autophagy for Treatment of RA

Chloroquine (CQ) is a well-known antimalarial drug that is widely used for the prophylaxis treatment of malaria, whereas the anti-inflammatory property of CQ also makes it a useful agent for the treatment of RA [76]. Nevertheless, hydroxychloroquine (HCQ) was found to show a more favorable therapeutic effect than CQ with less eye toxicity in a few decades ago, thus making it widely used for the treatment of RA until now. As a component of triple-drug therapy, HCQ is often used in combination with methotrexate and sulfasalazine due to inhibition of autophagy, which has been advocated as a safe, well-tolerated alternative to biologic therapy for RA [56, 61]. Resveratrol is a polyphenol derivative which exhibits a proapoptotic effect in a variety of human cancers by triggering mitochondria apoptosis pathway and autophagy [92]. A recent study has reported that resveratrol could suppress oxidative stress in adjuvant-arthritis (AA) rats and increase mitochondrial reactive oxygen species (mtROS) production by inhibiting autophagy to promote the apoptosis of fibroblast-like synoviocytes (FLSs) [93]. Metformin is an insulin-sensitizing drug originally approved for the treatment of T2DM. As an AMPK activator, metformin can directly activate ULK1 via phosphorylation modification and thus promote autophagy. Additionally, it has also been reported to reduce secretion of cytokines such as TNF-α from macrophages in vitro, and suppress clinical arthritis in mouse models of RA. However, it remains unclear whether this agent's ability to attenuate disease in animal models of RA should be attributed to activation of autophagy or to reduced TNF-α secretion by macrophages [32, 89]. Indeed, these findings demonstrate the critical role of autophagy in RA and that autophagy inducers such as metformin may be used as potential therapeutic approaches for the treatment of RA. In addition to these agents, there are also other interesting examples of autophagy-inducing agents from traditional Chinese medicine. For example, Astragalus polysaccharides, one of the primary bioactive ingredients of *Astragalus membranaceus*, has been found to attenuate the pathological progression of RA, increase cell apoptosis, and reduce the production of pro-inflammatory cytokines in IL-1β-stimulated FLSs by regulating autophagy via PI3K/AKT/mTOR pathway [55]. Artesunate, a semisynthetic derivative of artemisinin obtained from the plant *Artemisia annua*, is a well-known effective drug that is used to treat malaria and other diseases involving inflammation. Recently, artesunate has been found to inhibit chondrocyte proliferation and accelerate cell apoptosis and autophagy via suppression of the PI3K/AKT/mTOR signaling pathway in rat models with RA [20]. Triptolide is a bioactive compound derived from Tripterygium wilfordii Hook F, which has been used in folk medicine

as a treatment for a variety of inflammatory disorders including RA. Triptolide has been found to ameliorate adjuvant-induced arthritis (AA) by reducing neutrophil recruitment and suppressing the expression of IL-6 and TNF-α in vivo, as well as suppress the expression of pro-inflammatory cytokines in neutrophils, promote neutrophil apoptosis, and inhibit the migration, NETosis, and autophagy of neutrophils in vitro [30].

11.3.4 Small-Molecule Modulators of Autophagy for Treatment of MS

mTOR, as the gatekeeper of autophagy, plays important roles in the regulation of oligodendrocyte development and myelination process as well as several neuronal functions. Therefore, robust evidence has shown that rapamycin (represent by sirolimus in Table 11.2) ameliorates the clinical course and significantly reduces the hyperalgesia in both relapsing–remitting and chronic EAE models [16]. In addition, several studies have been reported that rapamycin mitigates EAE-induced autophagy suppression, inflammation, demyelination, and neuronal damage of EAE mice [19, 21]. Moreover, MCC950, an inhibitor of NLRP3, combined with rapamycin reduces both the clinical symptom of EAE and the release of cytokines in microglia, indicating that mTOR and autophagy activation might be potential therapeutics for treatment of MS [88]. Clioquinol, a metal chelator, inhibits the clinical course of EAE accompanied with decrease in demyelination and reduction of encephalitogenic immune cells infiltration. Clioquinol also remarkably inhibits EAE-associated blood–brain barrier disruption and MMP-9 activation, as well as further increase autophagy activation for clearance of aggregated proteins in astrocytes and neurons [11]. 1,25-dihydroxyvitamin D3, an active form of vitamin D, reduces inflammation, demyelination, and neuron injury in the spinal cord of mice, which is associated with increased autophagy level [100]. Different from these autophagy inducers, HCQ, the autophagosome–lysosome fusion inhibitor, has been reported to reduce the activation of human microglia both in vitro and in vivo, as well as ameliorate demyelination and delay the onset of EAE mice [38].

