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REVIEW

Involvement of autophagy in viral infections: antiviral function and subversion by viruses

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Abstract Autophagy is a cellular process involved in the degradation and turn-over of long-lived proteins and organelles, which can be subjected to suppression or further induction in response to different stimuli. According to its essential role in cellular homeostasis, autophagy has been implicated in several pathologies including cancer, neurodegeneration and myopathies. More recently, autophagy has been described as a mechanism of both innate and adaptive immunity against intracellular bacteria and viruses. In this context, autophagy has been proposed as a protective mechanism against viral infection by degrading the pathogens into autolysosomes. This is strengthened by the fact that several proteins involved in interferon (IFN) signalling pathways are linked to autophagy regulation. However, several viruses have evolved strategies to divert IFN-mediated pathways and autophagy to their own benefit. This review provides an overview of the autophagic process and its involvement in the infection by different viral pathogens and of the connections existing between autophagy and proteins involved in IFN signalling pathways.

Keywords Autophagy · Virus · Interferon · Virophagy

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Introduction

Cellular homeostasis requires a highly regulated equilibrium between protein synthesis and proteolysis, and its deregulation has been implicated in several pathologies. Two major pathways are involved in the degradation of macromolecules in eukaryotes: the ubiquitin/proteasome pathway and autophagy. The former is involved in the constitutive degradation of short-lived proteins, maintaining a continuous protein turn-over in the cell [1]. Autophagy, which literally means "to eat oneself", is necessary for the lysosomal degradation and recycling of long-lived proteins and entire organelles [2]. This process has been observed in all eukaryotes and is morphologically identical in plants, yeasts and animals. Autophagy can be classified into at least three different types: macroautophagy,

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microautophagy and chaperone-mediated autophagy (CMA). The last one is the only autophagic process that is not conserved through evolution and exits only in higher eukaryotes. The first CMA substrate identified was ribonuclase A and a particular motif in this protein, KFERQ, was shown necessary for its selective degradation. This motif is recognised by the chaperone protein Hsc70 and targeted to lysosomal membranes where Hsc70 interacts with the lysosomal membrane protein (lamp) type 2a. The substrates are then translocated to the lysosomal lumen where they are degraded. It is now known that all the CMA substrates contain a KFERK-like motif, which is recognised by the hsc70. This motif is composed of a basic (K, R), an acidic (D, E), a hydrophobic (F, I, L, V), and another basic or hydrophobic amino acid and is flanked by a Q on either side [3]. Microautophagy is characterised by the invagination of the lysosomal membrane that sequesters cytoplasmic constituents and, subsequently, degradation of the sequestered material. Macroautophagy is the major lysosomal route for the turnover of cytoplasmic constituents, and will hereafter be referred as autophagy. This process begins with an engulfment event of portions of the cytosol into a characteristic double-membrane vacuole, called the autophagosome. After maturation, autophagosomes fuse with lysosomes for the degradation of the sequestered material by lysosomal hydrolases. This last event allows the recycling of the degraded constituents [4, 5].

Autophagy is a highly regulated mechanism involving specific genes called Atg (autophagy-related genes). Although this process has been identified for more than 40 years, the first understandings of its molecular mechanisms dated from about 10 years, with the discovery of Atg genes in yeast and orthologs in humans [6]. Interestingly, it has been shown recently that the ubiquitin/proteasome pathway and autophagy are connected. Indeed, Atg7 knock-out mice present an accumulation of ubiquitinated aggregates. This observation suggests that, in addition to the proteasome pathway, ubiquitinated proteins can be removed by autophagy [7]. Moreover, it may also reflect a compensatory mechanism in the absence of autophagy.

Even if the origin of the lipids that compose autophagosomes has been an aspect extensively studied since the discovery of autophagy, this question is still hotly debated [8]. The first studies provided evidence that the source of autophagosomal membranes was both the Golgi and the endoplasmic reticulum and, at present, the endoplasmic reticulum is the most probable one [9]. Another possibility is that the membranes are unique or formed de novo [10]. Thus, it has been proposed that, in mammals, autophagosomes derived from a unique membrane of unknown origin, called the phagophore. Furthermore, a novel structure necessary for autophagosome formation, called the pre-autophagosomal structure (PAS), has been identified in yeast [11].

According to its essential role in cellular homeostasis, autophagy has been implicated in several pathologies including cancer, neurodegeneration and myopathies [12]. More recently, autophagy has been described as a mechanism of innate immunity against intracellular pathogens [13], and its implication in major histocompatibility complex (MHC) class II antigen presentation extends its function in adaptive immunity [13–15]. The term immunophagy has recently been proposed by Deretic [13] for the specialised role of autophagy in immunity. A new field of investigation is now emerging about the role of autophagy in viral infections. Besides its role as an intracellular host defence pathway against viruses, autophagy can also be used by the virus for its own profit to replicate more efficiently in the cells, or to control cell survival [16]. The term virophagy could be used to define the relationships that exist between autophagy and viral infections. In this paper, presented are the different molecular steps of the autophagic process, its implication in both cell survival and cell death and the relationships that are known between autophagy and viral infections.

