

Chapter 27

Autophagy and the Immune Response



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Abstract Innate immunity and adaptive immunity play critical roles in maintaining normal physiological functions and the development of diseases. In innate immune responses, heterogeneous autophagy can directly remove intracellular pathogens while activating PRRs, including TLRs and NLRs, to trigger their signal transduction pathways and promote NKT cell activation, cytokine secretion, and phagocytosis. In adaptive immune responses, the autophagy reaction has an important effect on the homeostasis, function, and differentiation of T lymphocytes, the survival, and development of B lymphocytes and the survival of plasma cells. This review highlights the key role that autophagy plays in the innate immune system and the acquired immune system. Further clarifying the mechanism by which autophagy regulates the immune system is essential for elucidating the precise mechanisms of various diseases and for developing new treatment methods.

Keywords Autophagy · Innate immunity · Adaptive immunity

Abbreviation

GAS	Group A streptococcus
GcAVs	GAS-containing autophagosome-like vacuoles
IRGs	Immunity-related p47 GTPases
SLRs	Sequestosome 1-like receptors
TLRs	Toll-like receptors
NLRs	NOD-like receptors
RLRs	RIG-I like receptors

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MAMPs	Microbe-associated molecular patterns
DAMPs	Damage-associated molecular pattern molecules
OPTN	Optineurin
NDP52	Nuclear domain 10 protein 52
GABARAP	GABA A receptor-associated protein
3-MA	3-methyladenine
SCVs	Salmonella-containing vacuoles
T3SS	Type III Secretion System
TBK1	TANK binding kinase 1
LIR motif	LC3 interacting motif
DAG	Diacylglycerol
Tecpr1	Tachylectin-II-like beta-propeller domain
AIEC	Adherent-invasive <i>E. coli</i>
LRSAM1	Leucine-Rich Repeat and Sterile α Motif-Containing-1
LAP	LC3-Associated Phagocytosis
TIMD4	T-cell immunoglobulin and mucin domain-containing 4
CLEC7A	C-type lectin domain family 7 member A, Dectin 1
NETs	Neutrophil extracellular traps
NOX2	NADPH oxidase 2
WIPI2	WD Repeat Domain Phosphoinositide-Interacting Protein 2
IRGM	Immunity-related GTPases
IFN- γ	Interferon gamma
HSV-1	Herpes simplex virus 1
KSHV	Kaposi's sarcoma-associated herpesvirus
ActA	Actin assembly-inducing protein
ARP2/3	Actin-Related Proteins 2/3
InlK	Internalin K
MVP	Major Vault Protein
SLAP	Spacious Listeria-Containing Phagosome
LLO	Listeriolysin O
GAPR1	Golgi-associated plant pathogenesis-related protein 1
FLIP	FLICE-like inhibitory protein
MHC	Major Histocompatibility Complex
AGO	Argonaute
eIF2 α	Eukaryotic Translation Initiation Factor 2 α
IRESs	Internal Ribosome Entry Sites
mTORC1	Mammalian Target of Rapamycin Complex 1
eIF4EBP1	Eukaryotic Translation Initiation Factor 4E binding protein 1
p70 ^{S6K}	Ribosomal Protein S6 kinase 70 kDa polypeptide 1
miRNA	MicroRNAs
DRiPs	Defective Ribosomal Initiation Products
ALISs	Aggresome-Like-Induced Structures
TAP	Transporter-Associated with Antigen Processing
LPC	Lysophosphatidylcholine

CRT	Calreticulin
PS	Phosphatidylserine
HSPs	Heat-Shock Proteins
HMGB1	High-Mobility Group Box 1
CLEC9AL	C-Type Lectin Domain Family 9 A Ligand
MHCs	MHC class II Compartments
OVA	Ovalbumin
UBA	The ubiquitin-associated domain
pDCs	Plasmacytoid Dendritic Cells
IRF7	Interferon regulatory factor 7
STK11, or LKB1	Serine Threonine Kinase 11, or Liver Kinase B1
AMPK	AMP-Activated Protein Kinase
DALISs	DC Aggresome-Like Lipopolysaccharide-Induced Structures
HSCs	Hematopoietic stem cells
iNKT cells	Invariant Natural Killer T cells
Vps34	Vacuolar protein sorting 34
CFLIP, or CFLAR	Cellular FLICE-like inhibitory protein
TCR	T cell receptor

With a more profound understanding of the molecular mechanisms of cellular autophagy and the immune system, the close relationship between the autophagy system and the immune system has been gradually revealed (Deretic et al. 2013; Jiang et al. 2019). In mammalian cells, autophagy involves multiple steps of innate and adaptive immune responses. Autophagy provides a primitive innate immune form of defense for eukaryotic cells against invading microorganisms. With the help of autophagy adaptor proteins, autophagy triggers a series of immune responses through pattern recognition receptors (PRRs). These steps constitute an essential process for eliminating intracellular microorganisms. Autophagy interferes with immune mediators such as cytokines by eliminating the endogenous inflammasome, thereby regulating innate immune signaling pathways (Levine and Kroemer 2019). Moreover, autophagy is also involved in the antigen-presentation process and further influences the adaptive immune response through the regulation of antigen-presenting cell homeostasis (Puleston and Simon 2014). This chapter focuses on the molecular immunological mechanisms underlying how autophagy regulates immune functions and the immune response.

27.1 The Restriction and Scavenging Effect of Autophagy on Pathogens

27.1.1 Xenophagy: The Direct Scavenging Effect of Autophagy on Intracellular Pathogens

Classified on the basis of their localization with respect to the host cell, extracellular bacteria and intracellular bacteria constitute the main types of bacteria. Most pathogenic bacteria are intracellular, which is strictly related to the chronic onset and recurrence of the disease. The pathogenicity of extracellular bacteria is related to virulence factors. The immune defense processes for fighting against these two types of bacterial infections are different. For infection with intracellular bacteria, the primary process consists of pathogen invasion, colonization of the tissue, amplification within the cell, and eventually, illness of the host. For example, *Salmonella typhimurium* can utilize a type III secretion system (T3SS) to secrete a large number of virulence proteins into the host cell. These virulence proteins are integrated into the host cytoskeleton to induce erythrocyte membrane shrinkage, facilitate the bacteria invading the cells, and form a vesicle called SCV containing the bacteria, thereby allowing the long-term survival of latent bacteria. Evidence suggests that some *Salmonella typhimurium* infections can block the formation of SCV and initiate mitochondrial division and autophagy. Unlike intracellular bacteria, extracellular bacteria cannot invade host cells. For example, *Pseudomonas aeruginosa* mainly relies on the secretion of virulence factors to infect the host and destroy cell structures to activate autophagy. It is still unclear what the molecular mechanism of autophagy induction by extracellular bacterial infection is. The exotoxin A (PEA) of the opportunistic pathogen *Pseudomonas aeruginosa* can induce oxidative stress damage in MLE-12 cells and activate autophagy. Vacuolating cytotoxin A (VacA) of *Helicobacter pylori* (Hp) interferes with endocytic pathways, lysosomal pathways, and host immune responses via cellular vacuolation and induces stress responses.

Approximately 30 years ago, the initial evidence suggested that inflammation can induce autophagy. In the last decade, studies have shown that autophagy plays a crucial role in the host defense system against pathogen invasion. The bacteria can be ubiquitinated after invading the cells and degraded through the autophagy pathway. This autophagic process is named “xenophagy”. Currently, autophagy has been found to be involved in the direct clearance of a variety of pathogens, including *Listeria*, *Shigella flexneri*, *Salmonella*, *Toxoplasma*, and Sindbis virus. However, parasites have acquired ways to circumvent host cell autophagic clearance during the evolutionary process. Parasites ultimately utilize autophagy of the host cell to proliferate. These phenomena reflect the multiple roles of autophagy in microbial infection.

The occurrence of xenophagy involves almost all molecules of the classical autophagy pathway. These molecules coordinate with each other to recognize, capture, and remove intracellular pathogens. Group A *Streptococcus* (GAS) was the

first bacterium found to be cleared by autophagy. GAS infects cells by endocytosis and then forms GAS-containing autophagosome-like vacuoles (GAS-containing autophagic small body-like vesicles) in the cytoplasm. The size of a common autophagosome is approximately 1 μm . However, the diameter of GcAV can reach 10 μm . The formation of GcAV depends on the autophagy core protein complex and the small GTP binding protein RAB7. After fusion of GcAVs with lysosomes to form autophagosomes, GAS is degraded and inactivated by lysosomes. GAS is inactivated in most cells by the xenophagy pathway described above.

Autophagy maintains intracellular metabolic homeostasis and is closely associated with microbial infections (Gomes and Dikic 2014). On the one hand, research evidence suggests that autophagy is involved in the direct clearance of multiple pathogens. On the other hand, parasites have evolved ways to circumvent autophagic clearance. When parasites start to proliferate, they rely on the autophagy of the host cells. This evidence suggests that autophagy has dual roles in microbial infection. Infectious diseases have become increasingly severe in recent years. Moreover, new infectious diseases continue to emerge. For example, super bacteria, SARS, Ebola virus, avian influenza virus, Middle East respiratory syndrome (MERS), and malaria, which have been afflicting people in the tropical regions, bring health threats and severe panic to the public. Antibiotics, interferons, and other medications have had essential roles in combating infectious diseases. However, with antibiotic overuse, bacterial resistance has become a serious problem. Viruses have also been shown to exhibit trends with increasing new mutations and drug resistance. According to WHO reports, the rates of drugs becoming ineffective are currently comparable to the speed of discovering new drugs. Targeting the intracellular autophagy process has been proven to be an effective way against intracellular infection. Studies of the molecular mechanisms between autophagy and pathogen-induced signaling pathways will continue to contribute to the discovery of new antibacterial methods with high efficiency and low drug resistance.

27.1.1.1 The Role and Molecular Mechanism of Xenophagy

Research has suggested that autophagy plays a key role during the clearance of pathogens such as bacteria and viruses. The host cells identify and clear the pathogens through autophagic degradation. This is similar to other types of selective autophagy, such as aggregate autophagy (aggrephagy) or mitochondrial autophagy (mitophagy). Autophagy receptors selectively recognize ubiquitinated pathogens in xenophagy. After an autophagy receptor interacts with LC3 or GABARAP, the pathogen is transported to autophagosomes. Therefore, the clearance of invading pathogens by xenophagy is ubiquitination dependent. The modification with ubiquitin provides “eat-me” signals during xenophagy. *Salmonella* can be modified inside the host cells by linear and K63-linked ubiquitin chains. K48-linked and K63-linked ubiquitination can modify *Mycobacterium marinum* in macrophages. In epithelial cells, the residual membranes of *Shigella* can be identified and modified with K48-linked ubiquitination. During bacterial and viral infection, the signals mediated by the host cell

receptors further trigger xenophagy. These receptors include Sequestosome 1-like receptors, pattern recognition receptors such as TLRs (Toll-like receptors) and NOD-like receptors, RLRs (RIG-I-like receptors), pathogen receptor CD46, and RAGE (receptor for advanced glycation end products, or AGEs). These receptors trigger xenophagy by recognizing a large number of MAMPs (microbe-associated molecular patterns) and DAMPs (damage-associated molecular patterns). The autophagy receptors SLRs play a key role in controlling pathogen infection. SLRs contain one or more LIR domains that are capable of linking autophagic adaptor proteins. SLRs mainly include p62 (sequestosome-1, SQSTM1), OPTN (Optineurin), NBR1 (neighbor of BRCA1 gene) and NDP52 (nuclear dot protein 52 kDa). SLRs can recognize ubiquitin-, galectin-, and membrane phospholipid-modified proteins. These modified proteins bind to the surface of invading microorganisms and damaged host cell surfaces. Moreover, SLRs assist in the connection between the modified proteins and ATG8 homologous proteins such as LC3 and GABARAP (GABAA receptor-associated protein) and then promote the autophagy receptor loaded with foreign microorganisms to enter newly formed autophagosomes. SLRs have different affinities for diverse types of ubiquitin chain-modified proteins, non-ubiquitin-modified proteins, or other ATG8 homologous proteins. These different affinities might be the reasons for the different abilities of pathogenic microorganisms to be recognized. For example, both NDP52 and p62 inhibit the proliferation of *Salmonella*, but only p62 can eliminate Sindbis virus.

Phagosome vesicles containing *M. tuberculosis*, *Salmonella* or *Helicobacter pylori* can be fused with autophagosomes. It is also possible that these pathogens are directly phagocytosed into autophagosomes. These bacteria have evolved the ability to interfere with autophagic degradation, which becomes a crucial part of their pathogenesis. For example, the fusion of autophagosomes with lysosomes is inhibited in macrophages infected with *Mycobacterium tuberculosis*, an intracellular pathogen. Macrophages cannot effectively induce mature autophagosomes to degrade and eliminate *Mycobacterium tuberculosis*. Autophagy inhibition provides a pivotal mechanism to explain the pathogenesis of *M. tuberculosis* infection. Gutierrez et al. have shown that activated autophagy can inhibit the intracellular survival efficiency of *M. tuberculosis*. Starvation- or rapamycin-induced autophagy promotes the fusion of phagosomes containing *M. tuberculosis* with lysosomes and delivers more *M. tuberculosis* into lysosomes for degradation. In an *M. tuberculosis*-infected mouse model, it was found that myeloid cells from *ATG5*-deficient mice can accumulate more *M. tuberculosis* and cause more severe necrosis in lung tissue.

