

Virus-Induced Autophagy in Innate Immunity

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Abstract

When a virus invades a host cell, autophagy is triggered to maintain cellular homeostasis. Virus-induced autophagy is a double-edged sword in the antiviral function of host innate immunity. Unlike apoptosis, autophagy plays a different role in cell survival or inducing cell death. This mini-review summarizes recent developments in our understanding of the antiviral roles of autophagy in host innate immunity.

Introduction

Autophagy means “self-eating” and is a remarkably conserved process cells utilize to remove and recycle intracellular components to maintain cellular homeostasis [1]. Classical autophagy comprises four major steps: vesicle regulation and nucleation; vesicle elongation and completion; autophagosome formation; and vesicle breakdown and degradation [2]. In the first step, many different pathways and proteins are involved in initiating autophagy. When nutrient deprivation or stresses occur, mammalian target of rapamycin (mTOR) kinase maintains UNC-51-like kinase 1 (ULK1) and autophagy 13 (Atg13) in hypophosphorylated state or inhibits the combination of ULK-1, leading to the upregulation of autophagy in mammals. Other proteins, including c-jun-N-terminal kinase 1 (JNK1), eukaryotic initiation factor 2 α (EIF2 α), and p53, promote autophagy by activating the class III phosphatidylinositol 3-kinase (PI3K) Vps34. HVps34 promotes the formation of phosphatidylinositol 3-phosphate (PI3P) recruiting PI3P-binding effectors. In the second step, ubiquitin-like conjugation systems play an important role in regulating vesicle elongation and completion. There are two systems: the Atg16-Atg12-Atg5 complex controls the curvature of the growing membrane; and cleavage of the microtubule associated protein light chain 3 (LC3) occurs. LC3 is the marker used most commonly for studying autophagy. Little is known of the third major step in autophagy. The last major step is lysosome degradation.

A broad spectrum of viruses induces the formation and accumulation of autophagosomes or autophagy-related vesicles, causing cell death, which activates cell self-defences via pattern-recognition receptors (PRRs) [3]. In addition, autophagy or autophagy-related proteins contribute to antiviral type I and type II IFN cytokines [4]. Virus-induced autophagy is a double-edged sword, functioning as a defence and offence via the regulation of inflammasomes and there is a homonymous relationship between apoptosis and cell survival [5].

Virus-Induced Autophagy

Upon invasion of cells, pattern-recognition receptors, as immune sensors, recognize viruses immediately and trigger autophagy, which induces an antiviral response. For example, TLR7 recognizes virus ssRNA and stimulates autophagy in RAW 264.7 macrophages via

MyD88, eliminating intracellular viruses [6]. A similar mechanism is shared with RSV-induced autophagy contributing to type I IFN production in dendritic cells [7]. Recently, ssRNA-induced autophagy was observed following the interaction of the nod-like receptor (NLR) receptor (NOD2) and autophagy-related-like 1 (ATG16L1) signaling [8]. RNA-dependent protein kinase (PKR) also acts as an antiviral warrior in autophagy. For example, PKR mediates the phosphorylation of eIF2 α and boosts beclin-1, causing autophagy in mouse embryonic fibroblasts (MEFs) and primary neurons infected with herpes simplex virus (HSV)-1 [9]. PKR-mediated autophagy has antiviral effects involving an inflammatory response in HSV-1 encephalitis [10]. Moreover, intracellular DNA sensors, such as absent in melanoma 2 (AIM2)-like receptors, can also trigger autophagy [11]. Furthermore, attenuated measles-induced autophagy can occur via cellular receptor CD46-Cyt-1. This protein binds to the beclin-1/VPS34/GOPC complex, which is related to autophagosome formation [12,13].