Overview

Molecular mechanisms of the autophagic process

In all eucaryotes, autophagy occurs as a house-keeping mechanism in normal growing conditions. In animal cells, it is subjected to suppression or further induction in response to different stresses, starvation, specific hormones or other stimuli [17–20]. The discovery of Atg genes has been essential in the molecular understanding of this process. A major challenge in the autophagy field is now to decipher the signalling pathways that act downstream the initial autophagy induction signals. Basically, the autophagic process can be divided into different steps: activation, autophagosome formation, targeting to and fusion with lysosomes and breakdown [21] (Fig. 1).

Activation

In mammalian cells, the autophagic Beclin 1 protein (ortholog of yeast Atg6) functions as part of a class III phosphatidylinositol 3-kinase (PI3K) complex and plays a crucial role in the early steps of autophagosome formation [22, 23]. The mammalian class III PI3K/Beclin 1 complex has its yeast counterpart that consists in the association of Vps34 (the ortholog of class III PI3K) with the autophagic proteins Atg6 and Atg14 [22]. The mammalian counterpart of Atg14 has not been identified so far. Recent data suggest that other proteins belong to this complex and thus regulate the outcome of the signalling via class III PI3K. Indeed,

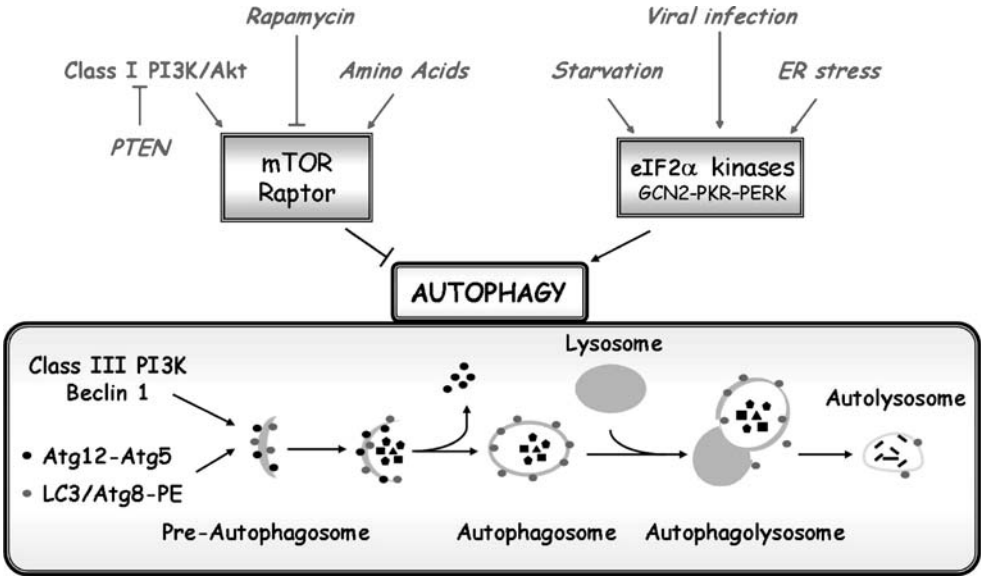

Fig. 1 The autophagic process and its regulation. Here, presented are two major pathways that regulate autophagy triggering. The mTOR pathway inhibits autophagy in response to Class I PI3K and amino acids. The eIF-2α kinases are positive regulators of autophagy in response to starvation, ER stress and viral infection. Autophagy triggering is dependent on the Class III PI3K signalling and on two conjugation systems. First, a pre-autophagosome is formed and sequesters cytoplasmic material. Its completion leads to autophagosome formation that fuses with lysosome to form autolysosome where the sequestered material is degraded

Bcl-2 can negatively regulate the activation of autophagy through its interaction with Beclin 1 [24]. Recently, an activator of autophagy, called UVRAG (UV irradiation resistance-associated gene), has been identified to be part of the PI3K/Beclin 1 complex and to represent an important signalling checkpoint in autophagy and tumour-cell growth [25].

Autophagosome formation

The autophagic process depends on two ubiquitin-like conjugation systems essential for autophagosome formation [26] (Fig. 2). In general, three different enzymes are required for the process of conjugation: an E1-activating enzyme, an E2-conjugating enzyme and an E3 ligase enzyme. The first conjugation system mediates formation of the conjugate Atg12/Atg5. Atg12 is activated by the E1-like enzyme Atg7, and then Atg12 is transferred to the E2-like enzyme Atg10. Finally, Atg12 is covalently linked to a specific lysine of Atg5. No E3-like enzyme has been discovered to date. Then, the conjugate Atg12/Atg5 interacts non-covalently with Atg16L (Atg16 in yeast) to trigger homo-oligomerisation, leading to the formation of a macromolecular complex of approximately 800 kDa (350 kDa in yeast) necessary for the formation of autophagosomes. This structure is associated with the outer side of the autophagosomes in formation and dissociates from membranes before the autophagosome completion. The second conjugation system is original because it consists in the conjugation of a protein to a lipid, resulting in the formation of the Atg8/PE (phosphatidyl-ethanolamin) conjugate. Atg8 is a soluble cytoplasmic protein that must be first proteolysed by Atg4 to enter the conjugation system. After maturation by Atg4, Atg8 is activated by the E1-like enzyme Atg7, and then transferred to the E2-like enzyme Atg3. Finally, Atg8 is covalently linked to PE. This conjugate is present in both sides of the autophagosomes and seems fundamental for its completion. After autophagosome completion, the Atg8 molecules present at the cytoplasmic face of this structure are recycled after cleavage by Atg4 and the remaining Atg8 proteins present in the inner membrane of autophagosomes are degraded after fusion with lysosomes. There are several orthologs of Atg8 in mammalian cells: MAP-LC3, GATE-16 and GABARAP, each representing a different subfamily [27]. Among these proteins, MAP-LC3 is the best characterised one and represents the most useful marker for autophagosome identification [28, 29]. In addition, there are four mammalian orthologs of Atg4, called autophagins, but only autophagin 1 (also called hAtg4B) cleaves specifically Atg8 and its different mammalian orthologs [30, 31]. Interestingly, it appears that these two conjugation systems are connected. Indeed, the Atg5/Atg12-Atg16 complex is necessary for the formation of the second conjugate [32]. Moreover, overexpression of Atg10 facilitates the maturation of MAP-LC3, overexpression of Atg3 facilitates the conjugation of Atg12 to Atg5, and excess amount of the Atg12-Atg5 conjugate inhibits the MAP-LC3 maturation [33].

