# Autophagy during viral infection — a double-edged sword

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Abstract | Autophagy is a powerful tool that host cells use to defend against viral infection. Double-membrane vesicles, termed autophagosomes, deliver trapped viral cargo to the lysosome for degradation. Specifically, autophagy initiates an innate immune response by cooperating with pattern recognition receptor signalling to induce interferon production. It also selectively degrades immune components associated with viral particles. Following degradation, autophagy coordinates adaptive immunity by delivering virus-derived antigens for presentation to T lymphocytes. However, in an ongoing evolutionary arms race, viruses have acquired the potent ability to hijack and subvert autophagy for their benefit. In this Review, we focus on the key regulatory steps during viral infection in which autophagy is involved and discuss the specific molecular mechanisms that diverse viruses use to repurpose autophagy for their life cycle and pathogenesis.

**Multivesicular bodies**
(MVBs). A specialized subset of endosomes that contain membrane-bound intraluminal vesicles.

**Unfolded protein response**
(UPR). A cellular stress response activated in response to an accumulation of unfolded or aggregated proteins in the endoplasmic reticulum.

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Autophagy is an evolutionarily conserved degradative process that is required to maintain host health and facilitates the capture and clearance of invading pathogens by the immune system[1,2]. Eukaryotic cells deliver dangerous and unwanted cytoplasmic material to lysosomes for degradation via three major routes: microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy[3]. During microautophagy, invaginations of the lysosomal membrane directly take up cytoplasmic material[4] or the material directly enters multivesicular bodies (MVBs) of late endosomes[5]. During CMA, a cytoplasmic chaperone mediates lysosomal-associated membrane protein 2A (LAMP2A)-dependent uptake of unfolded proteins[6]. Macroautophagy involves a specialized double-membrane vesicle (DMV) known as the autophagosome (BOX 1). Macroautophagy is the best-characterized form of autophagy, and we will hereafter refer to it simply as autophagy.

Autophagy begins with the sequestration of cytoplasmic proteins and damaged organelles into a cup-shaped double-membrane termed the isolation membrane (or phagophore), which is derived from various cellular compartments[7]. The immature isolation membrane grows to envelop the engulfed contents, thus forming an autophagosome, which subsequently fuses with the lysosome to form autolysosomes. At this stage, the contents undergo degradation to enable their recycling upon nutrient depletion[8]. Acidification is a key regulatory step in autophagosome maturation and requires the activity of vacuolar ATPases.

Autophagy is involved in various physiological processes, including starvation, cell differentiation and development, and degradation of aberrant structures, which ultimately maintains cellular homeostasis[9]. Moreover, autophagy is part of host stress responses such as the unfolded protein response (UPR)[10]. Given that viral infection and replication cause cell stress, autophagy is a frequent by-product of infection. However, autophagy during viral infection is not merely a passive process. As clearance of cytoplasmic components is a major function of autophagy, the innate immune system activates autophagy to degrade and dispose of invading viruses[11]. Furthermore, at later stages of infection, autophagy facilitates antigen processing and thereby the induction of adaptive immune responses[12,13]. There is a form of selective autophagy, xenophagy, that specifically recognizes intracellular microorganisms and targets them to autophagosomes for degradation[14]. Despite the seemingly hazardous conditions in autophagosomes for viruses, some viruses convert the autophagosome to their home for replication. The autophagosome provides a membrane-bound, protected environment to generate their progeny, and viruses can use autophagy-generated metabolites and energy for replication. Another type of autophagy, termed lipophagy, is a degradation pathway for lipid droplets in cells, and viruses can also hijack this process[15]. Lipid droplets are an ideal platform for virion assembly, and viruses can directly activate lipophagy to maintain the high level of ATP required for viral replication. In summary, current evidence suggests that viruses have evolved diverse strategies to either combat or utilize autophagy to promote their own life cycle. In this Review, we provide an overview of the function of autophagy as an antiviral defence mechanism and then focus on how viruses subvert and

# REVIEWS

The autophagic machinery was first described in yeast, and homologues were found in mammals. The core components consist of more than 30 autophagy proteins (ATGs)[145]. Autophagy is controlled by the sequential activities of three key protein complexes: the serine/threonine protein kinase ULK1 complex (comprising ULK1, FAK family kinase-interacting protein of 200 kDa (FIP200; also known as RB1CC1), ATG13 and ATG101)[146–148]; the phosphoinositide 3-kinase catalytic subunit type III (PI3KC3) complex (comprising Beclin 1, vacuolar protein sorting 34 (VPS34), VPS15 and ATG14L)[149,150] and the ATG16L1 complex (comprising ATG16L1, ATG5 and ATG12)[151]. Under nutrient-rich conditions, the serine/threonine kinase mechanistic target of rapamycin complex 1 (mTORC1) phosphorylates and inactivates the autophagy initiators ULK1 and ATG13. Nutrient starvation or rapamycin, which is an mTORC1 inhibitor, induces the formation of the active ULK1 complex, which ultimately phosphorylates and activates the Beclin 1–VPS34 complex[152,153]. The lipid kinase VPS34 creates phosphatidylinositol-3-phosphate (PtdIns3P)-rich regions on the surface of donor membranes, including the endoplasmic reticulum (ER), Golgi apparatus, ER–mitochondria contact sites, endosomes and the plasma membrane[154–158]. During autophagy initiation, PtdIns3P on the isolation membrane is recognized by the PtdIns3P-binding factors WD-repeat domain phosphoinositide-interacting protein 1 (WIPI1)–WIPI4 (REF.[159]). The binding of WIPI1 and WIPI2 to the PtdInsP3P-enriched phagophore is crucial for the recruitment of the ATG16L1 complex, which consequently facilitates LC3 lipidation. The ATG16L complex is generated through a ubiquitin-like (UBL) conjugation reaction in which ATG12 is conjugated to ATG5 by the sequential action of ATG7 and ATG10 (REF.[160]). ATG16L1 non-covalently binds to the ATG5–ATG12 conjugate to form a multimeric complex[151]. Another UBL molecule, LC3, is cleaved by the ATG4 protease at its C-terminal arginine to expose a glycine residue[161] and is subjected to a second UBL conjugation reaction involving ATG7 and ATG3 (REF.[162]). There are seven mammalian orthologues of ATG8 (LC3A, LC3B, LC3C, γ-aminobutyric acid receptor-associated protein (GABARAP), GABARAPL1, GABARAPL2 and GABARAPL3; in this Review, we refer to them as LC3). The ATG16L1 complex then conjugates LC3 to phosphatidylethanolamine (PE) to complete the reaction[163]. This lipidated form of LC3 mediates membrane tethering and fusion to extend the isolation membrane by recruiting membranes from multiple sources, leading to the formation of autophagosomes[164]. During the final maturation, autophagosomes are decorated with RAB7 and the tail-anchored SNAP receptor (SNARE) syntaxin 17 (STX17), which leads to fusion with lysosomes and degradation of sequestered substrates[109]. The Beclin 1–PI3KC3 complex subunit ATG14L directly binds the SNARE complex STX17–SNAP29 together with vesicle-associated membrane protein 8 (VAMP8). An adaptor called pleckstrin homology domain-containing protein family member 1 (PLEKHM1) binds to LC3, RAB7 and STX17. PLEKHM1 recruits the tethering complex transmembrane and ubiquitin-like domain-containing protein 1 (HOPS; also known as TMUB1) and brings the autophagosome and lysosome together[165]. At this stage, the Beclin 1–VPS34 complex is identifiable by the presence of ultraviolet radiation resistance-associated gene protein (UVRAG) instead of ATG14L[149]. BCL-2, B cell lymphoma-2; BIF, BAX-interacting factor; LAMP2, lysosome-associated membrane glycoprotein 2; PAS, pre-autophagosomal structure.
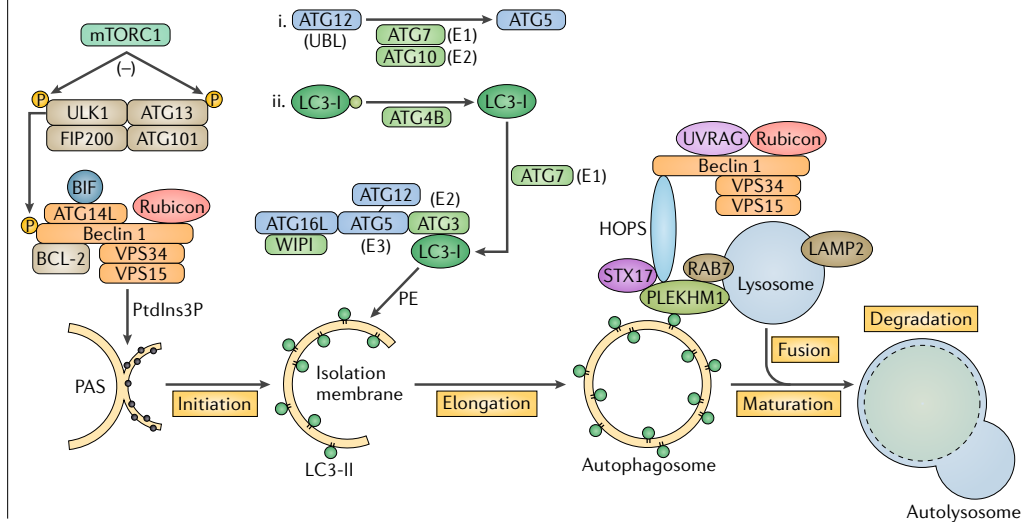


exploit multiple steps of the autophagic pathway to evade immune responses and facilitate viral replication.