11.4 Conclusion

In summary, as a universal and physiological phenomenon, autophagy has been found to play crucial roles in immunity, infection, and inflammation, and researches have expanded into all aspects of immune research in recent years. Although considerable progressions have been made in understanding autophagy-controlled innate and adaptive immunity, researches into autophagic regulation of inflammation, infection, and autoimmune disease have just started. Thus, further genetic studies may uncover

the potential role of the autophagy pathway in immune diseases through the identification of risk polymorphisms in various autophagy-related genes and those which regulate autophagy. Also, unraveling the detailed mechanisms between autophagy-related signaling pathways and immune-related diseases pathogenesis may help us to identify novel targets for the development of drugs used in clinical therapy. Although many small-molecule modulators of autophagy have been widely used for the treatment of immune diseases such as CD, SLE, RA, and MS, and showed certain therapeutic effects. However, some of these compounds also have significant disadvantages and limitations. Therefore, identifying and developing novel and better small molecules that can specifically control autophagy would be still urgently needed. At present, combination of computational methods and traditional medicinal chemistry approaches may contribute to, rapidly and efficiently, obtain compounds that regulate autophagy with more specificity. Meanwhile, artificial intelligence holds great promise in discovery, transformation, and application of small-molecule compounds. We believe that targeting autophagy would be a promising and beneficial therapeutic approach for treatment of immune-related diseases.

References

1. Alessandri C, Barbati C, Vacirca D, Piscopo P, Confaloni A, Sanchez M, Maselli A, Colasanti T, Conti F, Truglia S et al (2012) T lymphocytes from patients with systemic lupus erythematosus are resistant to induction of autophagy. FASEB J 26:4722–4732
2. Alirezaei M, Fox HS, Flynn CT, Moore CS, Hebb AL, Frausto RF, Bhan V, Kiosses WB, Whitton JL, Robertson GS et al (2009) Elevated ATG5 expression in autoimmune demyelination and multiple sclerosis. Autophagy 5:152–158
3. Aprahamian TR, Bonegio RG, Weitzner Z, Gharakhanian R, Rifkin IR (2014) Peroxisome proliferator-activated receptor gamma agonists in the prevention and treatment of murine systemic lupus erythematosus. Immunology 142:363–373
4. Arnold J, Murera D, Arbogast F, Fauny JD, Muller S, Gros F (2016) Autophagy is dispensable for B-cell development but essential for humoral autoimmune responses. Cell Death Differ 23:853–864
5. Baumgart DC, Sandborn WJ (2012) Crohn's disease. Lancet 380:1590–1605
6. Belkaid Y, Harrison OJ (2017) Homeostatic immunity and the Microbiota. Immunity 46:562–576
7. Bhattacharya A, Parillon X, Zeng S, Han S, Eissa NT (2014) Deficiency of autophagy in dendritic cells protects against experimental autoimmune encephalomyelitis. J Biol Chem 289:26525–26532
8. Cabrera S, Fernández AF, Mariño G, Aguirre A, Suárez MF, Español Y, Vega JA, Laurà R, Fueyo A, Fernández-García MS et al (2013) ATG4B/autophagin-1 regulates intestinal homeostasis and protects mice from experimental colitis. Autophagy 9:1188–1200