It was found that the ESX-1 secretion system increases the phagosome membrane permeability of host cells during macrophage infection by *M. tuberculosis*. This system promotes the exposure of bacterial DNA in host cells. The adaptor protein STING then recognizes ubiquitinated bacteria and degrades these bacteria through NDP52- or p62-dependent autophagic degradation. *Pseudomonas aeruginosa* can induce autophagy in MH-S alveolar macrophages. Silencing *BECN1* or treating with 3-MA (3-methyladenine) can increase the infection load of *P. aeruginosa* in MH-S cells. Rapamycin, an autophagy inducer, promotes the clearance of *P. aeruginosa* infection in MH-S cells.

The process in which individual viral components are degraded via autophagy is termed “viral phagocytosis” (virophagy). Unlike xenophagy, the targets of virophagy are newly synthesized viral components rather than the entire viral particle. Different autophagy receptors can recognize viral proteins and RNA-protein complexes in the host and then undergo autophagic degradation. For example, p62 recognizes the Sindbis virus capsid protein, but this effect is not dependent on ubiquitin modification.

27.1.1.2 The Role and Mechanism of Xenophagy in *Salmonella* Infection

Salmonella infection is typically associated with xenophagy. After infection of epithelial cells, most of the *Salmonella* hide in the host SCV (*Salmonella*-containing vesicle). However, due to damaged type III secretion system (T3SS), 15–20% of bacteria are modified with ubiquitin and then encapsulated by the autophagosome membrane. The autophagy receptors p62, NDP52, and OPTN are involved in the recognition of ubiquitin-modified *Salmonella* and fusion with autophagosomes (Fig. 27.1). Silencing the previously mentioned autophagy receptors leads to the proliferation of *Salmonella*, indicating the essential roles of these autophagy receptors in the inhibition of *Salmonella* infection. It was recently found that NDP52 binds LC3 and TBK1 (TANK-binding kinase 1) to promote the phosphorylation of serine residues at OPTN 177, thereby enhancing the binding ability of LC3B.

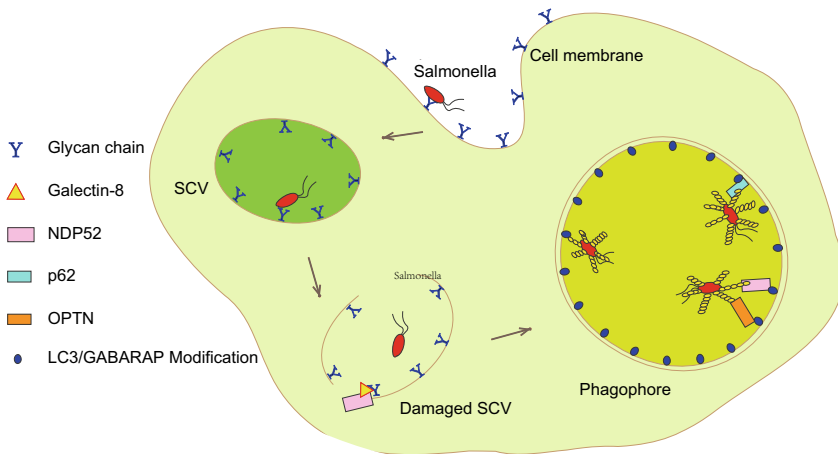


Fig. 27.1 Clearance of *Salmonella* by autophagy. When SCVs are damaged, the glycan chains of the host cells are exposed and recognized by Galectin-8. At this time, *Salmonella* is rapidly modified by ubiquitination. Autophagy receptors p62, NDP52, and OPTN recognize ubiquitin-modified *Salmonella* and transport them into the autophagosome

Galectin-8 is involved in maintaining the integrity of endosomes and lysosomes and recognizes bacterial infections by interacting with protein-sugar complexes. Galectin-8 recognizes the glycan chains of host cells exposed to the surface of damaged SCVs. It was found that host cells infected with *Listeria*, *Shigella flexneri*, and *Streptococcus pyogenes* can produce a large number of glycan chains. In addition, Galectin-8 can recognize damaged endosomes or lysosomes in the host cell. The glycan chain, the damaged endosomes or the damaged lysosomes are transiently bound to Galectin-8 and are then coupled to the NDP52 protein in a ubiquitination-dependent manner. Therefore, Galectin-8 plays essential roles in host defense in the early phase of pathogen infection.

The second messenger diacylglycerol (DAG), which mediates autophagic elimination of bacteria, does not depend on ubiquitination modification. DAG can directly localize to SCVs, which provides the possibility for the induction of autophagy pathways to clear *Salmonella*. DAG can cause damage to the SCV membrane, recruit protein kinase PKC δ , and then trigger autophagy via the JNK and NADPH oxidase pathways. It was found that bacterial autophagosomes independently colocalized with DAG or ubiquitin, suggesting two independent autophagy degradation pathways. Consistent with the ideas of Noda and colleagues, DAG can trigger autophagy on the membrane of an SCV surrounding *Salmonella*. Noda et al. suggested that the recruitment of LC3 does not depend on the formation of barrier membranes, but instead requires at least three levels of ATG recruitment that involve the ULK1 complex, ATG9L1, and the ATG16L complex. Therefore, ATG recruitment is most likely at the site of pathogen invasion in the host cells with the contribution of non-LC3/GABARAP autophagy receptor proteins. It was found that the Tecpr1 (tachylectin-II-like beta-propeller domain) receptor interacts with ATG5 and clears *Shigella* by xenophagy. ATG16L1 was then recruited to ubiquitin-modified *Salmonella* by interacting with ubiquitin chains to complete autophagy clearance.

Studying the clearance of *Salmonella* helps to understand the mechanism of xenophagy. However, there are still many unresolved questions. Ubiquitin can colocalize to invading bacteria. It is still not clear whether the ubiquitin-modified components are from bacteria or host cells. During infection with *Salmonella* or AIEC (adherent-invasive *E. coli*), LRR (leucine-rich repeat, E3 ligase-rich leucine-rich repeat) and LRSAM1 (sterile alpha motif-containing-1) are required for ubiquitination modification. The LRR of the LRSAM1 domain recognizes the bacterial protein, and the RING domain is involved in ubiquitination. However, the bacterial ligands recognized by LRSAM1 and their ubiquitinated substrates are still unknown. The E3 ligase Parkin was found to be involved in the clearance of *Mycobacterium tuberculosis* by the host cell through the ubiquitination autophagy pathway. Parkin-deficient mice are more susceptible to *Mycobacterium tuberculosis* infection, and Parkin-deficient flies are more susceptible to *Salmonella* and mycobacterial infections. However, it is still not clear what the binding target for Parkin is. Different autophagy receptors may recognize different “eat-me” signals. NDP52 is the only autophagy receptor that recognizes Galectin-8 binding-exposed polysaccharides. Different autophagy receptors have different affinities for polyubiquitin chains. *Salmonella* in the cytoplasm can be modified by at least two types of ubiquitin chains,

the linear chain and the K63-linked ubiquitin chain. Compared to the K48-linked ubiquitin chain, p62 and OPTN preferentially bind to K63-linked and linear ubiquitin chains. The posttranslational modifications close to the ubiquitin-binding region can affect the binding properties of autophagy receptors. The advantage of using multiple autophagy receptors in xenophagy is the recruitment of different effector proteins. For example, NDP52 recruits TBK1 to bind *Salmonella* in the host cell. TBK1 increases LC3 binding by phosphorylating OPTN and p62 and promotes autophagy by regulating autophagosome maturation.

27.1.2 The Restrictive Effect of Autophagy on Pathogens

27.1.2.1 LC3-Mediated Phagocytosis Inhibits the Propagation of Bacteria

After phagocytosis, LC3 rapidly modifies phagocytic vesicles with a monolayer membrane structure. This process is named LC3-associated phagocytosis (LAP). LAP relies on PI3K complexes and LC3 to form a conjugated system. LAP is independent of the ULK1 complex and does not form double-membrane vesicles. LAP is engaged in the macrophage clearance of *Rhizoctonia solani* and dead cell degradation and is also involved in the macrophage antigen-presentation process for fungi via TIMD4 (T-cell immunoglobulin and mucin domain-containing 4, T cell immunoglobulin mucin 4) or CLEC7A (C-type lectin domain family 7 member A, also known as Dectin 1) proteins.

27.1.2.2 Other Nonclassical Pathways of Autophagy Restriction on Pathogens

It was found that autophagy plays an essential role in the formation of neutrophil extracellular traps (NETs). Neutrophils are stimulated by exogenous microorganisms or chemicals to release extracellular fibrous structures composed of chromatin and granule proteins. These fibrous structures capture and kill a variety of extracellular microorganisms, including bacteria, fungi, and protozoa. Evidence has shown that similar events are also present in eosinophils and mast cells. NETosis is a cell death program in which chromatin release leads to host cell death. NETosis differs from apoptosis and necrosis. It requires three processes: oxidative stress, citrullinated histones, and autophagy. The importance of autophagy for NET formation has been supported by evidence that pharmacological autophagy interventions can regulate NET formation and affect the delivery of NET functional proteins. It was found that PMA relies on autophagy to induce NETosis. The autophagy inhibitor Wortmannin can inhibit NET formation in neutrophils.

Moreover, the autolysosome contains many active antibacterial molecules, including reactive oxygen radicals, antimicrobial peptides, proteolytic enzymes, thiol

reductases, and proton pumps. After triggering by inflammatory signals, phagocytic cells produce many active oxygen-free radicals to kill pathogenic microorganisms in phagolysosomes via NOX2 (NADPH oxidase 2). Additionally, autophagosomes contain defensin-like antimicrobial peptides that antagonize the action of microorganisms. These antibacterial peptides are mostly derived from products of cytoplasmic protein degradation such as ubiquitin and ribosomal precursor proteins, which can fuse with and kill bacteria in phagosomes. In *M. tuberculosis*-infected macrophages, it was found that the autophagy receptor p62 transports cytoplasmic proteins into autophagosomes, where these proteins are processed to kill *M. tuberculosis*. Moreover, acidic conditions activate cathepsin and chymase in autophagosomes, thereby enhancing the ability of autophagosomes to kill microorganisms and antigen processability.

It was found that some autophagy-related proteins have the ability to target the microorganism directly. For example, ATG5 can directly bind to the *Shigella* surface protein VirG. However, the interaction between ATG5 and VirG occurs on the presupposition that the *Shigella* protein IcsB does not occupy the VirG recognition epitope. It is dependent on the interaction between two ATG5 binding accessory proteins, TECPR1 and WIPI2 (WD-repeat domain phosphoinositide-interacting protein 2). WIPI2, involved in the formation of phagocytic vacuoles, is a paralog of mammalian phosphatidylinositol PtdIns3P-binding protein ATG8.

Other autophagy antibacterial pathways also involve the direct binding of the autophagy proteins Beclin-1, IRGM (immunity-related GTPases, immune-related GTPase family proteins) and bisphosphatidylglycerol (cardiolipin). Cardiolipin is a lipid found only in bacteria and mitochondria. IRGM, a genetic susceptibility factor for Crohn's disease, is partially localized to mitochondria. The E3 ubiquitin ligase Smurf-1 is a critical factor in host cell defenses against Sindbis virus and herpes simplex virus. Smurf-1 is involved in mitophagy, which in some respects resembles xenophagy.

IFN- γ -induced IRGs (IFN gamma-inducible immunity-related p47 GTPases) and Gbps (guanylate binding proteins) are involved in host cell xenophagy of *Chlamydia trachomatis* and toxoplasmosis. IRGs and Gbps can accumulate on the surface of PV (pathogen-containing vacuole) membranes, changing the electron density of the membranes and destroying them. Cell membrane deformation, perforation, and rupture lead to the death of the pathogens and eventually necrosis of the infected cells. This restriction of pathogens by IRGs and Gbps is independent of the formation of autophagosomes but is dependent on the autophagy proteins ATG3 and ATG5. It was found that silencing the *ATG3* or *ATG5* gene in host cells results in the inability of IRGs and Gbps to localize to PV membranes.