## Autophagy and innate antiviral immunity

*Pattern recognition receptors, autophagy and antiviral interferon responses.* The innate immune system is the first line of defence against invading microorganisms, including viruses. Germline-encoded receptors called pattern recognition receptors (PRRs) recognize pathogen-associated molecular patterns (PAMPs),

including virus-specific features, and activate the type I interferon (IFN) pathway to establish an antiviral milieu[16]. PRRs are found in different subcellular locations, which enables the innate immune system to detect infection throughout the viral life cycle.

The intracellular endosomal Toll-like receptors (TLRs) are primary detectors of viral PAMPs, including double-stranded (ds)RNA (TLR3), single-stranded (ss)RNA (TLR7 and TLR8) and DNA with unmethylated CpG sites (TLR9) (FIG. 1). Most TLRs recruit the
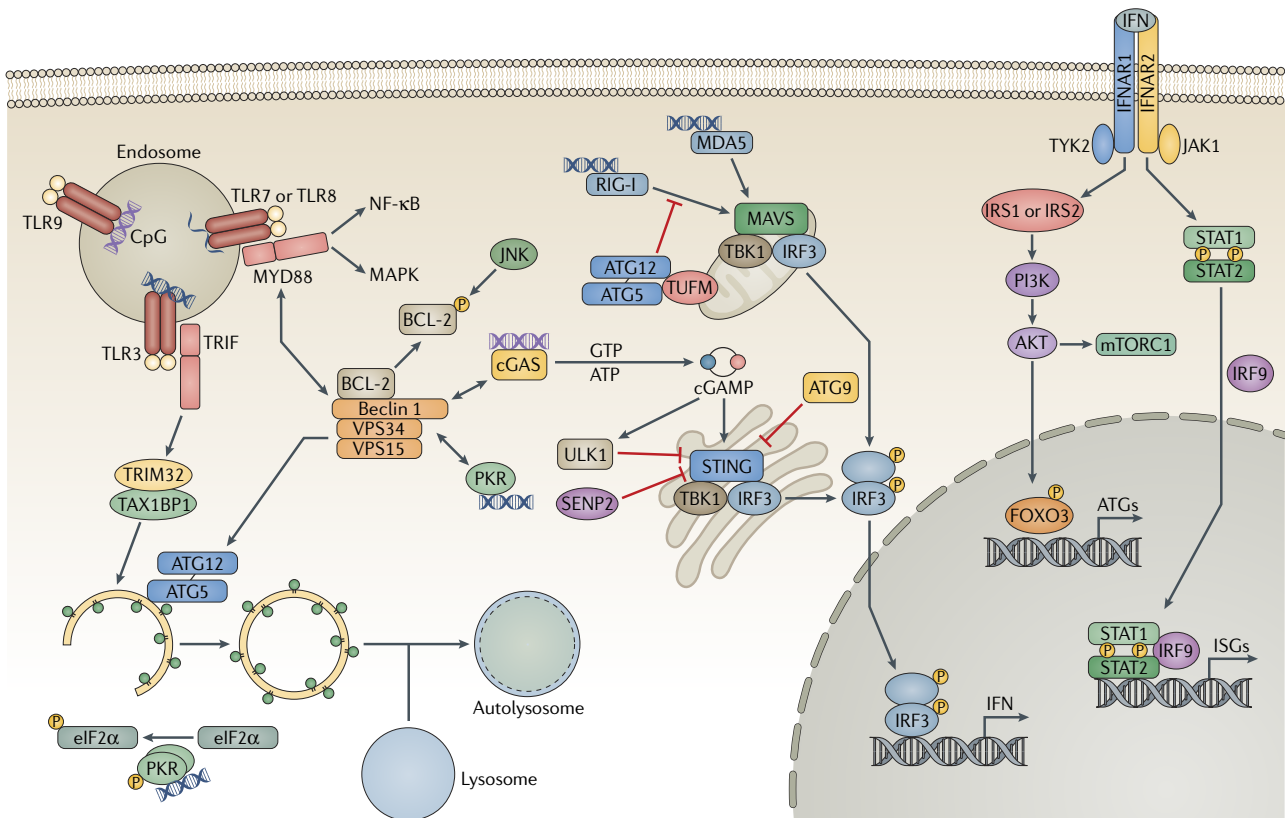
Fig. 1 | **Pattern recognition receptors and autophagy.** In host cells, various pattern-recognition receptors (PRRs) recognize viral pathogen-associated molecular patterns (PAMPs), which leads to the activation of transcription factors and the induction of the interferon (IFN) response. Endosomal Toll-like receptors (TLRs) recognize viral nucleic acids and recruit the adaptor proteins TIR domain-containing adaptor molecule 1 (TRIF) and myeloid differentiation primary response protein MYD88, relaying signals to the nuclear factor-κB (NF-κB) and mitogen-associated protein kinase (MAPK) pathways. Binding to MYD88 or TRIF causes Beclin 1 to dissociate from the B cell lymphoma-2 (BCL-2) inhibitory complex, resulting in the induction of autophagy. TRIF is targeted by selective autophagy by the tripartite motif-containing protein 32 (TRIM32)–TAX1-binding protein 1 (TAX1BP1) complex for degradation. JUN N-terminal kinase (JNK) phosphorylates BCL-2 to initiate Beclin 1-mediated autophagy. During RNA virus infection, retinoic acid-inducible gene I (RIG-I) senses double-stranded (ds)RNA and signals through mitochondrial antiviral-signalling protein (MAVS) to activate interferon regulatory factor 3 (IRF3), leading to the production of IFN. To suppress RIG-I signalling, the autophagy protein 5 (ATG5)–ATG12 complex disrupts the interaction between RIG-I and MAVS. Interferon-induced, dsRNA-activated protein kinase (PKR) phosphorylates eukaryotic translation initiation factor 2 subunit 1 (eIF2α) to induce autophagy. During DNA virus infection, the DNA sensor cyclic GMP–AMP (cGAMP) synthase (cGAS) produces the secondary messenger cGAMP, which activates stimulator of interferon genes protein (STING), IRF3 and IFN expression. Furthermore, cGAS competes with Rubicon for Beclin 1 binding, thus triggering autophagy. Beclin 1 also suppresses cGAMP production and activates ULK1 to phosphorylate STING for its degradation. ATG9 inhibits the aggregation of STING on Golgi apparatus-derived compartments to suppress the DNA sensing. STING undergoes desumoylation and chaperone-mediated autophagy (CMA)-mediated degradation. Finally, extracellular IFN is recognized by the interferon receptor (IFNAR) and activates JAK–STAT (Janus kinase–signal transducer and activator of transcription) signalling. JAK1 and non-receptor tyrosine-protein kinase TYK2 phosphorylate insulin receptor substrate 1 (IRS1) and IRS2, leading to the activation of the PI3K–AKT–mTOR (phosphoinositide 3-kinase–AKT–mechanistic target of rapamycin) pathway and forkhead box 3 transcription factor (FOXO3) as well as the expression of autophagy-related genes. ISGs, interferon-stimulated genes; MDA5, interferon-induced helicase C domain-containing protein 1; SENP2, sentrin-specific protease 2; TBK1, tank-binding kinase 1; TUFM, elongation factor Tu, mitochondrial; VPS34, vacuolar protein sorting 34.

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**Plasmacytoid dendritic cells** (pDCs). A subset of dendritic cells that are distinct from conventional dendritic cells and that produce large amounts of interferon.

adaptor myeloid differentiation primary response protein MYD88, whereas TLR3 and TLR4 recruit another adaptor, TIR domain-containing adaptor molecule 1 (TRIF; also known as TICAM1). Both adaptors activate nuclear factor-κB (NF-κB) for the synthesis of inflammatory cytokines and IFN regulatory factors (IRFs) for triggering IFN production[16]. TLR stimulation induces binding of MYD88 or TRIF to Beclin 1, which disrupts the Beclin 1–B cell lymphoma-2 (BCL-2) interaction and ultimately activates autophagy (FIG. 1). Conversely, autophagy can inhibit TLR signalling by promoting the selective degradation of TRIF[17] (FIG. 1). Furthermore, plasmacytoid dendritic cells (pDCs) that are deficient for autophagy protein 5 (ATG5) showed reduced

# REVIEWS

TLR7-dependent production of IFNs during infection with vesicular stomatitis virus (VSV) or Sendai virus (SeV)[18]. ATG5 also contributed to TLR9-induced IFNα production in pDCs infected with herpes simplex virus type 1 (HSV-1)[19]. During viral infection, TLR activation tends to induce autophagy to improve IFN production, whereas the negative regulation of autophagy helps terminate TLR signalling.