Besides recognizing viruses, host cell modifications caused by viruses can also trigger autophagy, such as endoplasmic reticulum (ER) stress and reactive oxygen species (ROS). During viral infections, synthesized viral proteins can activate the ER stress response. Hepatitis C virus (HCV) induced the phosphorylation of protein kinases RNA (PKR)-like endoplasmic reticulum kinase (PERK) and eIF2 α . Subsequently, the PERK siRNAs significantly reduced the LC3-II level. This indicates that PERK plays a critical role in the autophagic response induced by HCV. Malilal investigated the impaired autophagy induced by excessive ROS formation in A549 cells overexpressing CUG2 (A549-CUG2) infected with vesicular stomatitis virus (VSV) [14]. Under these conditions, the level of ISG15 protein decreased and S6 kinase was inactivated.

Role of Autophagy in Cell Survival in Viral Infection

Autophagy is a cellular survival strategy that was first proposed in 1977. Autophagy is an important pro-survival mechanism that counteracts apoptotic or necrotic death. Many recent studies have shown that virus-induced autophagy prevents or delays the death of the infected cells. For example, the knockdown of beclin-1 or Atg7 in HCV-infected human hepatocytes led to caspase-dependent apoptosis, while the inactivation of Atg4b resulted in the death of HCV-infected

human hepatoma cells. In addition, Japanese encephalitis virus (JEV) infects beclin-1- or Atg5-deficient cells, increasing cell death Figure 1.

As autophagy helps infected cells to live, this has the potential to allow the delivery of viruses to distant sites, promoting infection spread. For example, the knockdown of Atg7 and Atg12 completely abrogated the survival of γ HV68-infected endothelial cells, revealing that the autophagic machinery provides a cellular reservoir allowing viral persistence. The knockdown of Atg7 in HeLa cells infected with Sin Nombre virus (SNV) led to the cells succumbing to infection more rapidly [15].

Recent reports suggest that virus-induced autophagy supports the survival of the infected cells *in vivo*. Consider HSV-1, a mutant HSV-1 lacking ICP34.5, which binds beclin-1 and inhibits PKR-dependent autophagy, failed to inhibit autophagy and showed lower lethality. Similarly, Atg5-knockout mice were more sensitive to lethal SNV infection of the central nervous system.

In virus replication, one must consider the effect of autophagy as a key parameter determining the fate of the infected cell. There are three possible patterns of autophagy-virus replication cell death interaction. The first mechanism for the protective role of autophagy in infected cells could involve the xenophagic destruction of the virus and subsequent reduction of the viral burden-related cell death, which might occur in HSV-1 brain infection in mice. The second mechanism is that autophagy also inhibits viral replication/propagation via the suppression of apoptosis. For example, in Chikungunya virus (CHIKV)-infected fibroblasts, the apoptosis machinery was subverted, releasing the viral materials incorporated into the apoptotic blebs. Finally, the protective effect of autophagy in cells infected with flaviviruses, HCV, or JEV coincided with the promotion of virus replication [16] (Figure 1).

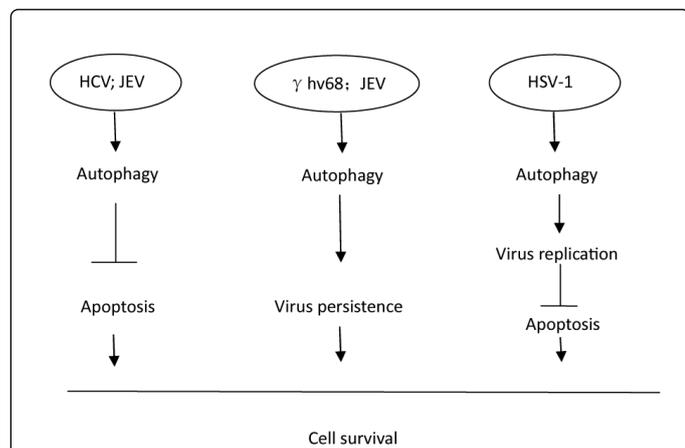


Figure 1: Mechanisms of virus-induced autophagy in cell survival. Autophagy inhibits apoptosis limiting virus replication and resulting in cell survival, or is required for virus persistence, enabling the survival of infected cells. Autophagy stimulates virus replication, ensuring the death of infected cells, allowing the survival of uninfected cells.