27.1.3 Pathogens Escape from Autophagy and Manipulate Autophagy

Bacteria employ various mechanisms to survive in the host cytoplasm and evade autophagic degradation. *Salmonella* and *Mycobacterium tuberculosis* inhibit phagosome maturation to survive and multiply in phagosomes. *Shigella flexneri*, *Legionella pneumophila*, herpes simplex virus (HSV-1) and Kaposi's sarcoma-associated herpesvirus (KSHV) can destroy autophagosomes or use the phagosome as a proliferation site. Studies have shown that *Shigella flexneri* quickly escapes to the cytoplasm and replicates after invading nonphagocytic cells. At the initial stage of infection, the autophagy core protein ATG16L1 is rapidly recruited by NOD1 and NOD2 to the invasion site of *Shigella flexneri*, activating autophagy by the RICK but not the NF- κ B pathway. After *Shigella flexneri* enters the cytoplasm, it utilizes the virulence protein IcsA to promote actin polymerization, thereby avoiding autophagy. The binding of the autophagy protein ATG5 to the IcsA protein isolates *Shigella flexneri* and promotes autophagy-mediated degradation. However, the T3SS (type III secretion system) of *Shigella flexneri* secretes another *Shigella* protein, IcsB. IcsB competes to inhibit protein binding of ATG5 to IcsA. After IcsB binds to the IcsA protein, the IcsA protein of *Shigella flexneri* is protected from ATG5 binding, which in turn causes *Shigella flexneri* to escape autophagic degradation. In addition to ATG5, other host factors also limit the effects of *Shigella flexneri*. For example, septin can capture *Shigella flexneri* and promote autophagic degradation. It was found that *Shigella flexneri* in the Septin cage colocalized with p62, NDP52, and LC3. Moreover, most *Shigella flexneri* and their vacuolar membrane residues that have escaped from autophagy can be recognized by Galectin-8, modified by ubiquitin and bound to autophagy receptor p62, and then isolated into autophagosomes. This isolation and degradation of *Shigella flexneri* residues help control the extent of downstream inflammatory reactions that would otherwise cause excessive damage to the host.

Similar to *Shigella*, *Listeria monocytogenes* can evade autophagic degradation after infecting the host. *Listeria monocytogenes* enters the cytoplasm from the vacuole and rapidly replicates in the cytoplasm. Birmingham et al. found that the colocalization of *Listeria* and LC3 was observed in the early phase of the infection. However, the bacteria were still able to avoid autophagic degradation. After the cytoplasmic *Listeria* is modified by ubiquitin, the bacteria can be recognized by p62, which binds to LC3, and then *Listeria* undergoes autophagosome phagocytosis. *Listeria monocytogenes*, phospholipase C and ActA (actin assembly-inducing protein) are three *Listeria*-derived proteins that inhibit autophagy in host cells and prolong the survival of *Listeria monocytogenes*. Once in the cytoplasm, ActA on the surface of *Listeria* cells interacts with actin monomer and ARP2/3 (actin-related protein 2/3) complex in the host cell to induce actin polymerization and drive motility. The above effects not only prevent *Listeria* from evading autophagic degradation but also inhibit the modification of the pathogen by ubiquitin. It was found that *Listeria* virulence factor K (Internalin K, InIK) can recruit host MVP (major vault protein). MVP is a major

component of the cytoplasm that makes up the nucleus of ribonucleoprotein particles. The interaction between InlK and MVP helps *Listeria* to camouflage and evade host autophagy recognition. *Listeria* replication occurs primarily in the cytoplasm of host cells. *Listeria* is also found in SLAP (spacious *Listeria*-containing phagosome) of the host cell that has a monolayer membrane structure.

LC3-positive SLAP, a nonacid degrading chamber, has a spacer-like autophagosome characteristic. *Listeria* isolated in SLAP grows more slowly than in the cytoplasm. However, it is believed that the presence of SLAP causes a long-term confrontation between *Listeria* and the host immune system. The formation of SLAP is also considered to be a cause of persistent bacterial infection. Evidence has shown that LLO (Listeriolysin O, cholesterol-dependent *Listeria* lysin O) is involved in the formation of SLAP and inhibits the fusion of phagosomes with lysosomes. Autophagy is involved in the formation of SLAP in the early stages of *Listeria* infection. Autophagy inhibitors inhibit SLAP formation.

On the other hand, autophagy degrades *Listeria* in SLAP vacuoles. This effect becomes more apparent when LLO activity is impaired. Inhibition of autophagy promotes excessive proliferation of *Listeria*. However, regardless of the mechanism by which *Listeria* evades autophagy, conditional knockout of *ATG5* in mouse macrophages and granulocytes increases the susceptibility of mice to *Listeria*. This evidence also indicates that autophagy plays a crucial role in antimicrobial responses.

Burkholderia pseudomallei is the causative agent of melioidosis. It is a common infectious disease with a high mortality rate in the tropics, especially in southeast Asia and northern Australia. *B. pyogenes* can adhere to nonphagocytic cells through bacterial surface type 4 pili. A small number of intracellular bacteria are recognized by the host cell through autophagy and are directly recruited into endosomes via LC3 rather than classical autophagosomes. However, most bacteria can evade autophagy and other defense mechanisms of the host cell, such as iNOS (inducible nitric oxide synthase), and replicate in the cytoplasm. These bacteria invade adjacent cells by fusion with cell membranes, resulting in the formation of multinucleated giant cells. Invasion of host cells by *Burkholderia pseudomallei* involves one or several proteins encoded by the TTSS3 (type III secretion system cluster 3) gene, which is partially involved in the escape of the bacterium from the endosome to the cytoplasm. For example, the *Pseudomonas* serotype III secreted protein BopA plays a crucial role in the escape process. In studies of mouse macrophage infection by *Burkholderia pseudomallei*, the bacterial *bopA* gene mutation enhances the colocalization of LC3, and the lysosomal marker protein LAMP1 reduced the number of intracellular viable bacteria. A large number of bacteria are killed in phagolysosomes. It was found that the *Burkholderia* VI-type cluster-related gene *bps0180* can induce autophagy in phagocytic and nonphagocytic host cells. In macrophage infection, the colocalization of *bps0180*-mutated *Burkholderia pseudomallei* strains and LC3 is decreased, and the number of viable bacteria in the cell is reduced. Introducing the wide-type *bps0180* gene can reverse the above phenomenon. These results indicated that the *bps0180* gene product supports the intracellular survival of *Burkholderia pseudomallei* in host cells.

Similar to bacteria, viruses have evolved a variety of mechanisms against autophagy. The ICP34.5 protein of type I herpes simplex virus (HSV), the M2 protein of influenza virus and the HIV-encoded protein Nef can block autophagy by inhibiting Beclin-1, which is the core protein for the whole autophagy system. Nef binds to the conserved domain of Beclin-1, which is the same binding region for the endogenous autophagy inhibitor GAPR1 (Golgi-associated plant pathogenesis-related protein 1). The mouse herpesvirus 68-encoded protein M11 (a homolog of the B-cell lymphoma 2 protein) inhibits the autophagy protein Beclin-1 via the BH3 domain. FLIP (FLICE-like inhibitory protein) of Kaposi's sarcoma-associated herpesvirus (KSHV) inhibits the E2-like enzyme ATG3 in the membrane of the LC3 binding region. The HIV Nef protein, the hepatitis C virus NS3 protein, and the measles virus Mev3 protein interact with the autophagy-related GTPase family protein IRGM. The effect of this interaction remains to be further studied. HBV can chronically infect human liver cells. The latent replication of HBV-infected hepatocytes is the primary cause of liver cancer development and drug resistance. Recent studies have indicated that the R-224 Smad-mediated noncanonical autophagy pathway helps HBV replication in host cells and promotes the establishment of chronic HBV infection.

27.2 Autophagy, Antigen Presentation, and Antigen-Presenting Cells

Antigen presentation refers to the process of efficiently delivering antigen via major histocompatibility complex (MHC) molecules. MHC molecules are classified into MHC class I molecules and MHC class II molecules. Almost all karyocytes express MHC class I on their surface. CD8⁺ T lymphocytes mainly recognize antigen peptides presented by MHC class I molecules. Upon infection, CD4⁺ T cells can recognize MHC II on the surface of antigen-presenting cells and antigenic peptides presented by MHC class II molecules of epithelial cells.

27.2.1 Autophagy and MHC Molecule Antigen Presentation

MHC class I molecules mainly present antigens derived from the cell, such as viral proteins, endogenous tumor antigens, and autoantigens from the cytoplasm or nucleus. Intracellular antigens are processed into immunogenic polypeptide fragments as antigenic peptides in the proteasome. The antigenic peptide is transported to the lumen of the endoplasmic reticulum with the help of a peptide chain transporter and forms a complex with MHC I. The MHC I/antigen peptide complex is expressed on the cell surface after passing through the Golgi. Autophagy affects the presentation of MHC I molecules in various aspects, such as restriction of antigen

peptide production, antigen peptide membrane localization, and the antigen presentation process.

27.2.1.1 Autophagy Regulates the Expression of MHC Class I Molecules on the Cell Membrane Surface

Autophagy can affect the production of MHC I-restricted antigen peptides and the expression of MHC I on the cell membrane surface by regulating protein translation (Van Kaer et al. 2017). Autophagy activation is often accompanied by eIF2 α (Eukaryotic Translation Initiation Factor 2 α) phosphorylation. Phosphorylated eIF2 α not only inhibits conventional 5'-cap ribosome transcription but also promotes binding of mRNA to IRESs (internal ribosome entry sites). Inhibition of mTORC1 (mammalian target of rapamycin complex 1) can inhibit eIF4EBP1 (eukaryotic translation initiation factor 4E binding protein 1) and p70^{S6K} (ribosomal protein S6 kinase 70 kDa polypeptide 1, RPS6KB1). Evidence has indicated that both eIF4EBP1 and p70^{S6K} are involved in 5'-cap general ribosome transcription. Rapamycin treatment can increase the diversity of the immunopolypeptides and produce more new antigens on the cell surface. Immunization of mice with these new antigenic peptides induced cytotoxic T lymphocytes to specifically kill rapamycin-treated lymphoma cells without killing untreated lymphoma cells. It is still not clear whether there is indirect activation between eIF2 α and the mTORC1 signal transduction pathway.

Moreover, the internalization and recycling of the plasma membrane affect the expression of MHC class I molecules on the cell membrane surface. MHC class I molecules can be internalized and degraded in the lysosome or recycled back to the cell membrane. In primary mouse DCs, silencing *ATG5*, *ATG7* or *Vps34* upregulates MHC class I expression on the cell surface. It was found that silencing *ATG5* and *ATG7* mainly reduced the internalization and degradation of MHC class I molecules. Enhanced MHC class I expression is not related to the protein recycling of the plasma membrane. Clathrin-mediated endocytosis and lipidated LC3 promote the binding of AP2-associated protein kinase 1 and MHC class I molecules to promote the internalization and degradation of MHC class I molecules. The above evidence suggests that autophagy can reduce the presentation of MHC class I antigens. Indeed, *ATG5*-deficient DCs are more potent at activating virus-specific T lymphocytes.

27.2.1.2 Autophagy Regulation of Antigen Presentation

Autophagy regulates the antigen presentation process of MHC class I molecules. The antigenic peptides presented by MHC class I molecules are mainly derived from newly synthesized antigenic peptides or DRiPs (defective ribosomal initiation products). The autophagy inhibitor 3-MA can inhibit the autophagic degradation of DRiPs in HeLa cells, promote its degradation by the proteasome pathway, and enhance the presentation of MHC class I antigens. After macrophage infection by HSV-1 (herpes

simplex virus-1), activation of autophagy contributes to the presentation of HSV-1 glycoprotein B-derived peptides to CD8⁺ T cells. Similarly, the presentation of the human cytomegalovirus latency-associated antigen UL138 requires autophagy. This process relies on endosomes but not proteasomes and antigen processing transporters. Autophagy plays a role in antigen presentation by DCs. The ability of *ATG5*-deficient DCs to present influenza virus and lymphocytic choriomeningitis virus was significantly reduced. *Vps34* deletion promoted the ability of DCs to present chicken ovalbumin, influenza virus, and lymphocytic choroiditis virus.

27.2.1.3 Autophagy Regulates the Steady-State Effects of miRNAs (microRNAs) on Immune Peptides

The abundant MHC class I molecular antigen peptides are mainly derived from the transcription products of miRNA response elements. RISC (RNA-induced signaling complex) recognizes complementary mRNA with miRNA as a template activates Argonaute and cleaves the RNA. It is easy to generate DRiPs from mRNAs under the action of RISC. Compared to full-length polypeptides, DRiPs are more readily loaded onto MHC class I molecules and become a significant source of MHC class I antigen peptides. The miRNA processing mechanism consists of two core components, Dicer and AGO2 (Argonaute RISC Catalytic Component 2). Dicer is a processing enzyme for miRNAs. AGO2 is the main effector protein of RISC. Dicer and AGO2 are critical for the formation of DRiPs, which in turn affects the diversity of immune polypeptides. The autophagy receptor NDP52 captures Dicer and AGO2 into the autophagy degradation pathway and affects the diversity of the immune polypeptides by altering miRNA homeostasis.

It was found that autophagy can directly affect DRiPs. DRiPs are modified by ubiquitin and processed in proteasomes. Otherwise, DRiPs can be captured by the autophagy receptor NBR1 and degraded by autophagy. Upon autophagy inhibition, a large number of ubiquitinated DRiPs accumulate in ALiSs (aggresome-like-induced structures), are proteasome degraded via the p62-dependent pathway, and then enter the classic MHC class-like molecule TAP (transporter associated with antigen processing)-dependent pathway. Autophagy activation does not accumulate DRiPs in ALiSs. Ubiquitinated DRiPs are recognized by the autophagy receptor NBR1 for autophagic degradation. Therefore, inhibition of autophagy in the clearance of DRiPs may promote MHC class I antigen presentation.