9. Cadwell K, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Kc W, Carrero JA, Hunt S et al (2008) A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. Nature 456:259–263
10. Cho JH, Brant SR (2011) Recent insights into the genetics of inflammatory bowel disease. Gastroenterology 140:1704–1712
11. Choi BY, Jang BG, Kim JH, Seo JN, Wu G, Sohn M, Chung TN, Suh SW (2013) Copper/zinc chelation by clioquinol reduces spinal cord white matter damage and behavioral deficits in a murine MOG-induced multiple sclerosis model. Neurobiol Dis 54:382–391

12. Clarke AJ, Ellinghaus U, Cortini A, Stranks A, Simon AK, Botto M, Vyse TJ (2015) Autophagy is activated in systemic lupus erythematosus and required for plasmablast development. Ann Rheum Dis 74:912–920
13. Cooney R, Baker J, Brain O, Danis B, Pichulik T, Allan P, Ferguson DJ, Campbell BJ, Jewell D, Simmons A (2010) NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. Nat Med 16:90–97
14. Dang J, Bian X, Ma X, Li J, Long F, Shan S, Yuan Q, Xin Q, Li Y, Gao F et al (2017) ORMDL3 facilitates the survival of splenic B cells via an ATF6α-endoplasmic reticulum stress-beclin1 autophagy regulatory pathway. J. Immunol 199:1647–1659
15. Dang S, Xu H, Xu C, Cai W, Li Q, Cheng Y, Jin M, Wang RX, Peng Y, Zhang Y et al (2014) Autophagy regulates the therapeutic potential of mesenchymal stem cells in experimental autoimmune encephalomyelitis. Autophagy 10:1301–1315
16. Dello Russo C, Lisi L, Feinstein DL, Navarra P (2013) mTOR kinase, a key player in the regulation of glial functions: relevance for the therapy of multiple sclerosis. Glia 61:301–311
17. Dong G, You M, Fan H, Ding L, Sun L, Hou Y (2015) STS-1 promotes IFN-α induced autophagy by activating the JAK1–STAT1 signaling pathway in B cells. Eur J Immunol 45:2377–2388
18. Dowling SD, Macian F (2018) Autophagy and T cell metabolism. Cancer Lett 419:20–26
19. Esposito M, Ruffini F, Bellone M, Gagliani N, Battaglia M, Martino G, Furlan R (2010) Rapamycin inhibits relapsing experimental autoimmune encephalomyelitis by both effector and regulatory T cells modulation. J Neuroimmunol 220:52–63
20. Feng FB, Qiu HY (2018) Effects of Artesunate on chondrocyte proliferation, apoptosis and autophagy through the PI3K/AKT/mTOR signaling pathway in rat models with rheumatoid arthritis. Biomed Pharmacother 102:1209–1220
21. Feng X, Hou H, Zou Y, Guo L (2017) Defective autophagy is associated with neuronal injury in a mouse model of multiple sclerosis. Bosn J Basic Med. Sci 17:95–103
22. Fernandez D, Bonilla E, Mirza N, Niland B, Perl A (2006) Rapamycin reduces disease activity and normalizes T cell activation-induced calcium fluxing in patients with systemic lupus erythematosus. Arthritis Rheum 54:2983–2988
23. Gros F, Arnold J, Page N, Décossas M, Korganow AS, Martin T, Muller S (2012) Macroautophagy is deregulated in murine and human lupus T lymphocytes. Autophagy 8:1113–1123
24. Hampe J, Franke A, Rosenstiel P, Till A, Teuber M, Huse K, Albrecht M, Mayr G, De La Vega FM, Briggs J et al (2007) A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. Nat Genet 39:207–211