The RIG-I like receptors (RLRs) retinoic acid-inducible gene I (RIG-I; also known as DDX58) and melanoma differentiation-associated protein 5 (MDA5; also known as IFIH1) sense viral RNAs in the cytosol and signal through interaction with mitochondrial antiviral-signalling (MAVS) via their caspase-recruiting domain (CARD), activating interferon regulatory factor 3 (IRF3), IRF7 and NF-κB[20]. Although autophagy enhances IFN production in virally infected cells, it also can limit RLR-triggered IFN production. ATG5 deficiency increases the production of IFN in response to RNA viruses such as VSV and inhibits viral replication[21]. The ATG5–ATG12 complex and its binding protein (elongation factor Tu, mitochondrial (TUFM)) suppress dsRNA-induced production of IFN by disrupting RIG-I signalling[22] (FIG. 1). In addition, interferon-induced, dsRNA-activated protein kinase (EIF2AK2; also known as PKR) binds to Beclin 1 within the vacuolar protein sorting 34 (VPS34) complex, which initiates the formation of autophagosomes. PKR-mediated phosphorylation of the eukaryotic translation initiation factor 2 subunit 1 (EIF2S1; also known as eIF2α) also induces autophagy[23] (FIG. 1). The RLRs provide an example of how autophagy can negatively regulate immune signalling.

The cytosolic DNA sensor cyclic GMP–AMP (cGAMP) synthase (cGAS) recognizes dsDNA during infection with bacteria or DNA viruses[24] and produces cGAMP. Subsequently, cGAMP binds to and activates the endoplasmic reticulum (ER)-associated stimulator of interferon genes protein (STING), leading to IRF3 activation and IFN production (FIG. 1). During HSV-1 infection, the DNA-sensing pathway and autophagy pathway converge: Beclin 1 interacts with and impairs the nucleotidyl transferase activity of cGAS, decreasing the amount of cGAMP available to stimulate STING[25]. Conversely, cGAS competes with the autophagy inhibitor Rubicon for Beclin 1 binding, thus triggering autophagy and stimulating the degradation of cytosolic viral DNA to avoid persistent immune stimulation[25]. In addition, cGAMP leads to the dissociation of ULK1 from 5′-AMP-activated protein kinase catalytic subunit α2 (AMPK), and activated ULK1 then phosphorylates STING, leading to its degradation[26] (FIG. 1). Loss of ATG9 causes aggregation of STING on Golgi apparatus-derived compartments and increases STING-dependent production of IFNs[27]. During the late phase of HSV-1 infection, STING is also targeted by CMA-mediated degradation to terminate IFN signalling[28]. Additionally, cGAS-dependent signalling induces autophagy, which promotes pathogen clearance and functions as a negative feedback loop to turn off signalling.

Downstream of PRR-detection of viral infection, newly synthesized IFN is secreted and binds to the IFN receptor (IFNAR), leading to the JAK1 and non-receptor tyrosine-protein kinase TYK2-mediated phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT2, which activate transcription of IFN-stimulated genes (ISGs) to restrict viral infection[29] (FIG. 1). Additionally, JAK1 and TYK2 can phosphorylate insulin receptor substrate 1 (IRS1) and IRS2[30,31], which activate the PI3K–AKT–mTOR (mechanistic target of rapamycin) pathway. Activated AKT has several targets, including forkhead box transcription factor 3 (FOXO3), which activates the transcription of a variety of autophagy-related genes[32] (FIG. 1). Upon viral infection, crosstalk between PRRs and autophagy leads to the activation and/or inactivation of various intracellular signalling pathways, which generates an optimal antiviral milieu.

***Autophagy-mediated restriction of viral replication.*** Autophagy can be harnessed to degrade viral components, viral particles or even host factors required for viral replication; thus, autophagy functions as an important innate antiviral response (BOX 2). However, this autophagic degradation of virions, which is also known as virophagy, can be subverted by several viruses.

During Sindbis virus (SINV) infection, Beclin 1 protects against SINV-mediated encephalitis[33]. Moreover, ATG5 deficiency results in delayed SINV clearance and accumulation of the autophagy receptor p62 (also called sequestosome 1 (SQSTM1)). Interestingly, p62 binds to a SINV capsid protein and targets the viral capsid to the autophagosome[34] (FIG. 2). There, SMURF1, an E3-ubiquitin ligase, is required for the colocalization of p62 with the SINV capsid protein and for virophagy[35]. Recently, Fanconi anaemia group C protein (FANCC) was found to interact with the SINV capsid protein to facilitate virophagy[36,37] (FIG. 2). SMURF1 and FANCC also target HSV-1 for virophagy, suggesting that those two proteins commonly function as virophagic factors[35,36].

Picornaviruses, including poliovirus, are detected by galectin 8, which restricts viral infection by initiating the autophagic degradation of the viral RNA genome[38]. Specifically, when poliovirus punctures the endosomal membrane to release its genome into the cytoplasm, β-galactosides are exposed and trigger galectin 8. This leads to the detection of permeated endosomes and marks them for autophagic degradation. In turn, poliovirus uses the host protein HRAS-like suppressor 3 (PLA2G16) to evade this detection and enable genome delivery (FIG. 2). Another picornavirus, coxsackievirus B3 (CVB3), uses the viral protease 2A to cleave p62 and inhibit virophagy[39] (FIG. 2).

The ability of hepatitis C virus (HCV) infection to induce autophagy has been widely demonstrated in cell culture and in the hepatocytes of chronically infected patients[40]. HCV can escape autophagic destruction, and it is thought to use autophagy for its own benefit (see below). However, a recent study demonstrated that an ER transmembrane protein, SHISA5 (also known as SCOTIN), interacts with the viral non-structural protein 5A (NS5A), which leads to its autophagic degradation and suppresses viral replication[41] (FIG. 2).

## Box 2 | Xenophagy

The precise mechanisms controlling selective autophagy are still unclear. The best evidence for the specificity of autophagy comes from the selective degradation of large cellular structures, pathogens, damaged organelles and protein aggregates. The removal of these components by selective autophagy is crucial for cellular homeostasis. According to the cargo, there are different types of autophagy: reticulophagy (endoplasmic reticulum)[97], mitophagy (mitochondria)[166], ribophagy (ribosomes)[167] and pexophagy (peroxisomes)[168]. 'Xeno' means 'stranger' or 'foreigner'; thus, xenophagy refers to selective autophagy targeting intracellular pathogens. Xenophagy targets and removes intracellular pathogens, including bacteria and viruses. A hallmark of xenophagy is its coordination by the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). The invading microorganisms are first phagocytosed and then delivered to autophagosomes. Here, pathogen specificity is mediated by xenophagy signalling receptors, such as p62 (sequestosome 1), next to of BRCA1 gene 1 protein (NBR1), calcium-binding and coiled-coil domain-containing protein 2 (NDP52; also known as CALCOCO2) and optineurin, which are a subset of PRRs called p62/SQSTM1-like receptors (SLRs)[169–172]. SLRs contain cargo recognition domains (CDRs), which recognize ubiquitin or galectin on intracellular pathogens, and LC3-interacting regions (LIRs), which recruit membranes to autophagosomes. Additionally, NDP52 interacts through its galectin-interacting region with galectin 8, which binds to cytoplasmically exposed β-galactoside glycans on pathogen-damaged endosomal membranes[173]. Autophagy can target invading pathogens in a process termed LC3-associated phagocytosis (LAP), which describes the recruitment of LC3 to phagosomes. LAP does not involve sequestration in double-membrane autophagosomes; instead, LC3 binds to and decorates a recently formed but intact phagosomal membrane.

There are many factors leading to the induction and resolution of xenophagy, necessitating clear experimental metrics to confirm xenophagy during viral infections. Although it is defined by its target specificity, xenophagy is identifiable by the usual hallmarks of autophagy. The crucial steps in the confirmation of autophagy are the autophagic protein response and turnover of autophagosomes. Protein response is determined based on the conversion of soluble LC3-I to lipidated LC3-II, which can be detected by immunoblot or immunofluorescent visualization of enhanced green fluorescent protein–LC3 puncta formation. Furthermore, red fluorescent protein–GFP–LC3 dual fluorescence can be used to visualize autophagosome maturation and lysosome fusion. Other markers of xenophagy include p62, ATGs, lysosome-associated membrane glycoprotein 1 (LAMP1), LAMP2 and upstream markers such as Beclin 1. The use of these markers, together with genetic knockdown of ATG effector proteins, will determine if a pathogen activates xenophagy during infection. Well-known autophagy inducers, including rapamycin, and inhibitors such as wortmannin, chloroquine, bafilomycin and 3-methyladenine can also be used to determine the effects of xenophagy.