In yeast, another complex is involved in autophagosome formation, which requires the autophagic proteins Atg9 and Atg2. Atg9, the only transmembrane autophagic protein so

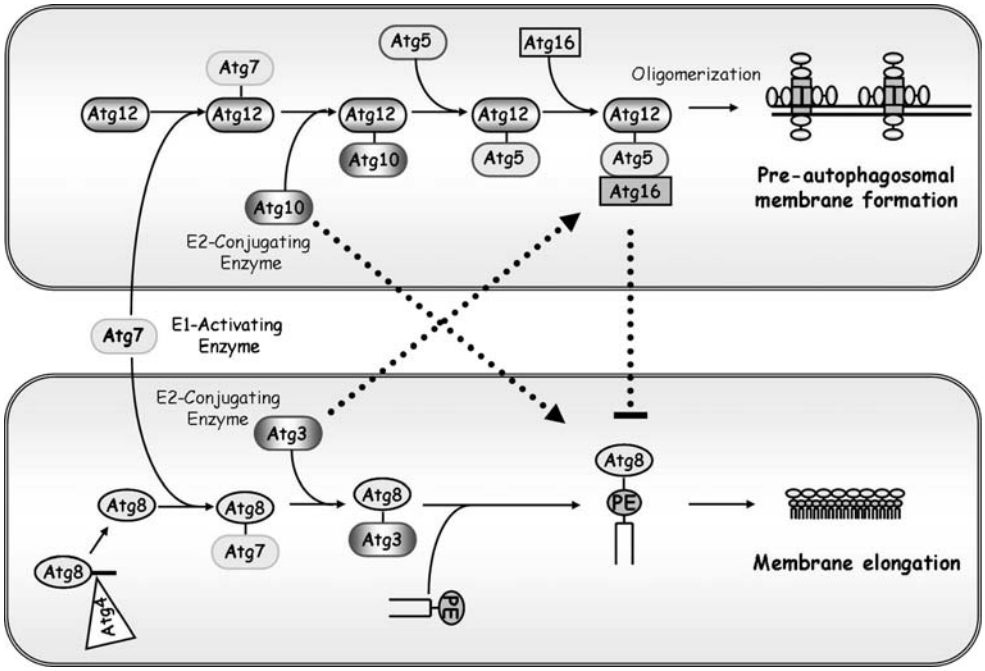

Fig. 2 Conjugation systems involved in autophagy. The first conjugation system mediates the formation of the conjugate Atg12/Atg5. Atg12 is activated by the E1-like enzyme Atg7, and then Atg12 is transferred to the E2-like enzyme Atg10. Finally, Atg12 is covalently linked to a specific lysine of Atg5. Then, the conjugate Atg12/Atg5 interacts non-covalently with Atg16 (Atg16L in mammals) to trigger homo-oligomerization leading to a macromolecular complex necessary for the formation of autophagosomes. This structure is associated with the outer side of the autophagosomes in formation and dissociates from the membranes before the autophagosome completion. The second conjugation system results in the formation of the Atg8/PE (phosphatidyl–ethanolamin) conjugate. Atg8 (LC3 in mammals) is first proteolysed by Atg4, activated by the E1-like enzyme Atg7, and then transferred to the E2-like enzyme Atg3. Finally, Atg8 is covalently linked to PE. This conjugate is present in both sides of the autophagosomes and seems fundamental for its completion. The two conjugation systems are connected: The Atg5/Atg12-Atg16 complex is necessary for the formation of the second conjugate. Over-expression of Atg10 facilitates the maturation of MAP-LC3, over-expression of Atg3 facilitates the conjugation of Atg12 to Atg5, and excess amount of the Atg12-Atg5 conjugate inhibits the MAP-LC3 maturation

far identified, is necessary for the formation of the PAS but is absent in the mature autophagosomes [34]. mAtg9, the human ortholog of the yeast Atg9 has been shown to traffic between the Golgi and endosomes, suggesting an involvement of the Golgi complex in the autophagic pathway [35].