27.2.2 Autophagy and MHC II Antigen Presentation

27.2.2.1 Overview

MHC class II molecular antigen presentation involves extracellular antigen capture by antigen-presenting cells and transportation to autophagosomes. Proteolytic peptides (such as cathepsins) derived from endosomes are used to produce immunogenic peptides. The peptides are then loaded onto the MHC class II molecules and presented on the surface of the antigen-presenting cells, thereby activating CD4⁺ T lymphocytes. MHC class II molecules are critical for the acquired immune response. These molecules present processed antigens to stimulate lymphocytes, thereby triggering an adaptive immune response. During infection, CD4⁺ T lymphocytes recognize antigenic peptides presented by antigen-presenting cells and MHC class II molecules that are also on the surface of epithelial cells. Antigen-presenting cells, such as DCs and macrophages, link innate immunity with adaptive immunity. These antigen-presenting cells phagocytose pathogenic microorganisms such as bacteria, fungi, parasites, and other particulate antigens into the phagocytic bodies by endocytosis. While clearing microorganisms in the phagocytic body, the cells proteolytically process and produce immunogenic polypeptides. These peptides bind to MHC class II molecules to form a stable complex, avoiding further degradation. MHC class I antigen peptide processing mainly occurs in the cytoplasmic proteasome. However, MHC class II exogenous antigen processing mainly occurs in endosomes. Microbial antigens are degraded into peptides by lysosomal proteases. These peptides are loaded onto MHC class II molecules and enter the MIICs (MHC class II compartments) with an acid endosome containing cathepsin via the endoplasmic reticulum. With the roles of the autophagic-lysosomal system in the presentation of MHC class II antigens, most researchers believe that autophagy is involved in the MHC class II antigen-presentation process.

27.2.2.2 The Effect of Autophagy on the Presentation Process of MHC II Antigens

Autophagy plays an essential role in the presentation of MHC class II-restricted antigenic peptides (Hayward and Dinesh-Kumar 2010; Munz 2016). Schmid et al. found that autophagosomes and MIICs have a high frequency of fusion in primary monocyte-derived DCs. More than 50% of MIICs receive proteins derived from autophagosomes. Inhibition of autophagosome formation by siRNAs significantly attenuates the antigen presentation ability of MHC class II molecules in eliciting acquired immune responses. This evidence indicates that autophagy is involved in the MHC class II molecular antigen presentation process. Studies in human B lymphoblastoid cells have revealed that some of the epitopes presented by MHC class II molecules are derived from host cells. Starvation-induced autophagy activation enhances the presentation of intracellularly derived antigens by MHC class II

molecules. In macrophages or DCs that had phagocytosed BCG (Bacillus Calmette-Guerin), autophagy induction by starvation or rapamycin increased the potency of MHC class II molecules to present *M. tuberculosis* antigens. The secretory antigen Ag85B of *Mycobacterium tuberculosis* colocalizes with LC3-positive autophagosomes, suggesting that autophagy can capture the antigens that have escaped the phagosome and deliver them to lysosomes for expression by MHC class II molecules. In studies of experimental mice injected with DCs infected with *M. tuberculosis*, rapamycin-pretreated DCs promoted their ability to initiate *M. tuberculosis*-specific CD4⁺ T lymphocyte responses. Blanchet et al. found LC3-positive autophagosome structures in DCs. These results suggest that autophagy participates in the activation of TLR receptor signaling and the antigen presentation process, thereby eliminating HIV-1. The HIV-1 Env protein activates mTOR to inhibit autophagy in DCs, thereby abolishing immune signals and enhancing the infection of CD4⁺ T lymphocytes. It was found that the interaction of influenza matrix proteins and LC3 elicits antigen-specific CD4⁺ T lymphocyte responses. Lee et al. found that autophagy is necessary for DCs to phagocytose extracellular antigens for presentation to CD4⁺ T lymphocytes in *ATG5*-silenced cells. During HSV-2 infection, the ability of *ATG5*-silenced DCs to activate CD4⁺ T lymphocytes and induce IFN- γ release was significantly reduced. In an HSV-2 virus lethal challenge study, *ATG5*-deficient mice were more likely to die. *ATG5* knockout mice failed to initiate the proliferative response of T lymphocytes with cytosolic antigen OVA (ovalbumin) immunization.

Moreover, phagosomes play an essential role in the processing of MHC class II antigens. It was found that the phagocytic bodies of macrophages can phagocytose antigenic substances by endocytosis and decompose the enzymes to produce MHC class II-related peptides in the phagocytic bodies. In *Mycobacterium tuberculosis*-infected DCs, MHC II and lipid antigen CD1b molecules are recruited at phagosomes, and MHC II molecules are rapidly transferred from phagosomes to the plasma membrane with DC maturation.

27.2.3 Autophagy and Cross-Presentation

It was found that MHC molecules have a cross-presentation function. MHC class I molecules can also present exogenous antigens, mainly by internalizing extracellular antigens into cells or loading antigen peptides onto endosomes expressing MHC I. The above process is named CP1 (type 1 cross-presentation). Similarly, intracellular antigens can also be presented by MHC class II molecules, a process known as CP2 (type 2 cross-presentation). Cross-presentation occurs primarily in XCR1⁺ subtype DCs. XCR1⁺ subtype DCs show more active autophagy than other subtypes of DCs. These results suggest that autophagy is highly activated in the cross-presenting cells.

Autophagy involves transferring microbes into the cells and processing microbial components. This indicates that autophagy can regulate the cross-presentation of MHC class I molecules. For example, DCs can control *Chlamydia trachomatis* infection in inclusion bodies. After the activation of DC, the inclusion bodies release

the bacteria into the cytoplasm. Cytoplasmic bacteria are captured into the autophagosome and degraded by cathepsin. The antigens are initially degraded by the protease inside the proteasome for further processing. The antigens are then loaded onto the MHC class I molecules via the endosomal pathway. Therefore, microbial peptide loading of MHC class I molecules is dependent on autophagy. This process requires neither TAP (transporter associated with antigen processing) nor N-terminal modification of the antigenic peptide. However, the transfer of the newly assembled MHC class I peptide complex to the cell surface by endocytosis is a necessary process. It is worth noting that the control of antigen presentation by autophagy is not limited to DCs but also involves other antigen-presenting cells, such as B lymphocytes and macrophages.

Autophagy transports multiple antigenic species into lysosomes, which constitute a major source of cell-derived MHC class II antigens. Autophagy activation promotes the production of antigenic peptides derived from the cytoplasm, mitochondria, and nucleus, indicating that autophagy facilitates the transport of these components into MIICs. It was found that MIICs from DCs contain autophagosome-like structures. These structures include not only molecules involved in antigen processing but also the autophagy marker proteins LC3 and ATG16L1. These findings suggest that autophagy is a major source of MHC class II antigens. Autophagy is primarily classified as an antigen processing pathway because autophagosomes are fused to antigen-loaded MIICs. Therefore, by participating in the MHC class II epitope presentation process, autophagy plays a crucial role in initiating the T lymphocyte immune response, thereby regulating the characteristics and intensity of acquired immune responses mediated by T lymphocytes.

27.2.4 Autophagy Affects the Immune Response of Antigen Donor Cells

Autophagy is a critical switch that determines the survival or death of ADCs (antigen donor cells). Autophagy is the primary mechanism of cell survival when cells are exposed to stress conditions such as nutrition deprivation, growth factor withdrawal, pathogenic microbial invasion, mechanical damage, malignant transformation, chemotherapy, or radiation therapy. Under long-lasting and robust stress conditions beyond the ability of autophagy to respond to maintain cell homeostasis, cell death will occur. Dead cells become new antigen donors and then induce inflammation and immune responses.

Autophagy not only participates in the direct killing of pathogenic microorganisms by host cells but also is also involved in the immune response against pathogenic microorganisms or tumor-associated antigens. In the absence of dead ADCs, autophagy tends to be a spontaneous and self-limiting defense mechanism in ADCs. However, when ADCs are unable to reestablish a steady state by autophagy, dead ADCs generated by autophagy stimulate immune responses through multiple

mechanisms. First, autophagy-enhanced ADCs include ATP and LPC (Lysophosphatidylcholine) and other “discover me” chemotactic signals. Autophagy is involved in the transport of ATP from a specific lysosomal chamber to a secretory chamber in a process that has not been defined to date. Autophagy is also involved in a caspase-dependent ATP extracellular secretion process at the end of apoptosis. Autophagy causes tumor cells to release more ATP chemotactic signals during cell death, thereby recruiting antigen-presenting cells into the tumor and promoting antigen uptake by these cells. Autophagy-deficient malignant cells cannot induce an effective antitumor immune response in response to chemotherapy because they cannot release enough ATP to recruit myeloid cells and lymphocytes into the tumor. Exogenous administration of ATPase inhibitors can reverse this defect and drive myeloid cells and lymphocytes into the tumor by maintaining extracellular ATP concentration, thereby producing an effective antitumor immune response to dead tumor cells. The above ATP-driven chemotactic response to immune cells involves the metabotropic P2Y2 receptor. It was found that autophagy can induce phagocytic “eat-me” signals such as CRT (calreticulin) and PS (phosphatidylserine). PS exposure plays a crucial role in activating phagocytosis. As a ligand for a variety of receptors, PS not only participates in the recognition of dead cells by phagocytic cells but also activates the phagocytic uptake.

On the other hand, the ATP released by the dead ADCs not only promotes the differentiation of myeloid-derived granulocyte-like precursor cells into DCs but also binds to the ion receptor P2RX7 on DCs and activates inflammatory vesicles, thereby promoting the secretion of IL-1 β by these cells. Dead ADCs can release intact autophagosomes, which contain not only many antigenic substances but also HSPs (heat-shock proteins), CRT, HMGB1 (high-mobility group box 1), and DRiPs. By expressing CLEC9AL (C-type lectin domain family 9 a ligand) on the surface of autophagosomes, antigen-presenting cells can use autophagosomes to cross-present a variety of antigens. Autophagosomes carrying antigenic material can be directly transferred from the dead ADCs to DCs for cross-presentation. Immunization with autophagosomes obtained by the generation of ADCs with proteasome inhibitor treatment can effectively stimulate specific immune responses in mice. During this process, proteasome inhibitor treatment of ADCs enhances the uptake of p62 compared to other autophagosome substrates. Autophagosomes obtained by purification and separation not only carry long-lived proteins but also load a variety of short-lived polypeptides, including DRiPs, CRT, and DAMPs. During cross-presentation by DCs, the nature of the antigen in the autophagosome depends on the interaction between its surface CLEC9AL and the DC CLEC9A. The antigenic substances carried by the autophagosome of ADCs by caveolae-mediated endocytosis can be transferred to a nonacid chamber. It was found that purified autophagosomes can directly induce B lymphocyte activation in a TLR2-MyD88-dependent manner. Although purified autophagosomes may be more effective in stimulating immune responses than whole-cell lysate-derived vaccines, it is still not clear that cross-presenting the autophagosome content of dead ADCs to antigen-presenting cells can occur in humans. In general, autophagy of ADCs under viral infection or malignant transformation can significantly promote the antigen phagocytosis and presentation ability

of antigen-presenting cells, suggesting that pharmacological induction of autophagy may be a strategy to activate anticancer immune responses.

27.2.5 Effect of Autophagy on Antigen-Presenting Cells

27.2.5.1 Autophagy and the TLRs of Antigen-Presenting Cells

Autophagy affects the antigen presentation process through TLR-mediated signaling pathways. Autophagy helps antigen-presenting cells to recognize danger signals such as microbe-associated molecular patterns (MAMPs), which in turn activates the antigen presentation process (Into et al. 2012). In the cytoplasm, MAMPs shuttle into the endosomal cavity in an autophagy-dependent manner and interact with the ligand binding domains of TLRs such as TLR7/9. Natural or synthetic TLR ligands can be recognized by autophagy receptors and sequestered in autophagosomes, which in turn bind to the same internalized TLRs. This event then triggers downstream signaling to promote the secretion of type I IFNs, thereby enhancing the antigen-presentation ability of APCs. Vesicular stomatitis virus could not activate the plasma cell-like DCs (plasmacytoid dendritic cells, pDCs) in which the *ATG5* gene was deleted. The TLR7 and TLR9 of these cells could not respond to stimulation by herpes simplex virus 1 and DNA-containing immune complexes, and the IRF7 (interferon regulatory factor 7) could not be further activated to produce IFN- α . The absence of ULK1 does not affect the above effects, indicating that this effect is primarily associated with LAP rather than the traditional autophagy pathway.

27.2.5.2 Autophagy and Immune Synapses

Autophagy may affect immune synapse formation and binding properties between antigen-presenting cells and T lymphocytes, which in turn affects the T lymphocyte response. The immune synapse formed between antigen-presenting cells and T lymphocytes activates STK11 (serine threonine kinase 11, or liver kinase B1, LKB1) and AMPK (AMP-activated protein kinase), thereby inhibiting mTOR and activating autophagy. Once DCs form an immune synapse with T lymphocytes, the primary role of autophagy is to degrade immune synapses and synaptic components, thereby activating STK11 and AMPK. The resulting reduced synaptic stability terminates T cell activation in a time-dependent manner. It was found that silencing *ATG16L1* or *IRGM* inhibits autophagy in DCs, increases the duration of the immune synapses between DCs and T lymphocytes, prolongs the activation time of T lymphocytes and results in Th17-type responses.