25. Henckaerts L, Cleynen I, Brinar M, John JM, Van Steen K, Rutgeerts P, Vermeire S (2011) Genetic variation in the autophagy gene ULK1 and risk of Crohn's disease. Inflamm Bowel Dis 17:1392–1397
26. Henry SC, Daniell X, Indaram M, Whitesides JF, Sempowski GD, Howell D, Oliver T, Taylor GA (2007) Impaired macrophage function underscores susceptibility to salmonella in mice lacking IRGM1 (LRG-47). J Immunol 179:6963–6972
27. Holvoet T, Devriese S, Castermans K, Boland S, Leysen D, Vandewynckel YP, Devisscher L, Van den Bossche L, Van Welden S, Dullaers M et al (2017) Treatment of intestinal fibrosis in experimental inflammatory bowel disease by the pleiotropic actions of a local rho kinase inhibitor. Gastroenterology 153:1054–1067
28. Homer CR, Richmond AL, Rebert NA, Achkar JP, McDonald C (2010) ATG16L1 and NOD2 interact in an autophagy-dependent antibacterial pathway implicated in Crohn's disease pathogenesis. Gastroenterology 139:1630–1641
29. Hooper KM, Casanova V, Kemp S, Staines KA, Satsangi J, Barlow PG, Henderson P, Stevens C (2019) The inflammatory bowel disease drug azathioprine induces autophagy via mTORC1 and the unfolded protein response sensor PERK. Inflamm Bowel Dis, pii izz039
30. Huang G, Yuan K, Zhu Q, Zhang S, Lu Q, Zhu M, Sheng H, Yu R, Luo G, Xu A (2018) Triptolide inhibits the inflammatory activities of neutrophils to ameliorate chronic arthritis. Mol Immunol 101:210–220

31. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M et al (2001) Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. Nature 411:599–603
32. Hyun B, Shin S, Lee A, Lee S, Song Y, Ha NJ, Cho KH, Kim K (2013) Metformin down-regulates TNF-α secretion via suppression of scavenger receptors in macrophages. Immune Netw 13:123–132
33. Igci M, Baysan M, Yigiter R, Ulasli M, Geyik S, Bayraktar R, Bozgeyik İ, Bozgeyik E, Bayram A, Cakmak EA (2016) Gene expression profiles of autophagy-related genes in multiple sclerosis. Gene 588:38–46
34. Kato H, Perl A (2018) Blockade of treg cell differentiation and function by the Interleukin-21-Mechanistic target of rapamycin axis via suppression of autophagy in patients with systemic lupus erythematosus. Arthritis Rheumatol 70:427–438
35. Kato M, Ospelt C, Gay RE, Gay S, Klein K (2014) Dual role of autophagy in stress-induced cell death in rheumatoid arthritis synovial fibroblasts. Arthritis Rheumatol 66:40–48
36. Kim EK, Kwon JE, Lee SY, Lee EJ, Kim DS, Moon SJ, Lee J, Kwok SK, Park SH, Cho ML (2017) IL-17-mediated mitochondrial dysfunction impairs apoptosis in rheumatoid arthritis synovial fibroblasts through activation of autophagy. Cell Death Dis 8:e2565
37. Klionsky DJ (2005) The molecular machinery of autophagy: unanswered questions. J Cell Sci 118:7–18
38. Koch MW, Zabad R, Giuliani F, Hader W Jr, Lewkonia R, Metz L, Wee Yong V (2015) Hydroxychloroquine reduces microglial activity and attenuates experimental autoimmune encephalomyelitis. J Neurol Sci 358:131–137
39. Kruse K, Janko C, Urbonaviciute V, Mierke CT, Winkler TH, Voll RE, Schett G, Muñoz LE, Herrmann M (2010) Inefficient clearance of dying cells in patients with SLE: anti-dsDNA autoantibodies, MFG-E8, HMGB-1 and other players. Apoptosis 15:1098–1113