Targeting to and fusion with lysosomes

After completion, autophagosomes can fuse in yeast with the vacuole, or in mammals with the lysosomes to form autolysosomes, using the same molecular fusion machinery. Before fusing with lysosomes, autophagosomes can also fuse with endosomes to form amphisomes, making a direct connection between the endo-lysosomal and autophagic pathways [36]. Membrane fusion can be delineated into different steps: tethering, docking and fusion of the membrane bilayers. Tethering and docking events are facilitated by multi-protein complexes, and the specificity of the events is provided by GTPases from the Rab family. In particular, the GTPase Rab7 is required for the final maturation of late autophagic vacuoles and thus for the progression of the autophagic process [37, 38]. Fusion is driven by assembly of pairs of membrane proteins called SNAREs on each fusion partner [39]. Using bafilomycin A1, an inhibitor of vacuolar H+ ATPase (V-ATPase), it has been shown that acidification of the lumenal space of autophagosomes or lysosomes by V-ATPase is an important step for the fusion between autophagosomes and lysosomes [40].

Breakdown

After the fusion step with lysosomes, the cytoplasmic material sequestered in autophagosomes is released in the lysosomal lumen and subsequently consumed by hydrolases. This event requires the acidic pH of the lysosome to efficiently break the inner membrane of the autolysosome. In yeast, the involvement of the lipase Atg15, which is delivered to the vacuole through the multivesicular bodies, is required for vesicle breakdown [41]. After degradation of the sequestered material, the constituents are recycled through lysosomal transporters toward the cytosol. Recently, the yeast protein Atg22 has been shown to mediate the

efflux of leucine and other amino acids resulting from autophagic degradation [42].

Signalling pathways involved in the regulation of autophagy

The mammalian serine/threonine protein kinase target of rapamycin (MTOR) is a key regulator that controls autophagy triggering and is often called "the gate keeper of the autophagic pathway" [43, 44]. This kinase senses environmental changes to modulate autophagy. Several proteins act positively or negatively on mTOR and, by this way, influence the autophagic process. The cascade upstream mTOR includes class I PI3K and Akt, whereas the phosphatase and tension homolog (PTEN) acts antagonistically to the class I PI3K to induce autophagy. Rapamycin, a specific inhibitor of mTOR, activates autophagy whereas amino acids are inhibitors of this process. However, the precise mechanisms involved in the negative regulation of autophagy by amino acids remain an open question.

Induction of autophagy by nutrient starvation is better characterised in yeast than in mammals. Indeed, it has been shown that under nutrient-rich conditions, the autophagic protein Atg13 is highly phosphorylated in response to the TOR kinase activation. This phosphorylation status provides a lower affinity of Atg13 for the autophagic serine/threonine protein kinase Atg1, repressing autophagy. In contrast, under nutrient-deficient conditions, or following treatment with the TOR inhibitor rapamycin, Atg13 is rapidly and partially dephosphorylated, resulting in an interaction of high affinity with Atg1. In these conditions, the autophagic activity is increased [45]. A putative ortholog of the yeast Atg1, called ULK1, has been identified in mammals. This protein seems to be involved in the localization of the mAtg9 [46]. It is worth noting that Atg13 is not conserved in mammals.

Finally, other signalling pathways independent of mTOR have been involved in the regulation of autophagy [47, 48].

Other regulators of the autophagic pathway are the eIF2α kinases. They belong to an evolutionarily conserved serine/threonine kinase family that regulates stress-induced translational arrest. It has been demonstrated that the yeast eIF2α kinase GCN2 and the eIF2α-regulated transcriptional transactivator GCN4 are essential for starvation-induced autophagy [49]. It is worth noting that GCN2 is conserved in mammals, suggesting that this protein may have a similar role in higher eukaryotes. Recently, endoplasmic reticulum stress induced by poly-glutamine 72 repeat (polyQ72) has been shown to induce autophagy after activation of the eIF2α kinase PKR-endoplasmic reticulum-related kinase (PERK) activation [50]. In contrast, another study has reported that the autophagic process triggered in response to endoplasmic reticulum stress is PERK-independent [51].

The mammalian interferon (IFN)-inducible eIF2α kinase PKR (protein kinase RNA-activated) plays also an important role in triggering autophagy [49]. As PKR is a protein essential in cellular response to viral infections, this point will be discussed in more details afterwards [52].

Dual role of autophagy in cell survival and cell death

Autophagy is a cellular mechanism essential for homeostasis. It has been extensively studied for its ability to maintain cells alive in response to starvation. This theory has been confirmed since then by studies demonstrating increased cell death in cells or organisms lacking gene products essential for autophagy [53]. A good example to illustrate this aspect is the demonstration of the role of autophagy during the early neonatal starvation period. Indeed, at birth, the placental nutrient supply is interrupted, and neonates face starvation until the first feeding by milk. It has been shown that autophagy is up-regulated in various tissues during this period, and those mice incompetent for autophagy (Atg5 knock-out mice) die within 1 day after birth [54]. In addition, autophagy has been shown to play a critical role in maintaining cellular bioenergetics and survival during growth factor deprivation in cells incompetent for apoptosis [55]. Furthermore, it has been shown that inhibition of autophagy triggers apoptotic cell death under conditions of nutrient depletion [56].

Despite its role in cell survival, an extensive body of literature highlights the fact that autophagy can also be considered as a cell death pathway (type II programmed cell death, PCD). Autophagic cell death can be distinguished from apoptosis (type I PCD) by morphological criteria, i.e. the presence of autophagosomes in dying cells. Cell death with autophagic features can occur in cells lacking critical apoptosis executioners, indicating that autophagy can compensate for defect apoptosis [57]. Autophagic cell death has also been described after treatment with chemotherapeutic drugs [58]. However, cell death, characterised by hallmarks of both type I PCD and type II PCD, is frequently observed; thus, making a clear-cut difference between them is difficult [59–62].