27.2.5.3 Autophagy and DCs

DCs have higher basal autophagy activity than other cells. Autophagy activity facilitates the processing of intracellular and extracellular antigens presented by MHC class I or class II molecules. In DCs, autophagy is mainly mediated by endosomes. The autophagosomes in MHCs have both molecular markers of antigen-presenting molecules and autophagosome molecular markers such as LC3 and ATG16L1. Endosomal-mediated autophagy involves direct phagocytosis of DALISs (DC aggregates-like lipopolysaccharide-induced structures). DALISs are p62- and polyubiquitin-labeled structures that are strictly related to protein synthesis. It was found that DRiPs entering DALISs can avoid degradation. Autophagy can affect the antigen presentation ability of DCs by direct phagocytosis of DALISs.

It was found that DCs deficient in the *ATG5* gene have impaired MHC class II molecular solubility and cell-associated antigen presentation. When a herpes simplex virus type 2 component is encountered, the CD4⁺ T lymphocyte response cannot be stimulated. The presentation by MHC class II molecules of citrulline peptides is not only restricted by the expression level of *ATG5* but also by the PI3K inhibitor 3-MA. This mechanism may be associated with the onset of rheumatoid arthritis characterized by citrulline autoantigens. The results of a study in which the thymus of *ATG5* gene deletion mice was transplanted into wild-type mice indicated that the autophagy-mediated antigen presentation by MHC class II molecules involved the differentiation of CD4⁺ T lymphocytes. Autophagy plays a role in the selection of thymus T lymphocytes. This effect is more pronounced in response to rare antigens and some antigens entering the autophagic cavity. However, it is not clear that the above mechanism is true for the antigens presented by peripheral DCs.

27.3 The Role of Autophagy in Innate Immunity

27.3.1 Overview

With the recent development of molecular biology, cell biology, and immunology techniques, the crosstalk between autophagy and innate immunity has been gradually revealed. Autophagy is essential for innate immunity and is involved in mediating a variety of innate immune pathways. Various stimuli, including hypoxia, bacterial infection, and organelle damage, induce autophagy, degrade intracellular damaged proteins and organelles, and eliminate bacteria and viruses in infected cells.

After recognition by pathogen recognition receptors (PPRs), pathogenic microbial components such as LPS, lipoprotein, flagellin, and nucleic acids activate transcription factors, causing upregulation of inflammatory cytokines, chemokines, type I interferons, and multiple anti-pathogenic genes, ultimately triggering innate immune responses. Abnormal activation of the innate immune response can cause inflammatory diseases such as autoimmune diseases and septic shock. The host has an

elegant innate immune regulatory system to prevent excessive or weak immune responses. Regarding the removal of pathogens, autophagy plays an essential role in innate immunity and adaptive immunity (Table 27.1). For example, nonphagocytic cells rely on the formation of Rab-7 bacterial autophagosomes during the clearance of *S. pyogenes* and *Q. rickettsia*. The transport proteins p62 and NBR1 bind to ubiquitin and LC3 through their UBA (ubiquitin-associated region) and LIR (LC3-interacting region) domains, respectively. Then, ubiquitinated proteins, damaged organelles, and intracellular pathogens are degraded by selective autophagy. It has been found that autophagy can selectively eliminate *Salmonella typhimurium*, *Shigella flexneri*, *Escherichia coli*, and *Streptococcus pyogenes* infection. *Listeria monocytogenes* secrete ActA on the cell surface of bacteria and then recruit Arp2/3

Table 27.1 Interaction between pathogens and autophagy pathways

Pathogen	Autophagy function
Bacterial	
<i>Mycobacterium tuberculosis</i>	Fusion of bacteria-containing phagosomes with autophagosomes
<i>Salmonella typhimurium</i>	Targeting damaged phagosomes
<i>Helicobacter pylori</i>	Targeting autophagosomes containing bacteria
<i>Pseudomonas aeruginosa</i>	Induce autophagy
<i>E. coli</i>	Invading strain and activate autophagy
<i>Bacillus anthracis</i>	Degradation of anthrax lethal toxin by autophagy
<i>Listeria</i>	Listeria, phospholipase C, and actin polyprotein A inhibit autophagy; autophagy clears intracellular bacteria
<i>Shigella flexneri</i>	P62 and NDP52 target bacterial and induce phagocytosis
<i>Vibrio cholerae</i>	Cholera toxin inhibits autophagy
Virus	
Sindbis virus	Degraded viral capsids induce autophagy
Vesicular stomatitis virus	During viral infection process of Plasma cell dendritic cells, autophagy transfers viral ligands to TLR7
Human immunodeficiency virus	HIV can induce autophagy-dependent cell death by gp41 in bystander T cells. The virus also inhibits autophagy in dendritic cells
Herpes simplex virus 1	HSV-1 protein ICP34.5 inhibits autophagy by interacting with Beclin-1
Human cytomegalovirus	hCMV virus protein TRS1 inhibits autophagy through interaction with Beclin-1
Measles virus	Viral infection induces autophagy through CD46 and GOPC proteins function
Protozoa	
<i>Toxoplasma gondii</i>	CD40-dependent activation of macrophages induces autophagy to kill <i>Toxoplasma gondii</i>

and Ena/rasp proteins to mimic host cell organelles, thereby avoiding ubiquitination and autophagic degradation. The effector of the *Shigella* type III secretion system damages the phagosome membrane. The damaged phagosome membrane is ubiquitinated, encapsulated, and degraded via the p62 pathway. Damaged phagosome membranes bind to proteins such as Galectin3, NOD1, Ipaf, ASC, Caspase-1, TRAF6, and NEMO, which are specifically related to NF- κ B-dependent cytokine storm. Therefore, degradation of bacteria by selective autophagy may have adverse effects on infection control.

Autophagy-related proteins (ATG) cooperate with each other and cause cell membrane changes during autophagosome formation. ATGs were found to be involved in the formation of autophagosomes and are responsible for Irga6 (immune-associated GTPase) transport between the endoplasmic reticulum-Golgi and vesicles. This effect contributes to the clearance of *Toxoplasma gondii* by macrophages. Moreover, autophagy plays a crucial role in the antigen presentation process. Autophagy promotes the binding of MHC II molecules located on the surface of autophagosomes to viruses or autoantigens and then presents them to CD4⁺ T cells. During the infection process of human herpes simplex virus 1, autophagy controls MHC I to present viral antigens to CD8⁺ T cells. In addition, autophagy plays an essential role in tissue barrier regulation, innate immune receptor-mediated immune responses, and immune effector regulation.

27.3.2 The Regulation of Autophagy in the Intestinal Mucosa

The intestinal mucosa is composed of intestinal epithelial cells, mucus layer, immune cells, microbial population, and endocrine and other defense systems (Fig. 27.2). Intestinal epithelial cells (IECs) constitute the initial physical and immune barrier of the intestinal mucosa, preventing the invasion of foreign pathogens and commensal bacteria in vivo (Goto and Kiyono 2012; Haq et al. 2019). The IEC maintains the homeostasis of the gut through various functions, such as apoptosis, autophagy, and endoplasmic reticulum stress. This protection plays a vital role under rapidly changing conditions in the environment. IECs are mainly composed of absorption cells, goblet cells, endocrine cells, Paneth cells, M cells, and undifferentiated cells. Intestinal inflammation may be associated with autophagy. However, the role of autophagy in intestinal epithelial cells remains unclear.

As the largest absorption organ, the gut is closely related to obtaining nutrients. Studies have found that amino acids play an essential role in regulating autophagy in intestinal epithelial cells. Glutamine can enhance autophagy under heat and oxidative stress. This upregulation of autophagy is directly related to cell survival. After stress occurs, glutamine deficiency induces apoptosis and increases protease and ADP ribosylase activity. Inhibition of mTOR or p38MAP kinase reduces the above enzyme activities, suggesting that autophagy protects cells and reduces apoptosis by enhancing glutamine activity. The endoplasmic reticulum is abundant in Paneth

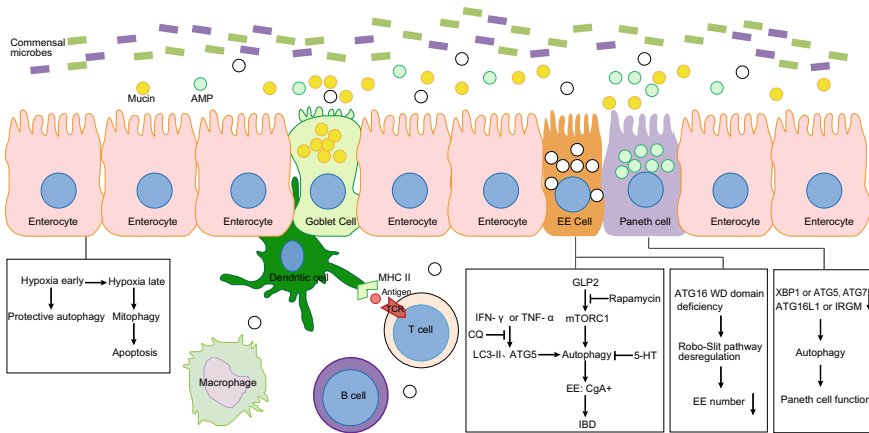


Fig. 27.2 Autophagy is involved in the regulation of the normal function of the intestinal mucosa and the pathogenesis of enteritis. The intestinal mucosa is a defense system consisting of intestinal epithelial cells, mucus layer, immune cells, microbial population, and endocrine cells. In the early stage of hypoxia, protective autophagy is activated in intestinal epithelial cells; in the late stage of hypoxia, mitophagy is activated and results in apoptosis. Endocrine cells and autophagy are mutually regulated, 5-HT inhibits autophagy, and GLP2 activates the mTORC1 signaling pathway to activate autophagy. Under the stimulation of IFN- γ and TNF- α , autophagy is activated, and the number of CgA⁺ EE cells increases, resulting in enteritis. ATG5 or ATG7 activates autophagy and is involved in maintaining the survival and function of Paneth cells

cells compared to other intestinal epithelial cells. Deletion of key autophagic proteins may disrupt organelle renewal and destroy endoplasmic reticulum homeostasis. XBP1 is a crucial transcription factor that maintains endoplasmic reticulum homeostasis. Paneth cells are abnormally sensitive to endoplasmic reticulum stress. However, the effect of autophagy deficiency is not fully understood by XBP1 deletion because Paneth cells die before they can exhibit autophagy deficiency. In vivo studies indicated that intestinal dysfunction is associated with autophagy deficiency.

In ATG16L1-deficient Paneth cells, electron microscopy results showed degradation of mitochondria and replacement of endoplasmic reticulum by many vesicular structures. In the ATG5- or ATG7-deficient mouse models, it was shown that the apical segmentation of the Paneth cell was separated from the basal part, and the Paneth cells disappeared. These effects result in a decrease in intestinal antimicrobial peptides and other secreted proteins. Clinical studies of patients with Crohn's disease showed that autophagy deficiency is related to the expression changes of various genes associated with intestinal mucosa homeostasis, injury healing, and pro-inflammatory response, suggesting that autophagy deficiency is closely related to human diseases. ATG16L1 and IRGM regulate the effects of autophagy. When ATG16L1 or IRGM is mutated, Paneth cells appear to be dysfunctional, leading to an impaired intestinal mucosal barrier. In the presence of hypoxic stress, secretory cells such as Paneth cells and goblet cells exhibited severe endoplasmic reticulum stress and reduced secretion, which may be an essential mechanism for endoplasmic

reticulum protein accumulation. The effects of autophagy on endoplasmic reticulum stress and exocytosis revealed that autophagy deficiency has various effects on intestinal secretory cells, affecting intestinal function.

Autophagy participates in the intestinal inflammatory process. It was found that in the *ATG5*- or *ATG7*-deletion mice, the overall morphology of intestinal epithelial cells of the colon and ileum appeared normal, but there were significant differences in intestinal Paneth cells. Compared with normal small intestinal epithelium, the expression of TNF- α and IL-1 β mRNAs were significantly increased in the intestinal epithelial cells of mice lacking *ATG7*, and NF- κ B pathway activation was significantly increased. This evidence suggests that autophagy regulates intestinal inflammation, reduces intestinal toxins, and maintains intestinal homeostasis via the NF- κ B signaling pathway. Neonatal necrotizing enterocolitis (NEC) studies have shown that autophagy-associated protein expression is increased and autophagy regulatory proteins Beclin-1 and LC3II are activated, accompanied by extensive degradation of p62 protein, indicative of autophagy activation in intestinal epithelial cells. Inhibition of autophagy can increase the inflammatory response through endoplasmic reticulum stress. In contrast, inflammatory gene expression was significantly reduced when autophagy was activated, or endoplasmic reticulum stress was inhibited. A large number of studies have shown that autophagy at the initial stage can remove damaged organelles and protect the intestinal mucosa, but excessive activation of autophagy produces a large number of autophagic vacuoles, which have different degrees of adverse effects on organelles, eventually leading to cell damage and even cell death.