40. Kuballa P, Huett A, Rioux JD, Daly MJ, Xavier RJ (2008) Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated Atg16l1 variant. PLoS One 3:e3391
41. Kuo SY, Castoreno AB, Aldrich LN, Lassen KG, Goel G, Dančík V, Kuballa P, Latorre I, Conway KL, Sarkar S et al (2015) Small-molecule enhancers of autophagy modulate cellular disease phenotypes suggested by human genetics. Proc Natl Acad Sci USA 112:E4281–E4287
42. Lai ZW, Kelly R, Winans T, Marchena I, Shadakshari A, Yu J, Dawood M, Garcia R, Tily H, Francis L et al (2018) Sirolimus in patients with clinically active systemic lupus erythematosus resistant to, or intolerant of, conventional medications: a single-arm, open-label, phase 1/2 trial. Lancet 391:1186–1196
43. Lapaquette P, Bringer MA, Darfeuille-Mi-chaud A (2012) Defects in autophagy favour adherent-invasive Escherichia coli persistence within macrophages leading to increased pro-inflammatory response. Cell Microbiol 14:791–807
44. Lapaquette P, Glasser AL, Huett A, Xavier RJ, Darfeuille-Michaud A (2010) Crohn's disease-associated adherent-invasive E. Coli are selectively favoured by impaired autophagy to replicate intracellularly. Cell Microbiol 12:99–113
45. Li X, Wang S, Chen Y, Liu G, Yang X (2014) miR-22 targets the 3′ UTR of HMGB1 and inhibits the HMGB1-associated autophagy in osteosarcoma cells during chemotherapy. Tumor Biol 35:6021–6028
46. Liang P, Le W (2015) Role of autophagy in the pathogenesis of multiple sclerosis. Neurosci Bull 31:435–444
47. Lin NY, Beyer C, Giessl A, Kireva T, Scholtysek C, Uderhardt S, Munoz LE, Dees C, Distler A, Wirtz S et al (2013) Autophagy regulates TNFα-mediated joint destruction in experimental arthritis. Ann Rheum Dis 72:761–768
48. Liu F, Fang F, Yuan H, Yang D, Chen Y, Williams L, Goldstein SA, Krebsbach PH, Guan JL (2013) Suppression of autophagy by FIP200 deletion leads to osteopenia in mice through the inhibition of osteoblast terminal differentiation. J Bone Miner Res 28:2414–2430
49. Lopez P, Alonso-Perez E, Rodriguez-Carrio J, Suarez A (2013) Influence of Atg5 mutation in SLE depends on functional IL-10 genotype. PLoS One 8:e78756

50. Ma Y, Galluzzi L, Zitvogel L, Kroemer G (2013) Autophagy and cellular immune responses. Immunity 39:211–227
51. Martinez J, Cunha LD, Park S, Yang M, Lu Q, Orchard R, Li QZ, Yan M, Janke L, Guy C et al (2016) Noncanonical autophagy inhibits the autoinflammatory, lupus-like response to dying cells. Nature 533:115–119
52. Massey A, Kiffin R, Cuervo AM (2004) Pathophysiology of chaperone-mediated autophagy. Int J Biochem Cell B 36:2420–2434
53. Massey DC, Bredin F, Parkes M (2008) Use of sirolimus (rapamycin) to treat refractory Crohn's disease. Gut 57:1294–1296
54. McCarroll SA, Huett A, Kuballa P, Chilewski SD, Landry A, Goyette P, Zody MC, Hall JL, Brant SR, Cho JH et al (2008) Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. Nat Genet 40:1107–1112
55. Meng Q, Du X, Wang H, Gu H, Zhan J, Zhou Z (2017) Astragalus polysaccharides inhibits cell growth and pro-inflammatory response in IL-1β-stimulated fibroblast-like synoviocytes by enhancement of autophagy via PI3K/AKT/mTOR inhibition. Apoptosis 22:1138–1146