The autophagic protein Beclin 1, which has first been identified as a Bcl-2 interacting protein, has haplo-insufficient tumour suppressor functions. Its gene mapped to a tumour susceptibility locus on chromosome 17q21 that is monoallelically deleted in 40 to 75% of cases of sporadic breast, ovarian and prostate cancers [63]. It has been recently shown that Bcl-2 negatively regulates Beclin 1-dependent autophagy and Beclin 1-dependent autophagic cell death, connecting the two types of PCD [24]. A new link between these death processes has been described by Crighton et al. [64], as a p53 target gene encoding a lysosomal protein, called DRAM (damage-regulated autophagy modulator), is also an inducer of autophagy.

As autophagy appears essential in the cell fate between life and death, it is not surprising that it has been implicated in numerous pathologies, including neurodegenerative diseases, infectious diseases and cancer. This dual role has been very well described in several reviews [44, 65–67].

Autophagy and viral infections

Both bacteria and viruses can be targeted to autophagic degradation [67, 68]. The best-characterised examples for autophagy of pathogens, also termed xenophagy, are engulfment of *Mycobacterium tuberculosis* in phagosomes [69], trapping of cytosolic group A *Streptococci* in autophagosomes [70] and immune escape by *Shigella* [71]. Moreover, it has been shown recently that *Staphylococcus aureus* can use autophagy for its replication before inducing an autophagic host cell death [72].

In this review, we focus on the role of autophagy in the replication of different viruses.

Autophagy is now recognised as a mechanism of immunity against microbes that invade eukaryotic cells. On one hand, evidences indicate that autophagy is involved in the delivery of cytosolic antigens to the MHC class II pathway. Indeed, antigens are processed before binding to MHC class II molecules within endosomal and lysosomal compartments of antigen-presenting cells for subsequent presentation to T cells [73]. Autophagy is also involved to digest endogenously synthesised viral proteins, allowing their processing for MHC II presentation and thus connecting autophagy with adaptive immunity [14]. On the other hand, the autophagic process allows the sequestration of pathogens inside the autophagosomes, leading to their destruction by lysosomes.

Beside its important role in the immune response against pathogens, very well described by Schmid et al. in [15], autophagy plays also a direct role in the life cycle of viruses.

Autophagy and viral infection in plants

In plants, the hypersensitive response (HR) is a complex, early defence response that causes necrosis and cell death at the infection site to restrict the growth of a pathogen. The HR is also thought to deprive the pathogens of a supply of food and thus to confine them to the initial infection site [74]. In addition, the HR could regulate the defence responses of the plant in both local and distant tissues [75].

It has been suggested that autophagy acts as an antiviral mechanism since Beclin 1- or Atg7-silenced plants present an accumulation of tobacco mosaic virus (TMV) at infection sites. Interestingly, induction of autophagy is not only restricted to the TMV infection sites but is also extended to uninfected adjacent tissues where autophagy protects cells from death. In this case, autophagy would play a pro-survival role by blocking pathogen spread and bystander cell death [76]. At present, no other studies connecting plant viruses and autophagy have been done.

Autophagy and viral infections in mammals

Autophagy has been first proposed as a protective mechanism against viral infection by degrading the pathogens in autolysosomes. Its antiviral role is strengthened by the fact that IFN signalling pathways are involved in autophagy induction. Indeed, IFNs are a family of multifunctional secreted cytokines that have been characterised by their ability to interfere with virus infection and replication [77]. However, several viruses have evolved strategies to divert IFN-mediated pathways and autophagy to their own benefit [16].

Table 1 summarised the different data available about the connections between autophagy and viral infections in mammals and higher plants.

Herpes simplex virus type I (HSV-1)

Herpes simplex virus type I (HSV-1) belongs to the herpes virus family. It is a double-stranded DNA virus that resides in a latent state in sensory neurons. During an attack, the virus grows down the nerves and out into the skin or mucous membranes where it multiplies, causing the clinical lesions [78].

It is well characterised that the HSV-1 neurovirulence ICP34.5 protein (infected cell protein 34.5) plays a crucial role in viral infection by inducing the dephosphorylation of the translation initiation factor eIF-2α, and thus negating the PKR antiviral activity [79, 80]. In accordance with the role of the PKR-eIF-2α pathway in the positive regulation of autophagy, wild type HSV-1-infected cells do not present any autophagosomes. However, HSV-1 mutants that do not express ICP34.5 are able to trigger autophagy in infected cells, leading to viral degradation. Moreover, the same mutant viruses that infect cells in which PKR is not expressed exhibit a normal viral replication, demonstrating that ICP34.5 is able to block autophagy by inhibiting the PKR antiviral activity in infected cells [81]. These results demonstrate that HSV-1 has evolved strategies to counteract cellular antiviral functions [82].