Enteroendocrine (EE) cells reside in the largest endocrine organ of the human body. The enterosteroids 5-hydroxytryptamine (5-HT) and glucagon-like peptides 1 and 2 (GLP-1, GLP-2) secreted by EE cells are closely associated with intestinal physiology and pathological function. The number of EE cells and the mode of secretion are related to enteritis. Clinical observations found that the density of polypeptide YY and chromogranin A-expressing (CgA⁺) cells increased in patients with lymphatic enteritis, and the number of GLP-secreting cells and 5-HT-secreting cells (intestinal chromaffin cells) in patients with inflammatory bowel disease (IBD) increased. Research evidence continues to show that autophagy is involved in the regulation of EE cell function. In the mouse colonic IBD model induced by IFN- γ and TNF- α stimulation, the number of CgA⁺ EE cells increased, while the colonic mucosa highly expressed the autophagy-associated proteins LC3-II and ATG5. It is suggested that autophagy is involved in regulating the production and differentiation of CgA⁺ cells. Deletion of the WD-40 domain of ATG16 in the *Drosophila* intestinal mucosa leads to dysregulation of the Robo-Slit signaling pathway, resulting in a decrease in the number of mature EE cells. This evidence suggests that *Drosophila* ATG16 promotes the differentiation of intestinal stem cells into EE cells. Hormones secreted by EE cells are also involved in the regulation of autophagy. In liver cancer cells and lacrimal gland cells, 5-HT is capable of inhibiting autophagy activity. C57BL/6 mice were injected with GLP2 to promote phosphorylation of the S6 ribosomal protein and the mTORC1 signaling pathway downstream of eukaryotic translation initiation factor 4E (eIF4e)-binding protein 1 (4E-BP1). Pretreatment with the mTORC1 inhibitor rapamycin blocked the phosphorylation of 4E-BP1 and

the S6 ribosomal protein. These results suggest that GLP2 can significantly activate the autophagic pathway in the mouse gut. The function and molecular mechanism of autophagy regulation in various types of cells during the development of enteritis need to be elucidated.

27.3.3 The Effect of Autophagy Regulation Mediated by Innate Immune Receptors on the Immune Response

27.3.3.1 Autophagy Is Necessary for TLR-Mediated Immune Effects

When the cells are under stress, autophagy often plays a cytoprotective role and inhibits the inflammatory response. Similarly, the inflammatory response associated with the TLR signaling pathway is also regulated by autophagy. This section will focus on the regulation of the TLR-mediated signaling pathway by autophagy, mainly its mitochondria-related functions.

Normally, autophagy inhibits the TLR signaling pathway. Several autophagy-related proteins have been found to negatively regulate TLR-induced responses. After stimulation of TLR4 with LPS, *ATG16L1*-deficient macrophages produce a large number of IL-1 β and IL-18 due to excessive activation of caspase-1. The recruitment of TLRs does not directly activate caspase-1, whereas the maturation of pro-IL-1 β /pro-IL-18 requires caspase-1. Extracellular ATP activates the P2X7 receptor, induces caspase-1 activation, and produces IL-1 β /IL-18 via the NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome. The above activation stress is associated with K⁺ efflux and impaired lysosomal function. Caspase-1 activity is high in *ATG16L1*-deficient cells, and ROS production can be promoted without the need for extracellular ATP. Thus, TRIF-mediated signaling pathways produce ROS that activates the NLRP3 inflammasome, whereas *ATG16L1*-mediated autophagy significantly inhibits ROS production.

Studies have shown that inhibition of autophagy by deletion of LC3II or Beclin-1 leads to abnormal mitochondrial aggregation while producing large amounts of ROS in macrophages. In cells with abnormal mitochondrial structure, TLR4 becomes more sensitive to LPS stimulation. Mitochondrial ROS production causes the NLRP3 inflammasome to be recruited to the mitochondria-linked ER membranes, where NLRP3 interacts with ASC (a key inflammatory body formation regulator) to promote the formation of the NLRP3 inflammasome. In addition, the NLRP3 inflammasome and overproduction of ROS promote the release of mitochondrial DNA into the cytoplasm, which is a coactivator of Caspase-1. Therefore, autophagy can negatively regulate the inflammatory response by protecting mitochondrial homeostasis. The TLR signaling pathway may be involved in mitochondrial autophagy, and TRAF6-mediated ECSIT polyubiquitination plays a vital role in this process.

Immune-associated p47 GTPases (IRGs) act to protect against intracellular pathogens. Murine Irgm1 (LRG47) GTPase abolishes intracellular *Mycobacterium*

tuberculosis by forming a sizeable autophagic lysosome that induces autophagy. Stimulation of TLR4 induces *Irgm1* expression, which in turn inhibits TLR4-triggered pro-inflammatory cytokine responses. The human *Irgm1* homolog, IRGM, also exerts an autophagy-mediated clearance effect of intracellular mycobacteria. IRGM has an affinity for mitochondrial cardiolipin lipids that localize to mitochondria and thereby affect mitochondrial division, mitochondrial membrane depolarization, and the induction of autophagy.

Interestingly, mitochondrial division is necessary for the control of intracellular mycobacteria by IRGM autophagy. IRGM also plays an essential role in the process of autophagosome maturation. Therefore, IRGM may regulate mitochondrial function downstream of TLRs. Proteins with a tendency to aggregate tend to be associated with cytotoxicity. For example, a mutated huntingtin protein contains a large amount of repetitive polyglutamine, which is cytotoxic. Aggregated Huntingtin proteins are recognized by autophagy receptors (such as p62 and HDAC6) and are considered markers of Huntington's disease. Polyglutamine mutants are usually present in a variety of forms, such as monomers, water-soluble oligomers, and insoluble inclusion bodies. Studies have found that soluble or small monomeric mutant Huntingtin proteins are more toxic, through forming a polymer or inclusion body escape cell scavenging effects. MyD88 exists in different forms, such as monomers, oligomers and inclusion bodies, and has similar characteristics to mutant Huntingtin. Although MyD88 can be incorporated into inclusion bodies and polymers via p62 and HDAC6, it is still unclear whether such polymeric structures affect the TLR signaling pathway. Silencing of p62 or HDAC6 revealed that p62 and HDAC6 are involved in the inhibition of TLR-mediated activation of p38 and JNK signaling pathways, but the effect on the NF- κ B signaling pathway is not yet apparent. The presence of these molecules impairs the recruitment of TRAF6 to the MyD88 signaling complex and promotes the recruitment of CYLD to the complex. Additionally, NDP52 can also affect the TLR signaling pathway. NDP52 mediates TRIF-TRAF6 complex polymerization and degradation, thereby inhibiting TLR3/4-induced activation of NF- κ B and IRF3 signaling pathways.

OPTN is involved in the regulation of the TLR signaling pathway, and OPTN competitively antagonizes the RIP1-NEMO interaction by binding to polyubiquitinated RIP1. OPTN interacts with the TRAF3-TBK1 complex to reduce the production of type I interferon. UBQLN1 inhibits TRIF signaling by degrading TRIF. These results indicate that the autophagy receptor negatively regulates TLR signaling, but it is not clear whether this negative regulation is involved in the protein aggregation process.

27.3.3.2 The Critical Roles of Autophagy in NLR-Mediated Immune Responses

The intracellular receptors NOD1 (nucleotide-binding oligomerization domain-containing protein 1) and NOD2 recognize the bacterial-specific polypeptides iE-DA (diaminopimelate) and MDP (cell wall phenol dipeptide), respectively, and play a vital role in the production of cytokines and antimicrobial peptides (Carneiro and

Travassos 2013). A study found that MDP activation of NOD2 can induce autophagy, thus promoting the binding of antigenic peptides to MHC II molecules by dendritic cells and ultimately eliminating intracellular bacteria. This effect requires the simultaneous involvement of NOD2 signaling, the downstream regulatory molecule RIPK2 and autophagy-related proteins, including ATG5, ATG7, and ATG16L1. The activation of NOD1 and NOD2 can stimulate the formation of autophagosomes in mouse embryonic fibroblasts (MEF), macrophages, and human lymphocytes. The above process does not depend on the adapter protein RIP2 and the transcription factor NF- κ B. The study found that NOD1 and NOD2, together with ATG16L1, were recruited to the bacterial invasion site on the membrane, induced autophagy, and eventually cleared intracellular bacteria. Although the downstream signaling pathways of NOD1 and NOD2 remain to be further studied, the above results suggest that autophagy plays an essential role in the antibacterial immune response induced by NOD1 and NOD2.

Agrewala et al. found that NOD-2 combined with the TLR-4 signaling pathway activates autophagy, enhances the ability of DCs to kill bacteria, and activates killer T cells. AngII (Angiotensin II) promotes NLRP3 inflammasome activation, IL-1 β secretion, and collagen production by activating ROS, which triggers pulmonary fibrosis. Administration of the autophagy agonist rapamycin attenuates the progression of pulmonary fibrosis described above. Autophagy eliminates ROS and inhibits the activation of the NLRP3 inflammasome, while the autophagy effector p62/SQSTM1 degrades ubiquitin-labeled IL-1 β , ultimately decreasing pulmonary fibrosis injury.

27.3.3.3 Autophagy Affects the RLR-Mediated Immune Response

RIG-I-like receptors (RLRs), including RIG-I, MDA-5, and LGP-2, are located in the cytoplasm and are responsible for recognizing viral dsRNAs. The viral dsRNA interacts with the linker molecule IPS-1 (IFN- β promoter stimulator 1) on the mitochondrial membrane through the CARD (caspase activation and recruitment domain), activating TBK1/IKK-i and IRF3/IRF7, which induces the production of inflammatory cytokines and type I interferons; it also produces a large number of cytokines via the NF- κ B activation pathway, which stimulates the host antiviral responses.

The ATG12 and ATG5 complexes directly bind to the CARDs of RLR and IPS-1, thereby inhibiting the production of type I interferon in the RLR-mediated antiviral process. In contrast, *ATG5*-deficient MEF cells showed enhanced expression and secretion of type I interferon after vesicular stomatitis virus (VSV) infection or dsRNA treatment and limited VSV proliferation. The study found that deletion of ATG7, which is required for the ATG12 and ATG5 complexes, promotes the expression of type I interferon-induced by dsRNA.

Autophagy deficiency disrupts cell homeostasis and affects the RLR-IPS-1 signaling pathway. *ATG5*-deficient MEF cells are deficient in autophagy, leading to dysfunctional mitochondrial aggregation, causing an increase in intracellular ROS.

The accumulation of ROS in the cell leads to an increase in the level of type I interferon after activation of the RLR, which ultimately enhances the clearance of VSV. Recent studies have found that HEK293T cells activate the RIG-I-IPS-1-TRAF6 signaling pathway during viral infection, resulting in the K63-linked polyubiquitination of Beclin-1 protein, which in turn activates autophagy to provide a defense against viral infection.

27.3.4 The Regulation of Immune Effector Molecules by Autophagy

27.3.4.1 Autophagy Regulates the Activation of Inflammasomes

The inflammasome is a cytosolic polyprotein complex that belongs to a new class of inflammatory signaling pathways. The inflammasome controls the maturation and secretion of various inflammatory factors, such as interleukin (IL) IL-1 β , IL-18, and IL-33. Members of the cytosolic NLR family (such as NLRP3 and NLRP1) interact with the autophagy receptor to form inflammasomes. In wild-type macrophages, the TLR agonist LPS is unable to induce inflammasome activation and secretion of IL-1 β . When the autophagy regulatory gene *ATG16L1* or *ATG7* is knocked out or chemical agents are used to inhibit autophagy, the LPS-dependent inflammasome is reactivated. These findings suggest that autophagy regulates the activation of the inflammasome while limiting the production of the inflammatory cytokines IL-1 β and IL-18.

Thus far, the mechanism by which autophagy inhibits the inflammasome is unclear. A possible molecular mechanism is direct degradation of the inflammasome by autophagy, as autophagy downregulates ROS production, which inhibits the inflammasome. Deficiencies in autophagy-related genes, such as *ATG5*, *ATG7*, *ATG12*, and *ATG16L1*, result in abnormal accumulation of mitochondria and a massive increase in ROS. ROS can be recognized by the inflammasome in innate immune cells, releasing pro-inflammatory factors, which in turn cause inflammation. Autophagy can reduce mitochondrial aggregation and apparent leakage of mitochondria and peroxisomes, thus negatively regulating intracellular ROS. These findings suggest that ROS-activated autophagy may be a negative feedback regulatory mechanism.