Human immunodeficiency virus 1 (HIV-1) is also subject to autophagic degradation. To overcome innate immunity, HIV-1 uses the virion infectivity factor Vif, which promotes the degradation of the HIV-1 restriction factor APOBEC3G[42]. In turn, histone deacetylase 6 (HDAC6) forms a complex with APOBEC3G and mediates autophagy-dependent Vif degradation, thereby inhibiting HIV-1 replication[43] (FIG. 2). In CD4+ T cells, autophagy selectively degrades the transactivator Tat, a protein that increases viral transcription through its ubiquitin-independent interaction with p62[44] (FIG. 2). In Langerhans cells, the restriction factor tripartite motif-containing protein 5α (TRIM5α) mediates assembly of the autophagy-activating complexes and targets langerin-bound, engulfed HIV-1 to autophagic degradation[45].

Autophagy also has antiviral activity independently of degradation. Norovirus (NoV) is a cause of human non-bacterial epidemic gastroenteritis[46]. In a mouse model of murine norovirus (MNV) infection, IFNγ-mediated antiviral defence requires the ATG5–ATG12–ATG16L1 complex that functions in autophagosome formation[47]. In ATG16L1 hypomorphic mice, MNV infection provoked a phenotype that resembled Crohn's disease[48]. Interestingly, the induction, fusion and degradative activities of autophagy were not required; instead, IFNγ-inducible GTPases, which were targeted to MNV replication complexes by LC3, inhibited viral replication[49] (FIG. 2).

Collectively, multiple host pathways detect viral infection to initiate autophagy-mediated innate defences. However, viruses have evolved to evade autophagy and to harness autophagy for their own life cycles.

*Autophagy-mediated viral antigen presentation.* The adaptive immune response is initiated by the presentation of protein fragments on major histocompatibility complex (MHC) molecules in antigen-presenting cells (APCs), which are then recognized by T cells. MHC class I molecules present intracellular antigens after they have been processed by the proteasome and transported into the ER by the antigen peptide transporter (TAP). By contrast, MHC class II molecules load extracellular antigens after lysosomal degradation in late endosomal MHC class II-containing compartments (MIICs). There is an additional mechanism for loading exogenous antigens onto MHC class I molecules in a process known as cross-presentation.

Autophagosomes constitutively form in MHC class II-positive dendritic cells, B cells and epithelial cells and fuse with multivesicular MIICs to deliver cytoplasmic proteins for MHC class II presentation and antiviral immunity[50]. Epstein–Barr virus (EBV) establishes a persistent infection by maintaining a latent state in memory B cells. EBV nuclear antigen 1 (EBNA1) is incorporated into autophagosomes and degraded before being presented on MHC class II molecules[13] (FIG. 2). Blocking of autophagy with a PI3K inhibitor or through knockdown of ATG12 reduced MHC class II presentation to CD4+ T cells. HIV-1 is also targeted to autophagosomes, processed by the autophagic machinery and presented on MHC class II molecules. HIV-1 Gag-derived proteins colocalize and interact with LC3, and autophagy promotes Gag processing[51] (FIG. 2). In turn, the HIV-1 envelope protein activates mTOR to shut down autophagy and impede the immune function of dendritic cells[52].

**Restriction factor**
An antiviral protein that is produced in the host and blocks viral replication.

**Langerhans cells**
Immune cells found in the epidermis that function as antigen-presenting cells that bind antigens entering through the skin.

**Crohn's disease**
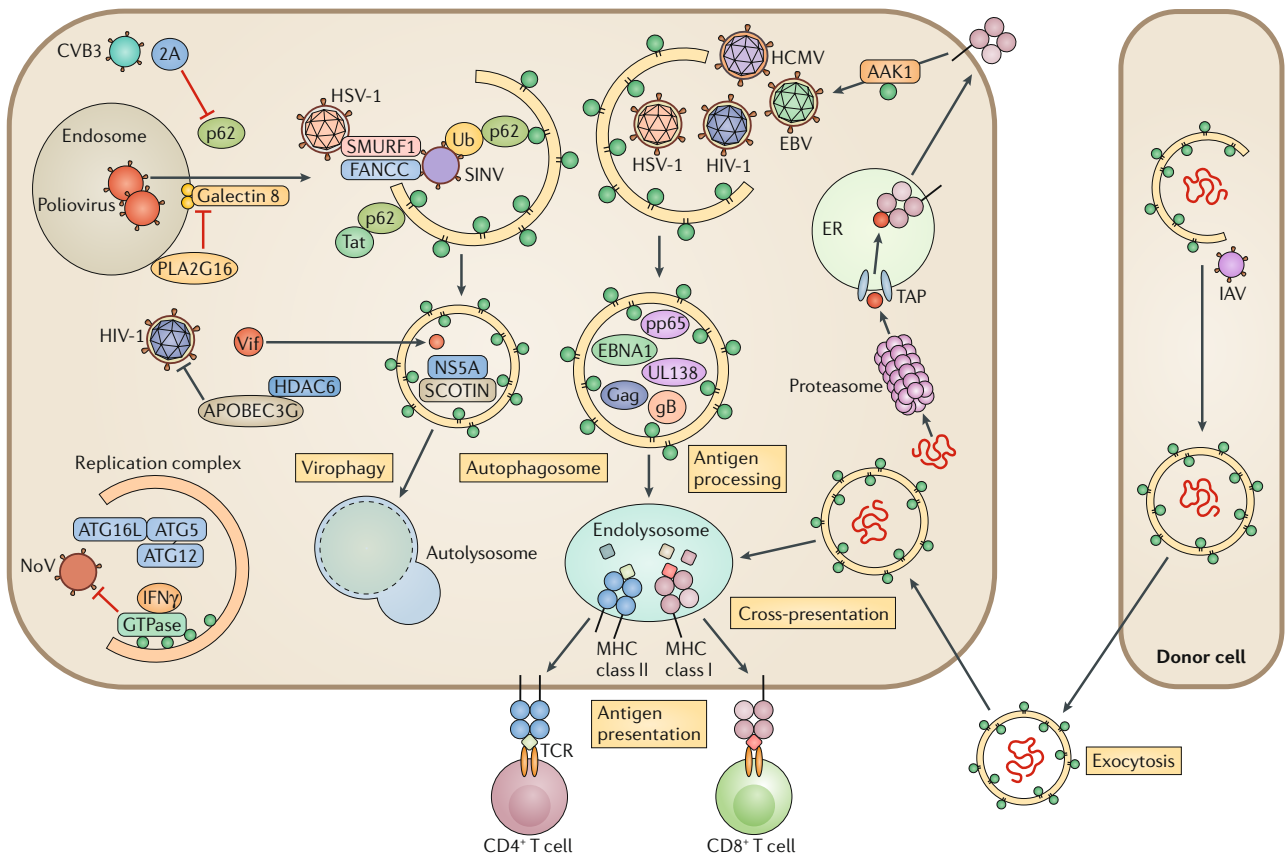A chronic inflammatory disease of the gastrointestinal tract.

Fig. 2 | **Autophagy-mediated antiviral immune responses.** Autophagy targets intruding viruses by directly eliminating them in autophagosomes. Adaptor protein p62 binds to ubiquitin-coated viral particles that are subsequently delivered to the autophagic machinery. For example, p62 binds to Sindbis virus (SINV) capsid protein and mediates selective autophagy by interacting with LC3 through its LC3-interacting region (LIR). Two host factors, E3 ubiquitin-protein ligase SMURF1 and Fanconi anaemia group C protein (FANCC), are involved in the virophagy of SINV and herpes simplex virus type 1 (HSV-1). During hepatitis C virus (HCV) infection, the host endoplasmic reticulum (ER) transmembrane protein SCOTIN associates with HCV non-structural protein 5A (NS5A) and leads to its degradation, restricting HCV replication. Poliovirus infection and membrane rupture are detected by galectin 8, targeting the virus to autophagosomes. Poliovirus uses HRAS-like suppressor 3 (PLA2G16) to escape from autophagic degradation. HIV-1 virion infectivity factor (Vif) is targeted by histone deacetylase 6 (HDAC6) for degradation. The autophagy protein 5 (ATG5)–ATG12–ATG16L1 complex is recruited in the replication complex and restricts norovirus (NoV) replication through interferon-γ (IFNγ)-inducible GTPases. In antigen-presenting cells (APCs), autophagy delivers intracellular and extracellular antigens to the endolysosome, where they are loaded onto major histocompatibility complex (MHC) class II molecules for presentation to CD4+ T cells. EBV nuclear antigen 1 (EBNA1) is the dominant CD4+ T cell antigen and is primarily processed in the autophagosome. HIV-1 Gag is also targeted by the autophagosome for processing. Autophagy regulates the internalization of MHC class I molecules from the cell membrane via AP2-associated kinase 1 (AAK1). UL138 from human cytomegalovirus (HCMV) and gB from HSV-1 are presented on MHC class I molecules in an autophagy-dependent pathway, when antigen peptide transporter (TAP)-dependent presentation is blocked. Autophagy also promotes an alternative pathway of class I presentation called cross-presentation. It modulates trafficking and processing of phagocytosed antigens from the endosome to MHC I, and autophagy induces antigen packaging in donor cells for release to APCs. 2A, protease 2A; CVB3, coxsackievirus B3; EBV, Epstein–Barr virus; IAV, influenza A virus; pp65, 65 kDa phosphoprotein; TCR, T cell receptor; Ub, ubiquitin.