Poliovirus and rhinoviruses

Poliovirus and rhinovirus belong to the picornavirus family. They are lytic non-enveloped viruses whose RNA genome is translated, replicated, and packaged in the cytoplasm of infected cells. The poliovirus, responsible for poliomyelitis,

Table 1 Currently known relationships between viruses and the autophagic process

Virus family	Genome	Virus	Viral protein involved	Presence of autophagosomes	Biological effect related to autophagy	Effect of autophagy on the viral replication level
Tobamovirus	ssRNA	TMV	?	+	Antiviral mechanism at the infection site Bystander uninfected cell protection from death	Decreased viral replication
Herpes virus	dsDNA	HSV-1	ICP34.5	–	Blockade of PKR-eIF-2α signalling pathway: inhibition of autophagy	Blockade of the antiviral action of autophagy by ICP34.5, leading to an increase in viral replication
Picornavirus	ssRNA	Poliovirus Rhinovirus	2BC and 3A	+	Autophagosomes as membrane support for viral RNA replication	Increased viral replication
Retrovirus	ssRNA	HIV-1	Env	+ in bystander CD4 T cells ? in infected CD4 T cells	Bystander uninfected CD4 T cell death Infected cells?	Nothing is currently known about the role of autophagy in the replication of HIV-1
Togavirus	ssRNA	Sindbis virus	?	?	Antiviral role of Beclin 1	Decreased viral replication
Parvovirus	ssDNA	B19	?	+	Autophagy triggering resulting in infected cell survival	Increased viral replication
Coronavirus	ssRNA	MHV	?	+	Autophagosomes as sites for viral replication	Increased viral replication
Reovirus	dsRNA	Rotavirus	NSP4	+	?	?
Flavivirus	ssRNA	Bovine Viral Diarrhea Virus	NS3	?	?	Use of autophagy proteins for the expression of viral proteins

invades the nervous system, and the onset of paralysis can occur in a matter of hours. Rhinoviruses are the most common viral infective agents in humans, and a causative agent of the common cold.

In poliovirus-infected cells, it has been observed, early after infection, an accumulation of membranous structures in the cytoplasm [83]. These structures are composed of double-membranes and contain markers of the entire secretory pathway, including the rough endoplasmic reticulum, the Golgi apparatus and lysosomes. This suggests that these double-membrane structures originate from a process analogous to the formation of autophagic vacuoles [84]. More recently, MAP-LC3 and LAMP-1 have been found localised in poliovirus- and rhinovirus-induced vesicles in infected cells. More precisely, the co-expression of two poliovirus-encoded proteins called 2BC and 3A triggers MAP-LC3 and LAMP1 co-localization. Moreover, inhibition of the autophagic process using siRNA directed against the autophagic proteins LC3 and Atg12 decreases both intracellular and extracellular virus yield. The authors suggested that poliovirus and rhinovirus infection induce the formation of autophagosome-like structures to serve as membrane scaffolds for RNA replication and to inhibit their maturation into degradative organelles [85]. Because of the presence of both LC3 and the poliovirus capsid protein VP1 in extracellular structures adjacent to poliovirus-infected cells, Jackson et al. speculate that viral particles may be released by a non-lytic pathway using autophagosome-like vesicles. It has also been suggested that autophagosomes, which can become single-membraned upon maturation, provide a mechanism for the non-lytic release of cytoplasmic viruses and possibly other cytoplasmic material [86]. Very recently, the Arf family of small GTPases, which controls secretory trafficking, has been shown to associate with the newly formed membrane structures used for viral RNA replication after poliovirus infection [87].

Human immunodeficiency virus type I (HIV-1)

The human immunodeficiency virus type I (HIV-1) is a member of the retrovirus family. HIV-1 infection usually leads to progressive decline in functionality and number of CD4 T lymphocytes, resulting in AIDS development [88]. As early as 1991, apoptosis has been proposed as a possible

mechanism responsible for CD4 T cell depletion in patients infected with HIV-1, and an extensive body of literature since then has supported this hypothesis [89]. In HIV-1-infected patients, both infected and uninfected cells undergo accelerated apoptosis, in vitro and in vivo. However, HIV-1-induced apoptosis in bystander uninfected immune cells is likely the major event leading to the depletion of CD4 T lymphocytes since the degree of cell loss largely exceeds the number of infected cells. Furthermore, the vast majority of T cells undergoing apoptosis in peripheral blood and lymph nodes of HIV patients are uninfected [90]. HIV-1 infection is mediated by the binding of envelope glycoproteins (Env) to the receptor CD4 and a co-receptor, mainly CCR5 or CXCR4. Notably, binding of Env, expressed on HIV-1-infected cells, to CXCR4, triggers apoptosis of uninfected CD4 T cells. Very recently, we have demonstrated that, independently of HIV-1 replication, Env-transfected or HIV-1-infected cells that express Env at the cell surface induce autophagy and accumulation of Beclin 1 in uninfected CD4 T lymphocytes, process dependent on the presence of CXCR4. Moreover, autophagy is a prerequisite to Env-induced apoptosis in uninfected bystander T cells, and CD4 T cells still undergo an Env-mediated cell death with autophagic features when apoptosis is inhibited [62, 91]. To the best of our knowledge, these findings represent the first example of autophagy triggered through binding of viral envelope proteins on target cells, without viral replication, and leading to apoptosis. At present, we do not know the significance of Env-induced Beclin 1 accumulation in CD4 T cell death and if autophagy has a role in HIV-1-replication, and these points are currently under investigation in the laboratory.

It has been shown that HIV-1 uses cytoplasmic late endosomal structures, identified as MVBs, for its budding step, suggesting that HIV-1 may use components of the autophagic machinery for its replication cycle [92]. In contrast, a recent study suggests that the initiating site for constitutive HIV-1 assembly and release is not the endosomal structure but the plasma membrane [93]. Thus, observation of HIV-1 particles in MVBs may be considered as a cellular defence mechanism that restricts the HIV-1 replication cycle by degrading assembled virions.