56. Moreland LW, O'Dell JR, Paulus HE, Curtis JR, Bathon St JM, Clair EW, Bridges SL Jr, Zhang J, McVie T, Howard G et al (2012) A randomized comparative effectiveness study of oral triple therapy versus etanercept plus methotrexate in early aggressive rheumatoid arthritis: the treatment of early aggressive rheumatoid arthritis trial. Arthritis Rheum 64:2824–2835
57. Murthy A, Li Y, Peng I, Reichelt M, Katakam AK, Noubade R, Roose-Girma M, DeVoss J, Diehl L, Graham RR et al (2014) A Crohn's disease variant in Atg16l1 enhances its degradation by caspase 3. Nature 506:456–462
58. Mutalib M, Borrelli O, Blackstock S, Kiparissi F, Elawad M, Shah N, Lindley K (2014) The use of sirolimus (rapamycin) in the management of refractory inflammatory bowel disease in children. J Crohns Colitis 8:1730–1734
59. Nikolich-Žugich J (2018) The twilight of immunity: emerging concepts in aging of the immune system. Nat Immunol 19:10–19
60. Nys K, Agostinis P, Vermeire S (2013) Autophagy: a new target or an old strategy for the treatment of Crohn's disease? Nat Rev Gastroenterol Hepatol 10:395–401
61. O'Dell JR, Leff R, Paulsen G, Haire C, Mallek J, Eckhoff PJ, Fernandez A, Blakely K, Wees S, Stoner J et al (2002) Treatment of rheumatoid arthritis with methotrexate and hydroxychloroquine, methotrexate and sulfasalazine, or a combination of the three medications: results of a two-year, randomized, double-blind, placebo-controlled trial. Arthritis Rheum 46:1164–1170
62. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH et al (2001) A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. Nature 411:603–606
63. Parkes M, Barrett JC, Prescott NJ, Tremelling M, Anderson CA, Fisher SA, Roberts RG, Nimmo ER, Cummings FR, Soars D et al (2007) Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. Nat Genet 39:830–832
64. Parzych KR, Klionsky DJ (2014) An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Sign 20:460–473
65. Patergnani S, Castellazzi M, Bonora M, Marchi S, Casetta I, Pugliatti M, Giorgi C, Granieri E, Pinton P (2018) Autophagy and mitophagy elements are increased in body fluids of multiple sclerosis-affected individuals. J Neurol Neurosurg Psychiatry 89:439–441
66. Paunovic V, Petrovic IV, Milenkovic M, Janjetovic K, Pravica V, Dujmovic I, Milosevic E, Martinovic V, Mesaros S, Drulovic J et al (2018) Autophagy-independent increase of ATG5 expression in T cells of multiple sclerosis patients. J Neuroimmunol 319:100–105
67. Pierdominici M, Vomero M, Barbati C, Colasanti T, Maselli A, Vacirca D, Giovannetti A, Malorni W, Ortona E (2012) Role of autophagy in immunity and autoimmunity, with a special focus on systemic lupus erythematosus. FASEB J 26:1400–1412
68. Ponticelli C, Moroni G (2017) Hydroxychloroquine in systemic lupus erythematosus (SLE). Expert Opin Drug Saf 16:411–419

69. Ramos-Barrón A, Piñera-Haces C, Gómez-Alamillo C, Santiuste-Torcida I, Ruiz JC, Buelta-Carrillo L, Merino R, de Francisco AL, Arias M (2007) Prevention of murine lupus disease in (NZBxNZW)F1 mice by sirolimus treatment. Lupus 16:775–781
70. Rioux JD, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW et al (2007) Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nat Genet 39:596–604