Autophagy limits MHC class I presentation by mediating internalization and degradation of MHC class I molecules. The AP2-associated kinase 1 (AAK1) associates with LC3 to facilitate this internalization[53] (FIG. 2). Dendritic cells that are deficient for ATG5, ATG7 or VPS34 had increased surface expression of MHC class I and induced CD8+ T cell activation[53,54]. Autophagy can also contribute to antigen presentation when TAP is inhibited. Both HSV-1 and human cytomegalovirus

(HCMV) encode TAP-blocking proteins[55]. Although macrophages infected with HSV-1 initially used the conventional MHC class I presentation pathway, a second pathway involving a vacuolar compartment was triggered later during infection. Specifically, a previously unknown type of autophagosome originated from the nuclear envelope and mediated presentation of HSV-1 antigens on MHC class I molecules[56] (FIG. 2). Similarly, during latent infection, the HCMV protein UL138 is

presented to CD8+ T cells in an autophagy-dependent pathway[57,58] and colocalizes with LAMP2-positive late endosomal structures[57] (FIG. 2). This suggests that autophagic viral cargos and antigens can access the MHC class I presentation machinery.

Autophagy also contributes to the delivery and processing of extracellular antigens for cross-presentation by MHC class I molecules in a cell-specific manner[59–61]. The autophagic machinery modulates endosomes to enhance cross-presentation and assists in packaging antigens for release by donor cells, including virus-infected, tumour or dying cells, to neighbouring dendritic cells[62]. Autophagy was also involved in cross-presentation of viral antigens during vaccination, which was promoted by a stress-dependent initiation of autophagy in dendritic cells[63]. Autophagy-dependent exocytosis transfers extracellular antigens in LC3-coated vesicles from donor cells to dendritic cells (FIG. 2). For example, mouse embryonic fibroblasts that are infected with influenza A virus (IAV) provide antigens to dendritic cells to stimulate IAV-specific CD8+ T cells[64]. Recently, the CD8+ T cell-specific antigens of HCMV 65 kDa phosphoprotein (pp65) were shown to be cross-presented by human B cells through a pathway involving autophagosomes rather than the TAP-dependent conventional secretory pathway[60] (FIG. 2).

During viral infection, the host uses autophagy for optimal antigen processing for MHC class II presentation and, when TAP is inhibited, MHC class I presentation. In addition, autophagy-mediated exocytosis of antigens in vesicular compartments supports cross-presentation on MHC class I molecules.

### Viral evasion of autophagic degradation

As previously discussed, autophagy can restrict viral infections. Persisting viruses, however, have evolved various strategies to escape or inhibit multiple steps of the autophagic pathway. HSV-1-encoded neurovirulence factor ICP34.5 interacts with Beclin 1 to inhibit autophagy[23] (FIG. 3). ICP34.5 also counteracts the antiviral role of PKR by recruiting host phosphatase PP1α to dephosphorylate eIF2α[65] (FIG. 3). TANK-binding kinase 1 (TBK1) was recently discovered as a target of ICP34.5 (REF.[66]). TBK1 regulates autophagosome maturation by phosphorylating the autophagy receptors p62 and optineurin. Thus, inhibition of TBK1 by ICP34.5 represents an additional strategy of HSV-1 to suppress autophagy (FIG. 3). Late during the HSV-1 life cycle, the RNA-binding tegument protein Us11 binds to PKR to prevent phosphorylation of eIF2α[65] (FIG. 3).

HCMV also counteracts autophagy and has a functional homologue of ICP34.5 called TRS1 (REF.[67]). Unlike ICP34.5, the PKR-binding domain of TRS1 is not involved in autophagy inhibition. Instead, TRS1 binds to Beclin 1 through its N-terminal region, and this binding is essential to inhibit autophagy. Recently, another HCMV protein, IRS1, was also found to block autophagy by interacting with Beclin 1 (REF.[68]) (FIG. 3).

To antagonize autophagy, γ-herpesviruses, including Kaposi's sarcoma-associated herpesvirus (KSHV) and murine γ-herpesvirus 68 (MHV68), have viral homologues of BCL-2, ORF16 and M11 (REF.[69]). These viral BCL-2 (vBCL-2) proteins mimic their cellular counterparts (cBCL-2) and attenuate autophagy through direct interaction with Beclin 1 (REF.[70]). Structural and biochemical analyses demonstrate that vBCL-2 has a markedly higher affinity for Beclin 1 and inhibits autophagosome formation more efficiently than cBCL-2 (REF.[71]). Moreover, vBCL-2 lacks the regulatory loop of cBCL-2 that is phosphorylated by JUN N-terminal kinase (JNK)[72]; therefore, it can continuously associate with Beclin 1 (REF.[73]) (FIG. 3). These data collectively show that vBCL-2 has evolved to be a highly potent autophagy inhibitor. Although EBV also encodes two vBCL-2 proteins (BHRF1 and BALF1), it is unknown whether they inhibit autophagy[74]. The K7 protein of KSHV promotes Rubicon–Beclin 1 interaction and inhibits the enzymatic activity of VPS34, which blocks the fusion of autophagosomes with lysosomes[75] (FIG. 3). In addition, KSHV vFLIP, a homologue of the cellular FLICE-like inhibitor protein (FLIP; also known as ORF71), suppresses autophagy[76] by preventing ATG3 from binding and processing LC3 during autophagosome elongation (FIG. 3). Interestingly, two KSHV vFLIP-derived peptides are individually sufficient for binding and releasing ATG3 from cellular FLIP, which results in robust autophagy and autophagic cell death[76].

HIV-1 also inhibits autophagy to prevent the sequestration of HIV-1 proteins within autophagosomes and their lysosomal degradation. Although HIV-1 infection induces autophagosome formation and HIV-1 Gag colocalizes with LC3, HIV-1 Nef blocks autophagosome maturation by interacting with Beclin 1 (REF.[51]) (FIG. 3). In infected macrophages, the interaction between HIV-1 and TLR8 and the Beclin 1-dependent dephosphorylation and nuclear translocation of transcription factor EB (TFEB) activates autophagy and lysosome biogenesis[77]. HIV-1 Nef interacts with Beclin 1 to sequester TFEB in the cytosol, thus inhibiting maturation of autophagosomes. These examples demonstrate the importance of deregulating the autophagy pathway to facilitate viral persistence.

### Autophagy supporting viral replication

*Viral manipulation of autophagosomes for replication.* RNA viruses exploit autophagy for their replication. Double-membrane compartments formed during autophagy can provide a physical platform for the viral replication machinery, locally concentrate essential intermediates and protect viral RNAs from detection by innate immune sensors and degradation.

The first example of viral reshaping of intracellular membranes came from polioviruses, which lack a membrane envelope[78]. The autophagy-inducer rapamycin increased poliovirus replication, whereas silencing of genes that are essential for autophagosome formation decreased it[79]. Electron microscopy of poliovirus-infected cells revealed DMVs that resembled autophagosomes and provided scaffolds for viral RNA replication. Further, poliovirus infection led to the accumulation of LC3 in puncta, and the expression of the viral proteins 2BC and 3A led to LC3 lipidation and DMV formation, which provides a mechanistic link between autophagy and poliovirus replication (FIG. 3). Other picornaviruses, such as