Sindbis virus

The Sindbis virus, which provokes encephalitis, belongs to the togavirus family. Its genome is a linear, single-stranded RNA. Sindbis-infected apoptotic cell death is closely linked to viral replication. Indeed, the antiapoptotic Bcl-2 protein protects neurons from virus-induced cell death and decreases viral replication in the central nervous system [94]. To explore the role of Bcl-2 in this context, a yeast two-hybrid screen has been performed and has permitted to identify the autophagic protein Beclin 1 as a Bcl-2 interacting protein in infected cells. The brains of mice infected with a recombinant virus that expresses Beclin 1 show fewer Sindbis virus RNA-positive cells, fewer apoptotic cells, and lower viral titers than the brains of mice infected with recombinant viruses that express a Beclin 1 protein deleted in its Bcl-2 interacting domain, or Beclin 1 containing a premature stop codon. These results provide evidence of an antiviral activity of the autophagic protein Beclin 1 [95]. However, no further studies have been done on the role of the entire autophagic process in the reduction in Sindbis virus infection.

The human B19 parvovirus

B19 belongs to the parvovirus family whose genome is a single-stranded DNA. It infects erythroid cells and causes several diseases including aplastic crisis in patients with haemolytic anemia, erythema infectiosum and hydrops fetalis [96]. B19 infection induces cell cycle arrest at G1 and G2/M phases and apoptosis mediated by a viral non-structural protein called NS1 [97]. Recently, it has been shown that autophagy is induced in human parvovirus B19-infected cells arrested in G2 phase, before they are competent for viral replication, leading to infected-cell survival. These results suggest that autophagy may benefit B19 in allowing viral multiplication before cell collapse [98].

Coronavirus

Coronaviruses are enveloped positive sense RNA viruses that replicate entirely in the cytoplasm of cells. They are the cause of many domesticated animal diseases and are responsible for up to 30% of human colds. A new human coronavirus has recently been identified as the causative agent of the severe acute respiratory syndrome (SARS). Murine hepatitis virus (MHV), which belongs to the coronavirus family, is frequently used to study the formation and function of the viral replication complexes. These complexes present a punctate perinuclear localisation and their number and size increase over the course of infection. Moreover, MHV RNA replication occurs on cytoplasmic double-membrane vesicles whose membranes are issued from the rough endoplasmic reticulum [99]. The co-localization of late endosomal proteins and LC3 with viral RNA-replication proteins on these membranes argues for their derivation from the autophagic pathway [99]. Importantly, the formation of autophagosome-like structures seems beneficial for MHV viral production. Indeed, the yield of extracellular virus is diminished 1,000-fold in clonal isolates of *Atg5*−/− mouse embryonic stem cells. In addition, expression of ectopic Atg5 in these cells restores

the wild-type yield [99]. These results demonstrate that Atg5, which is crucial for the autophagic pathway, is required for the production of infectious MHV virions. In consequences, the autophagic pathway may be required for the formation of double-membrane-bound MHV replication complexes and, by this way, may significantly enhance the efficiency of replication. However, the exact role of Atg5 in this process is not fully determined. It would be interesting to know if Atg5 benefits to MHV only by inducing the formation of autophagic membranes or if it has a more specific function in the viral replication cycle.

In SARS-infected cells, the early formation and accumulation of typical double-membrane vesicles, which probably carry the viral replication, have been observed by electron microscopy. Opposite to what was described for MHV, morphological and labelling studies argued against the previously proposed involvement of the autophagic pathway as the source for the vesicles, and instead suggested the endoplasmic reticulum to be the most likely donor of the membranes that carry the SARS replication complex [100].

Rotavirus

Rotavirus belongs to the reovirus family whose genome is a dsRNA. Infection with this virus is the first cause of infantile gastroenteritis. Rotavirus nonstructural protein 4 (NSP4) has functions in viral morphogenesis and pathogenesis. A recent report shows that inhibition of NSP4 expression by small interfering RNAs leads to alteration of the production and distribution of other viral proteins and mRNA synthesis, suggesting that NSP4 also affects virus replication.

In rotavirus-infected cells, it has been shown that NSP4 co-localises with LC3 in structures associated with the sites of nascent viral RNA replication. This report suggests that autophagy may be involved in rotavirus replication [101]. However, the precise role of this process in rotavirus infection needs further investigations.

Bovine viral diarrhoea virus (BVDV)

Bovine viral diarrhoea virus (BVDV) belongs to the flavivirus family whose genome is a ssRNA. The genomic RNA contains one long open reading frame that is translated into a polyprotein. This polyprotein is processed by cellular and virus-encoded proteases. BVDV-infection represents an economically important cause of disease of farm animals. The mucosal desease (MD) is the most severe clinical condition resulting from infection by BVDV. Both a cytopathogenic virus (cp-BVDV) and a non-cytopathogenic virus (noncp-BVDV) are required for induction of MD. Infection with noncp-BVDV occurs intra-utero, leading to a specific immunotolerance. The disease is induced by super-infection with a cp-BVDV or by generation of a cp-mutant of the persisting noncp-virus. In most cases, RNA recombination is responsible for the switch from a noncp- to a cp-virus.