Crosstalk between Autophagy and Apoptosis

Several pro-apoptotic proteins are thought to induce autophagy, including Bad, Bak, BNIP3, and Nix. These proteins regulate the

interaction between Bcl-2 and beclin-1, inducing autophagy, as shown in Figure 2. Noxa induces autophagy by displacing beclin-1 from Mcl-1, another Bcl-2 family member. In addition to triggering extrinsic and intrinsic apoptosis, p53 plays a significant role in autophagy. In the nucleus, p53 activates damage-regulated autophagy modulator (DRAM), stimulating autolysosome formation. PUMA, p53-inducible BH3-only protein, triggers mitochondria-specific autophagy via Atg5, Atg10, and Atg7.

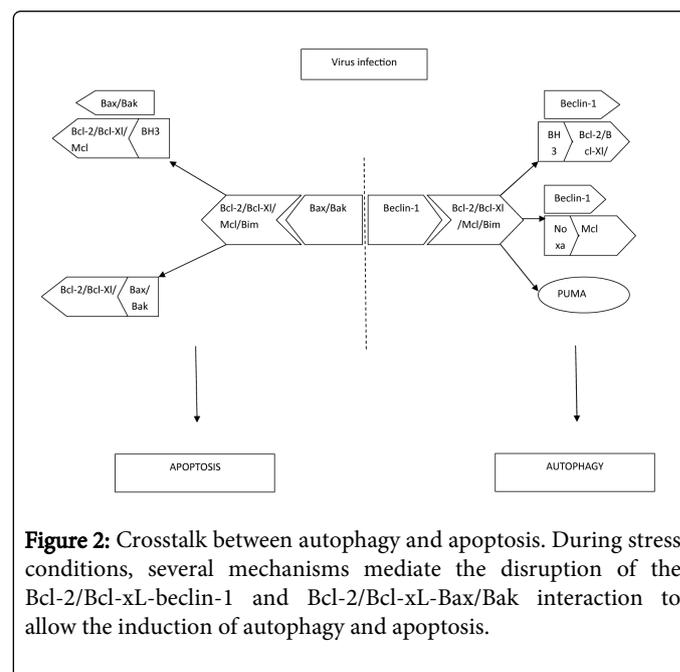


Figure 2: Crosstalk between autophagy and apoptosis. During stress conditions, several mechanisms mediate the disruption of the Bcl-2/Bcl-xL-beclin-1 and Bcl-2/Bcl-xL-Bax/Bak interaction to allow the induction of autophagy and apoptosis.

Autophagic proteins positively or negatively regulate mitochondrial apoptosis. In addition to its role in autophagy, mTOR has multiple effects on apoptosis that depend on specific cellular conditions and downstream targets, including Bad, Bcl-2, and p53.