**Puncta**
A structural marker that is involved in autophagosome formation by the dynamic process of LC3 association.

**LC3 lipidation**
A conjugation of phosphatidylethanolamine (PE) to LC3 to form LC3–PE, which is recruited to autophagosomal membranes.
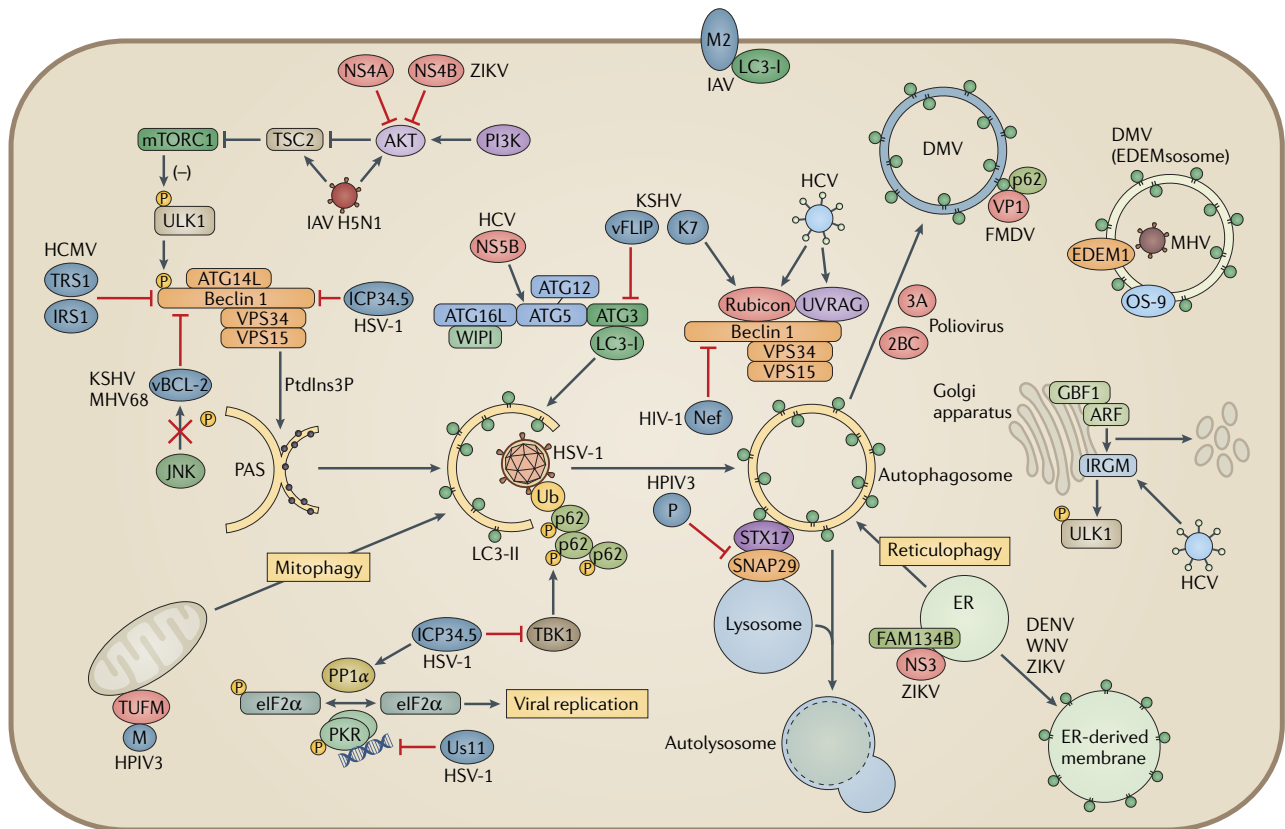
Fig. 3 | **Viral manipulation of autophagy.** Viruses interfere with autophagosome formation and fusion with the lysosome. Herpes simplex virus type 1 (HSV-1) neurovirulence factor ICP34.5 directly targets Beclin 1 to block autophagosome formation. ICP34.5 also recruits the phosphatase PP1α to dephosphorylate eukaryotic translation initiation factor 2 subunit 1 (eIF2α) and blocks tank-binding kinase 1 (TBK1)-mediated autophagosome maturation. HSV-1 tegument protein Us11 interacts with interferon-induced, double-stranded RNA-activated protein kinase (PKR) to prevent the phosphorylation of eIF2α and regulates autophagosome formation. Human cytomegalovirus (HCMV) encodes a functional homologue of ICP34.5 called TRS1, which can bind to Beclin 1 and inhibit autophagy. γ-Herpesviruses carry viral homologues of B cell lymphoma-2 (vBCL-2), which attenuate autophagosome formation by interacting with Beclin 1. vBCL-2 evades JUN N-terminal kinase (JNK) phosphorylation, allowing it to bind to Beclin 1 with high affinity. Kaposi's sarcoma-associated herpesvirus (KSHV) expresses another host protein homologue, vFLIP, that prevents autophagy protein 3 (ATG3) from processing LC3 during phagosome elongation. KSHV K7 interacts with Rubicon and inhibits fusion of the autophagosome with the lysosome. Viruses also control autophagosome formation and remodel vesicles for viral replication. Poliovirus induces autophagy to form a double-membrane vesicle (DMV) for replication. The poliovirus proteins 2BC and 3A increase LC3 lipidation and DMV formation. The foot-and-mouth disease virus (FMDV) capsid protein VP1 uses the adaptor p62 for autophagosome formation. Mouse hepatitis virus (MHV) hijacks the LC3-coated EDEMsosome by accumulating endoplasmic reticulum (ER) degradation-enhancing α-mannosidase-like protein 1 (EDEM1) and OS-9 in DMVs. Flaviviruses (dengue virus (DENV), West Nile virus (WNV) and Zika virus (ZIKV)) use ER-derived membranes for viral replication. ZIKV non-structural protein 4A (NS4A) and NS4B activate autophagy by inhibiting AKT and mechanistic target of rapamycin complex 1 (mTORC1). Furthermore, the viral protease NS3 cleaves reticulophagy regulator 1 (FAM134B) to block reticulophagy so that ZIKV can use the ER as a replication site. Influenza A virus (IAV) M2 binds to and relocalizes LC3 to the plasma membrane. A highly pathogenic IAV H5N1 strain modulates the AKT–tuberin (TSC2)–mTOR pathway to induce autophagy. Human parainfluenza virus type 3 (HPIV3) also directly inhibits the interaction between syntaxin 17 (STX17) and synaptosomal-associated protein 29 (SNAP29) by expressing phosphoprotein P, resulting in the accumulation of autophagosomes. HPIV3 matrix protein M interacts with elongation factor Tu, mitochondrial (TUFM), inducing mitophagy to inhibit the interferon response. Hepatitis C virus (HCV) has multiple targets to regulate the autophagic pathway. The RNA-dependent RNA polymerase NS5B binds to ATG5. By differentially inducing the expression of Rubicon and ultraviolet radiation resistance-associated gene protein (UVRAG), HCV NS4B temporally regulates the autophagic flux to enhance viral replication. Host immunity-related GTPase family M protein (IRGM) is also targeted by HCV for Golgi apparatus fragmentation and viral replication. Viral proteins inducing or inhibiting autophagy are displayed in red and blue, respectively. ARF, ADB ribosylation factor 1; GBF1, Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1; IRS1, insulin receptor substrate 1; MHV68, murine γ-herpesvirus 68; PAS, pre-autophagosomal structure; PI3K, phosphoinositide 3-kinase; PtdIns3P, phosphatidylinositol-3-phosphate; Ub, ubiquitin; VPS34, vacuolar protein sorting 34; WIPI, WD-repeat domain phosphoinositide-interacting protein.

CVB3 and foot-and-mouth disease virus (FMDV), also use autophagy for replication[80,81]. When CVB3-infected cells were treated with inhibitors of autophagosome maturation, virion production was increased and p62 protein levels remained unchanged[82], suggesting that viral replication increases the generation of autophagic membrane structures without progression to lysosomal degradation. FMDV also induces ATG5-dependent auto-phagosome formation and redistributes LC3 to punctate vesicles. However, this induction does not require the

PI3K activity of VPS34 and occurs very early during infection as ultraviolet-inactivated FMDV can still trigger autophagosome formation[81]. FMDV-induced autophagosomes contain the viral capsid protein VP1 that colocalizes with p62 (REF.[83]), suggesting that autophagosome formation is induced during FMDV entry (FIG. 3). In summary, picornaviruses use multiple mechanisms to modulate autophagy for their replication.

Coronaviruses (CoVs), including the severe acute respiratory syndrome (SARS)-CoV and mouse hepatitis virus (MHV), also induce the formation of DMVs. Several controversial studies concluded that CoVs induce autophagy but do not require the complete autophagy pathway[84–86]. Interestingly, MHV sequesters the non-lipidated, LC3-coated EDEMsosome (a vesicle involved in ER-associated degradation (ERAD)), into its replication and transcription complexes[87]. MHV also causes accumulation of two ERAD regulatory proteins, ER degradation-enhancing α-mannosidase-like protein 1 (EDEM1) and OS-9, in the DMVs, thereby hijacking the ERAD machinery for viral replication (FIG. 3).