71. Rockel JS, Kapoor M (2016) Autophagy: controlling cell fate in rheumatic diseases. Nat Rev Rheumatol 12:517–531
72. Saitoh T, Fujita N, Jang MH, Uematsu S, Yang BG, Satoh T, Omori H, Noda T, Yamamoto N, Komatsu M et al (2008) Loss of the autophagy protein Atg16l1 enhances endotoxin-induced IL-1beta production. Nature 456:264–268
73. Schmeisser H, Bekisz J, Zoon KC (2014) New function of type I IFN: induction of autophagy. J Interf Cytok Res 34:71–78
74. Schwerd T, Pandey S, Yang HT, Bagola K, Jameson E, Jung J, Lachmann RH, Shah N, Patel SY, Booth C et al (2017) Impaired antibacterial autophagy links granulomatous intestinal inflammation in Niemann-Pick disease type C1 and XIAP deficiency with NOD2 variants in Crohn's disease. Gut 66:1060–1073
75. Shaker ME, Ashamallah SA, Houssen ME (2014) Celastrol ameliorates murine colitis via modulating oxidative stress, inflammatory cytokines and intestinal homeostasis. Chem Biol Interact 210:26–33
76. Solomon VR, Lee H (2009) Chloroquine and its analogs: a new promise of an old drug for effective and safe cancer therapies. Eur J Pharmacol 625:220–233
77. Tekirdag KA, Korkmaz G, Ozturk DG, Agami R, Gozuacik D (2013) MIR181A regulates starvation- and rapamycin-induced autophagy through targeting of ATG5. Autophagy 9:374–385
78. Travassos LH, Carneiro LA, Ramjeet M, Hussey S, Kim YG, Magalhaes JG, Yuan L, Soares F, Chea E, Le Bourhis L et al (2010) Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. Nat Immunol 11:55–62
79. Van Der Vos KE, Eliasson P, Proikas-Cezanne T, Vervoort SJ, Van Boxtel R, Putker M, Van Zutphen IJ, Mauthe M, Zellmer S, Pals C et al (2012) Modulation of glutamine metabolism by the PI(3)K–PKB–FOXO network regulates autophagy. Nat Cell Biol 14:829
80. Van Loosdregt J, Rossetti M, Spreafico R, Moshref M, Olmer M, Williams GW, Kumar P, Copeland D, Pischel K, Lotz M et al (2016) Increased autophagy in $CD4^+$ T cells of rheumatoid arthritis patients results in T-cell hyperactivation and apoptosis resistance. Eur J Immunol 46:2862–2870
81. Vasheghani F, Zhang Y, Li YH, Blati M, Fahmi H, Lussier B, Roughley P, Lagares D, Endisha H, Saffar B et al (2015) PPARγ deficiency results in severe, accelerated osteoarthritis associated with aberrant mTOR signalling in the articular cartilage. Ann Rheum Dis 74:569–578
82. Weindel CG, Richey LJ, Bolland S, Mehta AJ, Kearney JF, Huber BT (2015) B cell autophagy mediates TLR7-dependent autoimmunity and inflammation. Autophagy 11:1010–1024
83. Wu H, Deng Y, Feng Y, Long D, Ma K, Wang X, Zhao M, Lu L, Lu Q (2018) Epigenetic regulation in B-cell maturation and its dysregulation in autoimmunity. Cell Mol Immunol 14:1–9
84. Wu H, Zeng J, Yin J, Peng Q, Zhao M, Lu Q (2017a) Organ-specific biomarkers in lupus. Autoimmun Rev 16:391–397
85. Wu ZZ, Zhang JJ, Gao CC, Zhao M, Liu SY, Gao GM, Zheng ZH (2017b) Expression of autophagy related genes mTOR, Becline-1, LC3 and p62 in the peripheral blood mononuclear cells of systemic lupus erythematosus. Am J Clin Exp Immunol 6:1–8
86. Xu K, Cai YS, Lu SM, Li XL, Liu L, Li Z, Liu H, Xu P (2015) Autophagy induction contributes to the resistance to methotrexate treatment in rheumatoid arthritis fibroblast-like synovial cells through high mobility group box chromosomal protein 1. Arthritis Res Ther 17:374