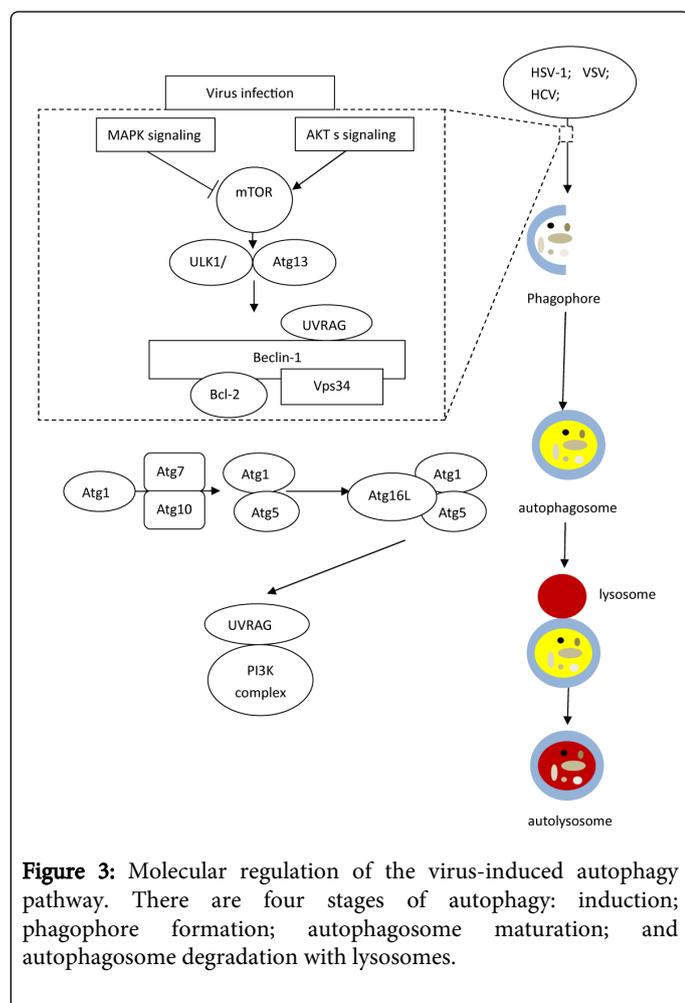
Apoptosis and autophagy play different roles in cell death. Apoptosis functions are emphasized to limit viral replication, but autophagy is a pro-survival cellular process. For example, the human flavivirus dengue virus type 2 can induce autophagy that protects the from death [17]. However, influenza A inhibits autophagy maturation to regulate cell death [18]. During virus infection, there is a complicated relationship between autophagy and apoptosis. CHIKV-induced autophagy delays apoptosis, since early cell death occurs in ATG5-deficient MEFs, resulting in fewer infected cells and limiting viral propagation. By contrast, the number of infected cells increases in ATG5^{-/-}MEFs. Therefore, autophagy, by preventing apoptosis, has different functions depending on the virus, promoting cell survival or inducing cell death.

Role of Autophagy in Innate Immunity

Autophagy can block the virus-triggered immune response when exaggerated immune inflammatory responses are harmful to the host. For example, the Atg5-Atg12 conjugate interacts with the caspase recruitment domains (CARDs) of retinoic acid-inducible gene I (RIG-I) and IFN- β promoter stimulator 1 (IPS-1), dampening the activity of RIG-I like helicases, which stimulate type I IFN production [19]. Tal et al. [20] found that VSV-induced autophagy could inhibit RIG-I signaling by clearing up mitochondrial ROS accumulation and

restricting inflammasomes activation. Furthermore, autophagy might negatively regulate the pro-inflammatory response of nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) proteins. For example, ASC (apoptosis-associated speck-like protein containing a caspase-activating and recruitment domain) and caspase-1 form a complex that contributes to the maturation of pro-IL-1 β . Interestingly, HPV16 early gene depletion-induced autophagy is associated with the senescence/senescence-associated secretory phenotype response, which promotes beneficial immune effects in bystander cells [20].

Some viruses successfully escape innate immunity by controlling autophagy. For example, the murine cytomegalovirus M45 protein interacts with subunit NF- κ B essential modulator (NEMO), which can impair the antiviral loop of inflammatory cytokine synthesis, allowing degradation by autophagy [21]. Interestingly, the M2 protein of influenza virus can both activate the inflammasome and inhibit autophagy. During influenza virus infection, activation of the domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome complex [22] depends on M2, which controls ion channels in the Golgi apparatus (Figure 3).



The influenza A M2 protein also blocks the fusion of autophagosomes with lysosomes [18]. The HIV-1-mediated down regulation of autophagy in DCs also impairs innate immune responses, as revealed by the markedly decreased TNF- α production upon Toll-like receptor (TLR) stimulation [18]. Furthermore, human

cytomegalovirus (HCMV) inhibits autophagy by means of two mechanisms to avoid degradation and evade the activation of innate immunity: by inhibiting antiviral PKR and blocking autophagosome maturation [23].

Our knowledge of virus-induced autophagy is still elementary, especially regarding innate and autophagy cross-talk. Virus pattern-recognition receptors (PRRs) and host pathogen-associated molecular patterns (PAMPs) are known to induce autophagy, but many mechanisms remain unclear. In addition, more attention should focus on innate signaling in autophagy. It is important to explore the function of autophagy in innate immunity during co-infection.

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