Flaviviruses take advantage of the fact that autophagy and ER processes are frequently connected[88]. Initially, the induction of autophagy during flavivirus infections was thought to be a consequence of the ER stress-derived UPR. However, dengue virus (DENV) and West Nile virus (WNV) non-structural proteins can induce autophagy independently of the UPR[89,90]. In human neural progenitor cells, Zika virus (ZIKV) infection led to ER rearrangement and the formation of vesicular clusters thought to be the sites of viral RNA replication and virion assembly[91,92]. ZIKV-infected primary fibroblasts contained multi-membrane structures that resembled autophagic vesicles[93]. Moreover, the increase of lipidated LC3 in ZIKV-infected placentae and lower titres of ZIKV in ATG16-deficient fetuses in mice support the proviral function of autophagy during ZIKV infection[94]. Co-expressed in fetal neural stem cells, ZIKV non-structural proteins NS4A and NS4B decreased AKT phosphorylation and subsequently inhibited mTOR activation, which induced autophagy[95] (FIG. 3). Recently, flaviviruses were shown to use the viral protease NS3 to directly cleave the ER-localized autophagic receptor reticulophagy regulator 1 (FAM134B; also known as RETREG1), thereby inhibiting FAM134B-mediated ER degradation (reticulophagy)[96,97] (FIG. 3).

HCV induces autophagosome formation but blocks lysosomal fusion, which benefits viral replication and virion production. The HCV RNA-dependent RNA polymerase NS5B interacts with ATG5 early during infection[98] (FIG. 3). HCV infection induces the expression of two autophagy regulatory proteins with different kinetics, ultraviolet radiation resistance-associated gene protein (UVRAG) and Rubicon, which stimulate and inhibit the maturation of autophagosomes, respectively[99]. HCV NS4B induces Rubicon early during infection to inhibit the maturation of autophagosomes, resulting in accumulation of autophagosomes in support of HCV replication[99]. Overexpression of UVRAG was shown to suppress HCV replication. Recently, an IFN-inducible GTPase, immunity-related GTPase family M protein (IRGM), was shown to regulate autophagy

and the formation of various intracellular membrane compartments[100]. Upon HCV infection, IRGM interacts with Golgi apparatus-specific brefeldin A-resistance guanine nucleotide exchange factor 1 (GBF1) and promotes AMPK-mediated GBF1 phosphorylation, thereby activating GTPase ADB ribosylation factor 1 (ARF1) for Golgi apparatus fragmentation and facilitating viral replication[101] (FIG. 3). Moreover, HCV triggers IRGM-mediated phosphorylation of ULK1 (REF.[102]) (FIG. 3). These studies suggest that HCV dynamically modulates autophagy to coordinate viral replication.

IAV also triggers the accumulation of autophagosomes for viral replication[103]. The virus matrix 2 (M2) ion channel protein is pivotal for IAV assembly and budding[104]. It also regulates autophagy through its LC3-interacting region (LIR)[105]. IAV relocates LC3 to the plasma membrane in an M2-dependent manner (FIG. 3), and disruption of the M2–LC3 interaction decreases virion budding and stability. NS1, a multifunctional protein of IAV, also stimulates autophagy indirectly by increasing the synthesis of HA and M2 (REF.[106]). A highly pathogenic avian H5N1 strain of IAV can also induce autophagy by inhibiting mTOR[107]. This virus reduces the phosphorylation of tuberin (TSC2), an upstream inhibitor of mTOR, and this modulation of the AKT–TSC2–mTOR pathway and the subsequent autophagic cell death might explain the high mortality of this virus (FIG. 3).

Finally, human parainfluenza virus type 3 (HPIV3) blocks autophagosome maturation and triggers the accumulation of autophagosomes[108]. Fusion of autophagosomes with lysosomes depends on binding of the adaptor protein synaptosomal-associated protein 29 (SNAP29) to syntaxin 17 (STX17), which is located on the outer membrane of complete autophagosomes, and to the SNAP receptor (SNARE) vesicle-associated membrane protein 8 (VAMP8) on the lysosome[109]. HPIV3 phosphoprotein (P) directly binds to the SNARE domains of SNAP29 and inhibits the interaction between STX17 and SNAP29 (FIG. 3), ultimately preventing autophagosome–lysosome fusion[108]. Furthermore, HPIV3 matrix protein (M) also interacts with TUFM and binds LC3 to induce TUFM-mediated mitophagy and inhibit the subsequent IFN response[110] (FIG. 3).

Viruses are under high pressure to block autophagosome–lysosome fusion; however, the mechanisms for achieving this vary considerably among viruses. Thus, how a virus interacts with the host autophagic machinery is often a reflection of the unique requirements that an individual virus has for its replication.

***Viral regulation of lipophagy for replication.*** All eukaryotic cells store lipids inside lipid droplets, which are organelles with a neutral lipid core of triglycerides surrounded by a monolayer of cholesteryl esters and coat proteins[111,112]. Lipid droplets were initially identified as a lipid reservoir that contributes to both energy metabolism and membrane biogenesis. Storage of cellular lipids is regulated by lysosomal degradation in a process termed lipophagy[111]. In starving cells, lipophagy degrades lipid droplets to supply mitochondria with fatty acids, which are oxidized to generate acetyl-CoA[113].

**ER-associated degradation**
(ERAD). A process through which misfolded proteins are transported from the endoplasmic reticulum to the cytoplasm for proteasomal degradation.

**SNAP receptor**
(SNARE). A key component of the cellular membrane fusion machinery that mediates vesicular fusion events in autophagosome–lysosome fusion.
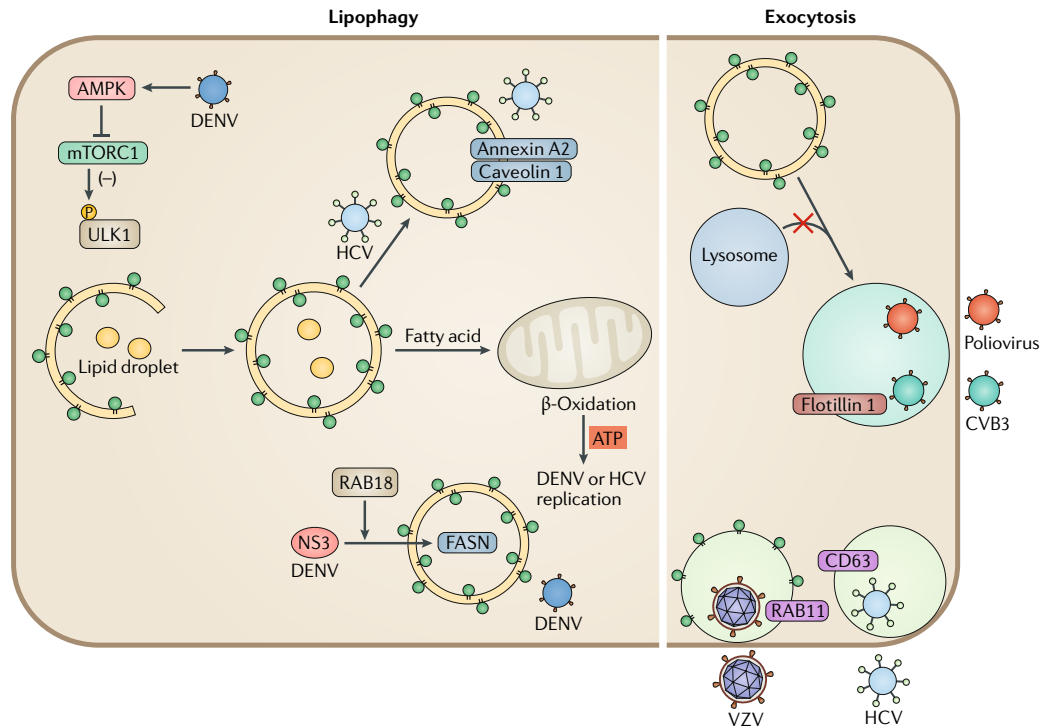
Fig. 4 | **Viral manipulation of lipophagy and exocytosis.** Dengue virus (DENV) infection activates 5′-AMP-activated protein kinase catalytic subunit α2 (AMPK), which in turn inhibits mechanistic target of rapamycin 1 (mTORC1) to induce lipophagy. Degradation of lipid droplets by DENV-mediated lipophagy contributes to β-oxidation and ATP production in mitochondria, which provides energy for viral replication. DENV viral protease NS3 sequesters fatty acid synthase (FASN) to the sites of DENV replication to increase fatty acid biosynthesis. Hepatitis C virus (HCV) also induces the formation of autophagosome-containing lipid cargos. HCV-induced autophagosomes comprise caveolin 1, caveolin 2 and annexin A2, which promote HCV assembly. Viruses also hijack the exocytosis pathway for the secretion of viral particles. Poliovirus uses phosphatidylserine (PS)-enriched autophagosome-like vesicles for non-lytic release. Coxsackievirus B3 (CVB3) exits cells through autophagosomes that contain the exosome marker flotillin 1. HCV is also released through exocytic pathways that are regulated by autophagy. Enveloped double-stranded DNA viruses replicating in the nucleus obtain their second envelope from endoplasmic reticulum (ER) and Golgi apparatus membranes. Varicella zoster virus (VZV) exits cells with autophagic membranes that contain lipidated LC3 and the endocytosis marker RAB11.