87. Xu K, Xu P, Yao JF, Zhang YG, Hou WK, Lu SM (2013) Reduced apoptosis correlates with enhanced autophagy in synovial tissues of rheumatoid arthritis. Inflamm Res 62:229–237

88. Xu L, Zhang C, Jiang N, He D, Bai Y, Xin Y (2019) Rapamycin combined with MCC950 to treat multiple sclerosis in experimental autoimmune encephalomyelitis. J Cell Biochem 120:5160–5168
89. Yan H, Zhou HF, Hu Y, Pham CT (2015) Suppression of experimental arthritis through AMP-activated protein kinase activation and autophagy modulation. J Rheum Dis Treat 1:5
90. Yang Z, Klionsky DJ (2010) Eaten alive: a history of macroautophagy. Nat Cell Biol 12:814–822
91. Yin L, Liu J, Dong H, Xu E, Qiao Y, Wang L, Zhang L, Jia J, Li L, Geng X (2014) Autophagy-related gene16L2, a potential serum biomarker of multiple sclerosis evaluated by bead-based proteomic technology. Neurosci Lett 562:34–38
92. Yu W, Fu YC, Wang W (2012) Cellular and molecular effects of resveratrol in health and disease. J Cell Biochem 2012(113):752–759
93. Zhang J, Song X, Cao W, Lu J, Wang X, Wang G, Wang Z, Chen X (2016) Autophagy and mitochondrial dysfunction in adjuvant-arthritis rats treatment with resveratrol. Sci Rep 6:32928
94. Zhang M, Yao C, Cai J, Liu S, Liu XN, Chen Y, Wang S, Ji P, Pan M, Kang Z et al (2019) LRRK2 is involved in the pathogenesis of system lupus erythematosus through promoting pathogenic antibody production. J Transl Med 17:37
95. Zhang Y, Morgan MJ, Chen K, Choksi S, Liu ZG (2012) Induction of autophagy is essential for monocyte-macrophage differentiation. Blood 119:2895–2905
96. Zhang YM, Cheng FJ, Zhou XJ, Qi YY, Zhao MH, Zhang H (2015) Rare variants of ATG5 are likely to be associated with chinese patients with systemic lupus erythematosus. Medicine 94:e939
97. Zhang YM, Zhou XJ, Cheng FJ, Qi YY, Hou P, Zhao MH, Zhang H (2017) Autophagy-related gene LRRK2 is likely a susceptibility gene for systemic lupus erythematosus in northern Han Chinese. Oncotarget 8:13754–13761
98. Zhao J, Dong JN, Wang HG, Zhao M, Sun J, Zhu WM, Zuo LG, Gong JF, Li Y, Gu LL et al (2017) Docosahexaenoic acid attenuated experimental chronic colitis in interleukin 10-deficient mice by enhancing autophagy through inhibition of the mTOR pathway. JPEN J Parenter Enteral Nutr 41:824–829
99. Zhao J, Sun Y, Shi P, Dong JN, Zuo LG, Wang HG, Gong JF, Li Y, Gu LL, Li N et al (2015) Celastrol ameliorates experimental colitis in IL-10 deficient mice via the up-regulation of autophagy. Int Immunopharmacol 26:221–228
100. Zhen C, Feng X, Li Z, Wang Y, Li B, Li L, Quan M, Wang G, Guo L (2015) Suppression of murine experimental autoimmune encephalomyelitis development by 1,25-dihydroxyvitamin D3 with autophagy modulation. J Neuroimmunol 280:1–7
101. Zhou XJ, Lu XL, Lv JC, Yang HZ, Qin LX, Zhao MH, Su Y, Li ZG, Zhang H (2011) Genetic association of PRDM1-ATG5 intergenic region and autophagy with systemic lupus erythematosus in a Chinese population. Ann Rheum Dis 70:1330–1337
102. Zhu L, Wang H, Wu Y, He Z, Qin Y, Shen Q (2017) The autophagy level is increased in the synovial tissues of patients with active rheumatoid arthritis and is correlated with disease severity. Mediat Inflamm 2017:7623145