Several types of cellular insertions, which code for ubiquitin, different ubiquitin-like proteins, a protein of unknown function or a part of LC3, have been found. Naturally occurring noncp-viruses express the fusion protein NS2-3, but further processing gives rise to both proteins NS2 and NS3, only present in cp-BVDV. Interestingly, it has been shown that LC3 inserted in the polyprotein of a naturally occurring mutant BVDV served as a proteolytic processing signal by a cellular protease related to Atg4, allowing the expression of the viral NS3 protein. These data provide evidence of the use of autophagy proteins for the expression of viral proteins, linked to the pathology [102].

Interferon system and autophagy

IFNs are a family of multifunctional secreted cytokines that have been characterised by their ability to inhibit virus infection and replication [77]. They can be divided in two sub-groups: IFN type I (α,β) and IFN type II (γ). After their secretion, IFNs bind to specific cell surface receptors and act in paracrine and autocrine ways to induce expression of more than 200 genes called IFN-stimulated genes (ISGs). The large majority of them are still not characterised and their involvement in the IFN system is very fragmentary. ISGs are the effectors of the IFN functions, namely antiviral, antiproliferative, immunomodulatory and apoptotic functions [77]. All these effects of IFNs seem to converge towards the most known IFN activity that is an antiviral function. In consequence, it is not surprising that many viruses have evolved strategies to circumvent the IFN antiviral response and even use IFN-induced processes to replicate more efficiently [103].

Interestingly, beside the fact that IFNγ can induce autophagic vacuole formation, several proteins known to be involved in triggering autophagy are involved in the IFN response [104]. Among them, we can mention the double-stranded RNA-dependent PKR, the death-associated protein kinase and DAPK-related protein-1 (DAPK and DRP-1), the TNF-related apoptosis inducing ligand (TRAIL) and the Fas-associated death domain (FADD).

dsRNA-dependent protein kinase (PKR)

PKR is a serine–threonine protein kinase inactive at a basal level and whose activity can be stimulated by different inducers. It has been first associated with the antiviral action of IFNs [105]. It can be activated by dsRNA, a

common intermediate in the replication of many viruses [106]. After interaction with dsRNA, PKR undergoes conformational changes that trigger its autophosphorylation and thus its activation. The major substrate of PKR is the α-subunit of eukaryotic translation initiation factor eIF2α, resulting in inhibition of protein synthesis [107]. However, PKR can be activated without viral dsRNA or viral infection by different stresses or through protein-protein interactions. For instance, PKR can be activated by interacting with the protein PKR activating protein (PACT), leading to apoptosis [108].

The role of PKR in autophagy triggering is mediated by its capacity to inhibit protein translation through eIF-2α phosphorylation. At present, HSV-1 is the only virus known to inhibit induction of PKR-dependent autophagy, through action of the viral protein ICP34.5 [82]. As a large number of viruses are able to inhibit dsRNA-mediated PKR activation and eIF2α phosphorylation, it would be interesting to analyse their role in autophagy regulation [68].

Death-associated protein kinase: DAPk and DRP-1

DAPk is a calcium–calmodulin (CaM)-regulated serine/threonine kinase involved in IFNγ-mediated cell death [109]. Four additional kinases of the family have been identified according to homologies with DAPk in the catalytic domain [110]. DRP-1 is one of the closest family members, as its catalytic domains share approximately 80% identity to those of DAPk. It has been shown that expression of DAPk or DRP-1 in cells induces morphological changes associated with membrane blebbing and mediates a caspase-independent cell death pathway with formation of autophagic vesicles that are characteristic of type II programmed cell death [111].

TNF-receptor apoptosis induced ligand (TRAIL)

TRAIL, also called Apo2L, initiates apoptosis of tumour cells by binding to either of its receptors, DR4 or DR5.

Using an in vitro morphogenesis model, in which MCF-10A human mammary epithelial cells form hollow acini-like structures, it has been demonstrated that TRAIL is required for the induction of autophagy. The authors demonstrated that both apoptotic cell death and autophagic cell death are required to eliminate the cells during the lumen formation [112].

Fas-associated death domain (FADD)

FADD protein belongs to the death-inducing signalling complex (DISC) that transmits apoptotic signals through activation of caspase-8 [113]. It has been demonstrated that FADD is involved in IFNγ-induced autophagic cell death. In this study, Pyo and collaborators have demonstrated that Atg5 mediates IFNγ-induced vacuole formation and subsequent cell death through interaction with FADD [104]. Moreover, recent data has identified a novel cell death pathway activated by FADD that combines apoptosis and autophagy and that is selectively inactivated at the earliest stages of epithelial cancer development [114].

Conclusion

Considering these data, it becomes clear that autophagy is a fundamental and general process playing a role in viral infections. Indeed, this process is involved in both adaptive and innate immunity, contributing to clearance of intracellular pathogens, and in cell survival or cell death. Moreover, viruses are able to evolve strategies to counteract or to exploit autophagy to their own profit. Indeed, several viruses can block autophagy triggering to avoid their destruction in autolysosomes. In contrast, other viruses use autophagosomes to their own replication. Unravelling the specific role of autophagy in different viral infections would be a crucial step in the understanding of the pathogenesis.

Finally, knowledge of the relationships between autophagy and the antiviral IFNs open new routes of investigation. As a large number of IFN-inducible proteins have still no known biological function, it would be very interesting to determine their role in autophagy triggering.

The interplay between the autophagic pathway and the viral life cycle is thus complex and needs a better comprehension to provide new antiviral therapeutics.

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