Recent studies have found that trimethylamine N-oxide produced by intestinal microbial metabolism inhibits the expression of *ATG16L1*, LC3-II, and p62 proteins to reduce autophagy and upregulate the nucleotide-binding domain of intestinal epithelial cells, the activity of alanine-rich family proteins and the NLRP3 inflammasome. The GCN2 (generally controlled nonrepressed kinase) knockout mouse model showed increased expression of IL-1 β , enhanced Th17 cell responses, and increased production of reactive oxygen species, resulting in a severe enteritis response. Ravindran et al. found that enteritis was strictly related to the inhibition of autophagy in

IECs and APCs. Short-chain fatty acids (SCFAs) produced by the anaerobic glycolysis of carbohydrates, such as food residues, are the primary source of energy for IECs and maintain the normal function of the intestinal barrier. As HDAC inhibitors, SCFAs block the activity of the NLRP3 inflammasome and reduce autophagy, thereby alleviating LPS injury in the intestinal barrier.

27.3.4.2 The Effect of Autophagy on the Production and Release of Cytokines

Studies have found that autophagy can directly affect the transcription, processing, and secretion of some cytokines. It is worth mentioning that the disruption of the normal autophagy pathway increases the secretion of the pro-inflammatory cytokines IL-1 α , IL-1 β , and IL-18. Regulation of IL-1 β processing and secretion is mainly dependent on the activation of caspase-1, which subsequently affects the formation of the inflammasome (Piccioli and Rubartelli 2013). The secretion of IL-1 β is usually divided into two phases: first, IL-1 β precursor transcription is induced by LPS, and then inflammatory body assembly and caspase-1 activation are induced by ROS, uric acid crystals or ATP stimulation. Autophagy regulates the secretion of IL-1 β by at least two different mechanisms. Knockout of *ATG7*, *ATG16L1* or the autophagy gene *BECN1*, or treatment with the autophagy inhibitor 3-MA, can inhibit autophagy in macrophages or dendritic cells. Inhibition of autophagy enhances the processing and secretion of IL-1 β by TLR agonists. The study found that the above effects in murine macrophages and dendritic cells are dependent on TRIF (TIR-domain-containing adaptor inducing interferon- β), mitochondrial ROS and/or mitochondrial DNA and are partially dependent on NLRP3. However, the effects mentioned above in human peripheral blood mononuclear cells may be dependent on the p38-MAPK signaling pathway but not TRIF. TNF- α production is dependent on mitochondrial ROS and then induces oxidative stress. In the absence of *ATG7* in mouse intestinal epithelial cells, LPS can promote large-scale transcription of IL-1 β . In LC3B-deficient mice, LPS can produce higher levels of IL-1 β and IL-18. Clinical studies have found that the *ATG16L1* polymorphism is closely related to the risk of Crohn's disease. Sodium dextran sulfate (DSS) is more likely to induce colitis in mice lacking *ATG16L1*. These results indicate that autophagy may be an essential mechanism for controlling inflammatory responses in vivo.

It was found that autophagy can control inflammasome activation and IL-1 β production. Under the stimulation of LPS and ATP or alum, rapamycin-induced autophagy can inhibit the secretion of IL-1 β by mouse dendritic cells. Furthermore, after treatment with rapamycin and LPS or PAM3Cys, the level of IL-1 β precursor in macrophages is reduced. These findings indicate that autophagy can specifically target IL-1 β precursors to undergo lysosomal degradation and to regulate the expression of IL-1 β precursors inside the cell. Rapamycin-induced autophagy can also effectively reduce the increase of serum IL-1 β induced by LPS in mice. Both IL-1 α and IL-1 β induce autophagy, which may be a negative feedback loop that regulates IL-1-induced inflammatory responses. For example, IL-1 and IL-23 can promote the

secretion of IL-17 by lymphocytes, and autophagy plays a vital role in regulating Th17 cell responses and participates in the development of autoimmune diseases such as multiple sclerosis.

Similar to IL-1 β , IL-18 also binds to the complex (inflammasome) through NALP1, NALP3, and PAF, hydrolyzes procaspase-1 after recognition of PAMPs or DAMPs, and procaspase-1 hydrolyzes pro-IL-18 to produce IL-18, which then triggers an inflammatory response. Recent studies have found that the inflammatory cytokine IL-6 is involved in autophagy. Thorburn et al. found that autophagy activation promotes the secretion of IL6 in CD44⁺/CD24^{low/-} breast cancer stem cells, thereby maintaining breast cancer stemness. In glioma cells, hypoxia activates protective autophagy. Highly expressed IL-6 inhibits autophagy by regulating the STAT3-miR155-CREBRF-CREB3-ATG5 signaling pathway, thereby promoting the development of glioma.

27.4 Autophagy Participates in the Adaptive Immune Response

27.4.1 Autophagy Participates in T Lymphocyte Activation and Responses

27.4.1.1 Overview

T lymphocytes have been widely demonstrated to express autophagy-related genes and have autophagic activity. The constitutive autophagic activity of mouse and human CD4⁺ and CD8⁺ T lymphocytes is low. Stimulation of T lymphocyte receptors or HIV infection induces autophagy activation in vitro. The study found that thymic epithelial cells in the thymic cortex of mice have high levels of autophagic activity, suggesting that autophagy plays a role in the development and selection of T lymphocytes (Merkley et al. 2018).

27.4.1.2 The Effect of Autophagy in the Homeostasis and Function of T Lymphocytes

It was found that the timely removal and reduction of mitochondrial load by autophagy are essential for maintaining the function of normal hematopoietic stem cells (HSCs) and are essential to produce myeloid and lymphoid progenitor cells. After naive T lymphocytes leave the thymus, their maturation is dependent on autophagy-mediated mitochondrial content reduction. After knocking out the thymic stroma via *ATG5*, the selection of CD4⁺ T lymphocytes specific for multiple organ inflammatory responses changed, indicating that autophagy plays a role in T lymphocyte selection and central tolerance. In contrast, the selection of CD8⁺ T lymphocytes

did not change. A series of gene knockout models have been established to study the effects of autophagy on T lymphocytes *in vivo*. In fetal liver chimeric *ATG5^{-/-}*, *ATG7^{flox/flox}* Lck-Cre, *Vps34^{flox/flox}* (Vacuolar Protein Sorting 34) CD4-Cre mice, it was found that autophagy-deficient T lymphocytes can develop normally in the thymus but peripheral T lymphocytes are affected. The number of T lymphocytes in the spleen and lymph nodes of *ATG5^{-/-}*, *ATG7^{-/-}*, *ATG3^{-/-}*, or *Vps34^{-/-}* mice was significantly reduced compared to wild-type mice. These T lymphocytes are unable to achieve efficient proliferation under specific activation stimuli. Autophagy-deficient T lymphocytes do not adequately regulate and control intracellular organelle quality, while the mitochondrial burden of T lymphocytes increases, which leads to positive feedback with increased oxidative stress and cell death.

It was found that autophagy is involved in regulating the energy metabolism of T lymphocytes. Typically, ATP production is increased when T lymphocytes are activated, and lysosome inhibitors can inhibit the increase of ATP. Autophagy-deficient T lymphocytes supplemented with exogenous energy with methyl pyruvate restores some of their functions. Autophagy is involved in the development of iNKT (invariant Natural Killer T) cells. In transgenic mice with the T lymphocyte gene *vps34* knocked out, thymic iNKT cells were arrested at the G0 phase. A similar study found that autophagy also plays a crucial role in maintaining the homeostasis and function of Foxp3⁺ regulatory T lymphocytes. A growing body of evidence suggests that autophagy regulates the homeostasis and function of T lymphocytes at multiple levels.

27.4.1.3 The Effects of Autophagy in the Homeostasis and Function of T Lymphocytes

Autophagy affects the survival of T lymphocytes, and the autophagy of naive T lymphocytes is low. The autophagic activity of T lymphocytes is mainly inhibited by CFLIP protein (cellular FLICE-like inhibitory protein, also known as CFLAR, cell type FLICE-like inhibitory protein). The autophagy of activated T lymphocytes increases upon the induction of TCR (T cell receptor) signals and CD28 costimulatory signals. T lymphocytes maintain their activation status with the help of autophagy: on the one hand, autophagy counteracts the proapoptotic effects produced by upregulated CD95 (also known as FAS) and CD95L (also known as FasL) on TCR stimulation; on the other hand, autophagy eliminates impaired mitochondria in T lymphocytes and maintains normal oxidative stress levels to inhibit apoptosis. Compared with wild-type mice, the number of T lymphocytes in the spleen and lymph nodes of T lymphocyte autophagy-deficient mice was significantly higher.

Naive T lymphocytes develop into mature T lymphocytes in the thymus, which is accompanied by a series of changes in surface marker proteins. MHC molecules play an essential role in the differentiation and development of T lymphocytes. Naive T lymphocytes must come into contact with thymic epithelial cells expressing MHC I or MHC II antigens to differentiate into CD8⁺ or CD4⁺ T lymphocytes, respectively.

Autophagy selectively determines the maturation of T lymphocytes by controlling the MHC I or MHC II antigen peptides presented on the cell surface.

Autophagy is involved in T lymphocyte activation by maintaining homeostasis of endoplasmic reticulum calcium flux. T lymphocyte activation is dependent on a stable calcium ion flux of the endoplasmic reticulum, and autophagy promotes the homeostasis of the endoplasmic reticulum calcium ion flux. After TCR stimulates T lymphocyte activation, the calcium influx of the endoplasmic reticulum increases. However, calcium influx is impeded in *ATG7*-deficient T lymphocytes, and calcium ions bind to and accumulate on an endoplasmic reticulum-like structure and fail to respond to TCR stimulation.

27.4.1.4 The Effect of Autophagy in T Lymphocyte Differentiation

Autophagy affects T lymphocyte differentiation. Part of this effect is achieved by controlling innate immune cells. For example, autophagy-deficient macrophages secrete IL-1 α and IL-1 β in large amounts and induce Th17-type T lymphocyte immune responses under the synergistic effect of IL-6 and TGF- β . Marrow-like cells in *ATG5*-deficient mice have higher levels of IL-17 after infection with *M. tuberculosis*. The use of *M. tuberculosis* antigen to stimulate the lymphocytes of lung cells of *ATG5*-deficient mice in vitro also showed a similar phenomenon of increased IL-17 expression in CD4⁺ T lymphocytes. Additionally, autophagy defects lead to a prolonged duration of binding of DCs to T lymphocyte immune synapses, as well as tilting the T lymphocytes toward the Th17 type.

On the other hand, different cytokines present in the immune environment can induce the corresponding differentiation direction of T lymphocytes. As mentioned above, autophagy has a regulatory effect on cytokine production and secretion, and it is bound to affect the direction of T lymphocyte differentiation. For example, rapamycin induces autophagy to inhibit IL-1 secretion, and IL-1 itself activates autophagy, so autophagy itself constitutes a negative feedback mechanism that regulates IL-1-induced inflammation. IL-1 synergizes with IL-23 to drive Th17 polarized differentiation, so autophagy plays an essential role in regulating Th17-type lymphocyte responses.

Recent studies have found that the number and short-term activation of CD4⁺ T cells in mice knocked out for *ATG5* are not significantly different, but the ability to produce antibodies is significantly reduced. T cell-specific knockout of *ATG1611* in mouse enteritis animals showed a type II immune response and a lack of Foxp3⁺ regulatory T (Treg) cells. Knocking out *ATG1611* specifically in Foxp3⁺ Treg cells reduced the activity of intestinal Foxp3(+) Treg cells. Autophagy inhibits the ability of Th2 cells to expand. During selective autophagy, p62 inhibits the secretion of IL-9 by Th9 cells by degrading the transcription factor PU.1 of Th9 cells. The autophagy inhibitor chloroquine reverses the above effects and considerably promotes the secretion of IL-9, thereby exerting an antitumor effect.

27.4.1.5 Autophagy and CD8⁺ T Lymphocytes

CD8⁺ T lymphocytes are essential cells for the body to eliminate viral infections. Scientists at the Emory University Vaccine Center in the United States vaccinated volunteers to detect the genes change in the blood. It was found that the *gcn2* gene was rapidly activated, producing a large number of CD8⁺ T lymphocytes involved in the immune response (Ravindran et al. 2014). The protein encoded by *gcn2* is a sensor for detecting amino acid levels in the cell and is involved in the regulation of autophagy. When DCs are infected by yellow fever virus, the amino acids in the cells are consumed in large quantities, and the autophagy of DCs is activated by GCN2, which enhances the antigen presentation ability of DCs to CD8⁺ T lymphocytes. The absence of the *gcn2* gene impairs the ability of DCs to activate CD8⁺ T lymphocytes, and mice deficient in the *gcn2* gene are unable to produce an effective immune response to yellow fever vaccine and influenza vaccine. Recent studies have found that herpes simplex virus 1 can disrupt the function of activated CD8⁺ T cells by interfering with macroautophagy in mouse DCs.

27.4.2 *The Effects of Autophagy on B Lymphocytes and Antibody Immune Response*

27.4.2.1 Overview

Autophagy is involved in maintaining the survival of B lymphocytes and B1 lymphocytes in the precursor phase and is critical for the function, survival, and homeostasis of plasma cells. Highly secreting plasma cells require autophagy to maintain their endoplasmic reticulum function. Autophagy has a significant protective effect on the bone marrow plasma cell bank and participates in and maintains long-term humoral immune memory.