How autophagy selectively identifies and sequesters lipid droplets remains unknown; however, lipid droplets that are targeted for degradation are decorated with LC3, similar to autophagosomes[111]. Although lipophagy has been primarily studied in the context of lipid metabolism-related diseases, there are also multiple viruses that exploit lipophagy.

DENV infection increases the number of lipid droplets per cell, and, thus, pharmacological inhibition of droplet formation markedly reduces DENV replication. DENV-induced lipid droplets contain viral capsid proteins, suggesting that they provide a platform for nucleocapsid formation and viral replication[114]. Moreover, DENV infection induces lipophagy, which depletes stored triglycerides and increases β-oxidation and energy production for viral replication (FIG. 4). In autophagy-deficient cells, adding exogenous free fatty acids rescues DENV replication, but the drug etomoxir, which prevents the transport of fatty acids into the mitochondria, blocks this process[115]. These results suggest that lipophagy increases the release of free fatty acids and the amount of ATP, which is crucial

for DENV replication. Furthermore, DENV induces AMPK kinase activity, which in turn inhibits mTOR complex 1 (mTORC1), and this regulation is required for virus-induced lipophagy[116].

Similar to DENV, HCV also induces the production of lipid-filled autophagosomes[117]. HCV inhibits autophagosome maturation, and this leads to the accumulation of cholesterol. Lipid droplets are important for the assembly of HCV virions. HCV-induced autophagosomes contain lipid raft-associated proteins, such as caveolin 1, caveolin 2 and annexin A2 (REF.[118]) (FIG. 4), and depletion of cholesterol, a major component of lipid rafts, reduces viral replication[119]. Lipophagy is less well studied than other aspects of autophagy during viral infection, but it likely has a crucial role in viral replication.

***Viral exocytosis and autophagy.*** Vesicle exocytosis involves the release of cellular contents from the *trans*-Golgi network (TGN) or fusion of recycling endosomes with the plasma membrane[120]. Exocytosis and autophagy both involve membrane trafficking and

**Lipid raft**
A small heterogeneous cellular compartment enriched with sphingolipids and cholesterols.

fusion events and share molecular machineries, such as GTPases for tethering and SNARE proteins for fusion. Moreover, exosomes that originate from MVBs contain lipidated LC3, suggesting that autophagosomes are redirected to MVBs for exocytosis. Similar to their use of autophagic membranes, viruses can hijack the secretion machinery for exocytosis.

The enveloped dsDNA herpesviruses undergo two envelope-acquisition events during lytic replication[120]. The second envelope originates from ER and Golgi apparatus membranes, and at the cell surface, this outer membrane fuses with the plasma membrane[121]. Two herpesviruses are known to use autophagic membranes for their second envelope acquisition. EBV causes the accumulation of LC3-coated membranes during lytic replication in B cells[122]. If LC3 lipidation is inhibited, EBV genomes accumulate in the cytosol, likely owing to impaired second envelope acquisition. Similarly, varicella zoster virus (VZV) exits cells with autophagic membranes[123]. VZV infection increases the autophagic flux, whereas inhibition of autophagy reduces the spread of infectious virus. Specifically, both lipidated LC3 and RAB11 are found on some infectious VZV particles (FIG. 4), and the viral glycoprotein gE colocalizes with LC3 in the cytoplasm[123].

As discussed above, picornaviruses accumulate autophagic membranes to support their viral replication and release. As non-enveloped RNA viruses, picornaviruses are packaged inside phosphatidylserine-enriched autophagosome-like vesicles and are released non-lytically within these membranes, enabling multiple viral transmissions[124] (FIG. 4). Indeed, ATG-silencing inhibited the non-lytic spread of poliovirus[125], and autophagic membranes have similar functions for the release of other picornaviruses[79,83]. Mature CVB3 seems to exit cells in autophagic membranes[80], as CVB3-containing vesicles are associated with both lipidated LC3 and the exosome marker flotillin 1 (REF.[80]) (FIG. 4).

Flaviviruses use autophagic membranes both for replication and for exocytosis through MVBs[126,127] (FIG. 4). Autophagic membranes enable viral particles to reach the extracellular space either in exosomes or viral envelopes. However, it is unclear how this trafficking is coordinated.

## Autophagy and viral pathogenesis

Ideally, cells use autophagy to control viral replication, destroy viral particles and activate immune signalling, all of which should prevent disease. However, the success of many viruses depends on the subversion and sequestration of host autophagic responses. For example, in neurons, autophagy is involved in HSV-1 replication and reactivation of latent infection[128]. During reactivation in neurons, HSV-1 can relocate to the central nervous system, and, there, the expression of the autophagy inhibitor ICP34.5 is involved in causing fatal encephalitis[129,130]. Strikingly, 50% of mice infected with wild-type HSV-1 developed neuropathogenesis, whereas an ICP34.5-mutant caused no symptoms[131]. ICP34.5-mediated inhibition of Beclin 1-dependent autophagy is a crucial regulatory step for HSV-1 pathogenesis in the central nervous system.

Numerous cancer risk factors, such as ageing, obesity and chronic inflammation, disturb autophagy[132]. Accordingly, as improved autophagy correlates with tumour-suppression, its downregulation by viral infection may facilitate cancer development. Viruses that are associated with cancer development, including EBV, hepatitis B virus (HBV), HCV and KSHV, have evolved multiple strategies to avoid autophagy-mediated elimination, which can facilitate tumour progression. During reactivation, EBV replication and transcription activator (Rta) transiently activates autophagy through extracellular-signal-regulated kinase (ERK) signalling[133], but early lytic viral products block autophagy and prevent degradation[134]. In Burkitt's lymphoma cells, the inhibition of autophagy increases EBV transcription and replication, which links the modulation of autophagy to EBV-associated malignancies[135]. The HBV small surface protein increases the risk of liver cancer by triggering the UPR and autophagy. Moreover, HBV X protein (HBx) binds to VPS34 and stimulates autophagy initiation. HBx also interacts with V-ATPase and impairs lysosome acidification, resulting in hepatocyte death[136]. Similar to HBV, HCV infection frequently leads to chronic liver diseases, liver cirrhosis and hepatocellular carcinoma. HCV-infected hepatocytes have high UPR levels, which activates autophagy, sustains viral replication and induces disease progression[137,138]. As mentioned before, the KSHV vFLIP and vBCL-2 target ATG3 and Beclin 1, respectively, and these interactions block autophagosome formation and vesicle nucleation, thereby supporting KSHV replication and disease progression.

As previously discussed, many RNA viruses enhance autophagy for viral replication and disease progression. For example, autophagy is involved in ZIKV transmission from mother to fetus[94,139]. Genetic or pharmacological inhibition of autophagy reduced ZIKV infection in the fetus, suggesting that blocking autophagy can be a promising treatment to limit the vertical transmission of ZIKV[94].

Autophagy is also involved in DENV pathogenesis. Sub-neutralizing antibodies from prior DENV infection may improve secondary DENV infection via facilitating viral binding and entry into Fcγ receptor-bearing cells, in a process termed antibody-dependent enhancement[140]. Infection that involves antibody-dependent enhancement induces the expression of ATG5 and ATG12 earlier in the infection process compared with direct DENV infection[141]. Induction of autophagy in antibody-enhanced DENV infection facilitates viral replication[142]. Antibody-dependent enhancement primes the autophagic machinery before viral entry, leading to greater DENV pathogenesis.

In pancreatic cells, CVB3 modulates autophagy to induce MVB formation[143]. A genetic deficiency of autophagy in pancreatic acinar cells led to the reduction of CVB3 infection and protected mice from disease, suggesting the temporary inhibition of autophagy as a possible therapeutic approach[144].

Collectively, hosts attempt to balance the induction of autophagy to avoid viral infection and pathogenesis. As obligate parasites, viruses can either strongly block or activate the autophagy pathway, which can upset this balance and lead to pathogenesis.

# REVIEWS

## Conclusions

As a catabolic pathway of mammalian cells, autophagy controls viral infections at multiple levels by causing the destruction of viruses, regulating inflammatory responses and promoting antigen presentation. Moreover, viruses manipulate autophagy for their immune evasion, replication and release from infected cells. The broad mechanisms that viruses use to interfere with autophagy are striking. Viruses that establish either acute or persistent infections often target similar host pathways, but they do so in unique ways and with different functional outcomes. Further, a virus may block certain aspects of autophagy while requiring others. For example, herpesviruses have genes that potently block autophagosome maturation, yet autophagosomes are the source of their outer membrane. Many RNA viruses exist in an intriguing balance in which they both cannot live with autophagy and cannot live without it. Each virus uses unique strategies to fine-tune autophagy signalling so that it can simultaneously escape destruction while harnessing the structural and nutrient benefits that autophagy provides. However, the exact functions of ATG proteins and the detailed mechanisms that govern autophagy during viral infection are still being elucidated. Further research is needed to clarify the detailed functions of autophagy for immunity against viral infections and for viral pathogenesis.

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