27.4.2.2 Autophagy Is Required for the Survival and Development of B Lymphocytes

Autophagy is not essential for the survival of most mature B lymphocytes. Autophagy defects mainly affect the survival of B lymphocytes and B1 lymphocytes in the precursor phase (Miller et al. 2008). B1 lymphocytes are a self-renewing B lymphocyte subset, which is different from traditional bone marrow-derived B lymphocytes (B2 lymphocytes), which are non-bone marrow-derived and T lymphocyte-independent cells. B1 lymphocytes are capable of secreting many types of autoantibodies. Studies have found that the survival of B1 lymphocytes depends on autophagy involvement. Transplantation of fetal liver progenitor cells from ATG5-deficient mice to lethal dose-radiated mice increased B lymphocyte death in bone marrow-reconstructed

mice and impaired the pro-B lymphocyte transition to pre-B lymphocytes, eventually leading to a significant reduction in the number of peritoneal and peripheral antibody-secreting B lymphocytes B-1a cells. Another study on the specific knockout of mouse *ATG5* in CD19⁺ (mature B lymphocyte marker protein) B lymphocytes found that, although there were normal numbers of mature B lymphocytes and normal marginal zone B lymphocytes/follicles B in the mouse, the number of B-1a cells of antibody-secreting B lymphocytes was significantly reduced, suggesting that autophagy plays a crucial role in maintaining secreting B-1a B lymphocytes. Recent studies on pro-B cells, found that in the pre-B cell-specific knockout of *ATG5* transgenic animals, autophagy does not affect the transformation between the two cells but plays an essential role in maintaining the basal level of surrounding mature B cells. Autophagy of B cells in the kidney promotes the secretion of antinuclear antibodies and increases the number of long-lived plasma cells, thereby maintaining normal humoral immune responses.

27.4.2.3 The Roles of Autophagy in Plasma Cell Differentiation and Antibody Response

Plasma cells are B lymphocytes in the terminal activation phase, which are the primary effector cells for acquired humoral immune responses. B lymphocytes present in the secondary lymphoid organs, such as the spleen and lymph nodes, differentiate into short-lived plasma cells after encountering an antigen. Repeated stimulation of T lymphocytes can produce memory B lymphocytes and long-lived plasma cells, which can survive for the lifetime of an individual in a specialized bone marrow niche, maintain basal antibody levels and retain immune memory against specific antigens, thereby enabling the timely generation of antibodies that protect against pathogens and toxic substances.

Plasma cells are specialized antibody-secreting cells in which a large number of antibodies are synthesized, assembled, and secreted. The differentiation from B lymphocytes to plasma cells is an intrinsic remodeling process mediated by cellular stress that is related to proteomic plasticity, involving the complicated relationships between cell pressure, metabolism, and cell renewal. The regulation of antibody production involves the study of protein folding and assembly in this specialized secretory cell, as well as the analysis of the components of the bone marrow long-lived plasma cell and its surrounding environment. It helps to understand the mechanism of the generation of antibody memory and the formation of plasma cell degenerative diseases such as monoclonal gamma globulin disease and multiple myeloma. Autophagy plays an essential role in plasma cell differentiation, which involves the balance between the endoplasmic reticulum, differentiation, and antibody production. Autophagy is an intrinsic factor that determines the fate of long-lived plasma cells and the long-term immunity of the body.

As professional antibody-secreting cells, plasma cells are capable of synthesizing, assembling, and secreting large amounts of antibodies. B lymphocytes must remodel their proteome structure, rapidly silence B lymphocyte functions in a genetically

encoded manner, inhibit the expression of the transcription factors PAX5 and BCL-6, and regulate IRF4 and PRDM1/Blimp-1 expression by inducing transcription, resulting in the establishment of plasma cell function. In the early stage of plasma cell differentiation, the critical misfolding reaction protein XBP-1 can increase the protein secretion load by driving the expansion and folding ability of the endoplasmic reticulum. The process of assembling and processing a large number of antibodies through the endoplasmic reticulum is also closely related to metabolic stress and oxidative stress, and the antioxidant response is one of the adaptive mechanisms to address these stressors. Plasma cells undergo proteasome pressure, and their strong secretion capacity also means degradation of a large number of antibody production byproducts via the proteasome degradation pathway. However, the proteasome component in short-lived plasma cells is significantly reduced, resulting in excessive consumption of free ubiquitin and accumulation of polyubiquitinated proteins. In addition, proapoptotic factors are stabilized, thereby establishing an intrinsic mechanism to reduce the death threshold and a temporary restriction on the antibody response. For the above reasons, plasma cells are particularly sensitive to apoptosis induced by proteasome inhibition. Even malignant transformed tumor cells, such as multiple myeloma cells, are equally sensitive. The proteasome inhibitor bortezomib can be used to reduce antibody-mediated autoimmune diseases.

Autophagy is a conservative self-digestive physiological process. As a major recovery pathway, autophagy is a possible resource for cell remodeling. Moreover, the differentiation process of various cells, including adipocytes, red blood cells, and lymphocytes, is dependent on autophagy. Pengo et al. found a robust autophagy-inducing effect during plasma cell differentiation. LPS was used to stimulate primary cultured B lymphocytes, and the number of LC3-positive autophagosomes and the acidic lysosomal fraction increased as the stimulation time was prolonged (Pengo et al. 2013). Bafilomycin or lysosomal inhibitor NH_4Cl could further increase the number of LC3-positive autophagosomes. These results indicate that the autophagic flow is in an activated state in the early B lymphocyte activation process. The expression of LC3, ATG7, ATG9, and ATG4a increased, and the expression of the autophagy receptor p62 increased rapidly at the beginning of B lymphocyte activation and then gradually decreased. Compared with CD19^+ B lymphocytes, CD138^+ (specific markers of plasma cells) lymphocytes isolated from spleen have more LC3-positive fluorescent autophagic plaques, suggesting that autophagy participates in the differentiation of B lymphocytes into plasma cells.

Pengo et al. found that knocking out *ATG5* specifically in B lymphocytes inhibits autophagy (Conway et al. 2013). LPS can stimulate the differentiation of B lymphocytes into plasma cells. By comparing the changes in the *ATG5*-deficient and wild-type B lymphocyte proteomes, the authors found that *ATG5*-deficient B lymphocytes contained a large accumulation of antibody and unfolded protein in the endoplasmic reticulum during plasma cell differentiation, while mitochondria and ribosomal components did not show a significant change. The endoplasmic reticulum showed significant enlargement and increased activity in *ATG5*-deficient B lymphocytes, which was reflected in the accumulation of mRNAs encoding spliced XBP-1, total XBP-1, and BiP. Treatment with the lysosomal protease inhibitor leupeptin or

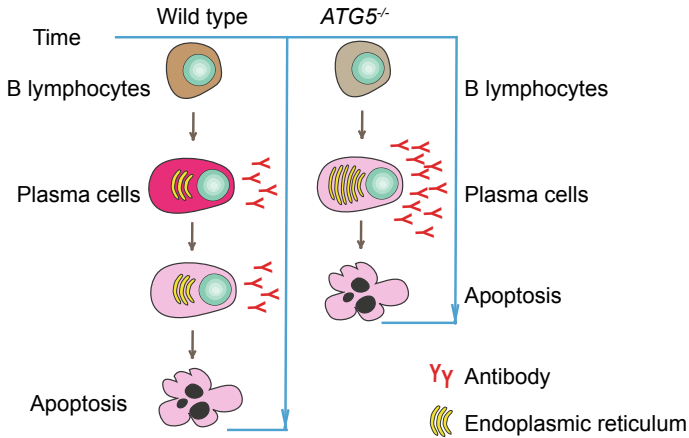


Fig. 27.3 Effects of autophagy on plasma cell biology and antibody secretion. Knocking out *ATG5* in plasma cells causes autophagy deficiency, increased endoplasmic reticulum, and rapid secretion of large amounts of antibodies, ultimately resulting in premature plasma cell apoptosis

E-64d can also cause an accumulation of unfolded protein in the endoplasmic reticulum of B lymphocytes. Blimp-1, a key transcriptional regulator of plasma cells, is induced by endoplasmic reticulum stress and drives antibody expression. However, during increased endoplasmic reticulum stress responses, *ATG5*-deficient B lymphocytes have higher Blimp-1 mRNA and IgM μ chain levels. Treatment with the endoplasmic reticulum stress inducer tunicamycin of wild-type B lymphocytes also caused increased expression of Blimp-1 and IgM μ chain. *ATG5*-deficient B lymphocytes are capable of synthesizing and secreting a large amount of antibody (Fig. 27.3), which also indicates that autophagy has a certain limiting effect on the synthesis of antibody proteins.

Significant energy changes occur during plasma cell differentiation. Autophagy needs to meet the high metabolic requirements required for plasma cell differentiation and antibody secretion. In resting B lymphocytes, autophagy is not critical for ATP production. After three days of LPS stimulation, the ATP produced by *ATG5*-deficient B lymphocytes could not reach 50% of the level in wild-type B lymphocytes, suggesting that more *ATG5*-deficient B lymphocytes have undergone apoptosis. These results indicate that autophagy is involved in maintaining the energy and survival of B lymphocytes during plasma cell differentiation.

27.4.2.4 Autophagy Prolongs Antibody Secretion

It was found that specifically knocking out *ATG5* in B lymphocytes produced lower levels of IgM and IgG antibodies only in mice immunized with T lymphocyte-dependent antigens and non-T lymphocyte-dependent antigens. In a mouse model of antigen-specific immunity, parasitic infection, and mucosal inflammation, knockout

of *ATG5* in mouse B lymphocytes can significantly reduce the antibody response. Although in vitro experiments have shown that the synthesis and secretion of antibodies by *ATG5*-deficient B lymphocytes are increased due to the lack of protection from autophagy, more B lymphocytes undergo apoptosis, which attenuates the effect of enhanced antibody secretion. To validate the above hypothesis, the researchers immunized mice with hapten NP-Ficoll, which is capable of continuously activating B lymphocytes. Two weeks after NP-Ficoll immunization, the number of plasma cells in B lymphocyte-specific *ATG5* knockout mice did not change compared with wild-type mice, but more NP antibodies were produced. This finding suggests that a single *ATG5*-deficient plasma cell has higher antibody secretion activity than a wild-type plasma cell. Batista et al. found that under viral infection, autophagy is highly activated in B cells and germinal center cells. Unlike the classical autophagy signaling pathway activated by rapamycin, GC B cells mainly undergo nonclassical autophagy signaling pathways. This indicates that autophagy regulates the activation state of B cells.

27.4.2.5 Effects of Autophagy on Long-Lived Plasma Cells of the Bone Marrow

Autophagy reduces the expression of the transcriptional repressor Blimp-1 and immunoglobulin by inhibiting the endoplasmic reticulum stress response and its signaling, reducing the excessive consumption of energy. This mechanism allows the in vivo antibody response to persist and is an essential intrinsic mechanism to maintain the niche of bone marrow-derived long-lived plasma cells. Bone marrow-derived CD19⁻CD138^{hi} plasma cells have more LC3-positive fluorescent plaques. However, compared to wild-type mice, the total amount of antibody in the circulation and the number of bone marrow-derived plasma cells did not change in B lymphocyte-specific *ATG5* knockout mice. This phenomenon may be due to the reemergence of many *ATG5* transcripts and LC3-positive fluorescent plaques in bone marrow-derived plasma cells B lymphocyte-specific *ATG5* knockout mice compared with the B220^{hi}CD138^{lo} cells derived from spleen, suggesting a partial recovery of autophagy in the bone marrow. However, 11 months after immunization with NP-CGG antigen, NP antigen-specific, bone marrow-derived, long-lived plasma cells in B lymphocyte-specific *ATG5* knockout mice were reduced by 90% compared to wild-type mice. The reason for the decrease in long-lived plasma cells in the bone marrow is due to the increase in plasma cell death because of impaired autophagy function and the decrease in MHC II-restricted antigen-presenting ability dependent on autophagy. Finally, the interactions between B lymphocytes and T lymphocytes are blocked.

Long-lived, donor-reactive memory B cells (Bmems) generate homologous antibodies that regulate the posttransplant immune response. Knocking out *ATG7* to block B cell autophagy can inhibit the type II allergic reaction, does not affect the type I allergic reaction, and inhibits the antibody production frequency in Bmems. This finding suggests that targeted autophagy has the potential to clear the Bmems

response. The Epstein–Barr virus (EBV) tumor protein EBNA3C is an essential protein for the transformation of primitive B cells and the maintenance of lymphocyte expansion. EBNA3C affects the cell cycle and apoptosis by regulating ubiquitination-mediated protein degradation and gene transcription. Recent studies have found that tumor viruses (such as EBV) promote cell survival by tampering with autophagy. EBNA3C upregulates ATG3, ATG5, and ATG7 gene transcription to activate autophagy (Bhattacharjee et al. 2018). Under conditions of nutrient deprivation, EBNA3C up-regulates tumor suppressor genes such as p27Kip1 to activate autophagy. These findings suggest that EBNA3C acts as a survival mechanism by regulating autophagy and provides a potential therapeutic approach for EBV-induced B-cell lymphoma.

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