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A game of survival: herpesvirus strategies of autophagy manipulation

Gra o przetrwanie: strategie manipulacji autofagii przez herpeswirusy

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Summary

Viruses are a very "clever" group of pathogens and well known for disrupting multiple processes in host cells. One of them is autophagy, a conserved mechanism that relies on degradation of intracellular structures in lysosomes. Autophagy can be triggered in response to viral infections and its aim is to digest viral particles, thereby limiting virus replication and spread. Induction of autophagy during viral infections is mediated by different regulatory pathways, but the biggest participation belongs to PKR and eIF2alpha. In this review we focused on the herpesvirus interactions with autophagic machinery. The *Herpesviridae* family presents various strategies to manipulate autophagy induction or suppression of that process may depend on cell type and stage of infection. Research carried out in the past 10 years has demonstrated the impact of herpesviruses on autophagy not only during productive infections, but in latency infections also.

Key words: **autophagy • herpesvirus • productive infection • latency**

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Abbreviations: **AMPK -** 5' adenosine monophosphate-activated protein kinase; **ADAR -** adenosine deaminase; **APOBEC3G -** apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G protein; **Atg -** autophagy related genes; **BBD -** beclin 1 binding domain; **BHV-4 -** bovine herpesvirus type 4; **BoHV-1** - bovine herpesvirus type 1; **CAMKK2 -** calcium/calmodulin-dependent protein kinase kinase 2; **EBV -** Epstein-Barr virus; **EBNA1 -** Epstein-Barr nuclear antigen 1; **eIF2α** - phosphorylation of eukaryotic initiation factor 2α; **GOPC** - Golgi-associated PDZ and coiled-coil motif-containing protein; **HHV-1/HSV-1** - Human herpesvirus type 1/herpes simplex virus 1; **HHV-3/VZV** - Human herpesvirus type 3/varicella zoster virus; **HHV-5/CMV -** Human herpesvirus type 5/cytomegalovirus;

HHV-8/KSHV - Human herpesvirus type 8/Kaposi's sarcoma associated herpesvirus; **IRF3** - interferon regulatory factor 3; **JNK** - c-Jun N-terminal kinase; **LAT** - latency associated transcript; **LC3** - microtubule-associated protein light chain 3; **LMP1** - latent membrane protein 1; **MDBK** - Madin Darby Bovine Kidney cells; **mTOR** - mammalian target of rapamycin kinase; **MX1** - myxovirus resistance 1 protein; **NEDA** - nuclear envelope-derived autophagy; **NLRs** - nucleotide oligomerization domain-like receptors; **NLRX1** - nucleotide-binding oligomerization domain, leucine rich repeat containing X1; **OAS1** - 2′-5′-oligoadenylate synthetase 1; **OIS** - oncogene-induced senescence; **PAMPs** - pathogen-associated molecular patterns; **PERK** - protein kinase RNA-like endoplasmic reticulum kinase; **PRRs** - pattern recognition receptors; **PKR** - protein kinase R; **STAT3** - Signal Transducer and Activator of Transcription 3 protein; STING, stimulator of interferon genes; **TBK1** - TANK-binding kinase 1; **TLR** - Toll-like receptors; **TSC2** - tuberous sclerosis protein 2; **TUFM** - mitochondrial Tu translation elongation factor.

Introduction

The term autophagy (gr. *autos*- self, *phago*- eat) was used for the first time by Christian de Duve over 40 years ago, observing the degradation of mitochondria and other intracellular components inside lysosomes of rat liver that was treated with glucagon. Since then many studies concentrating on autophagy have led to a better understanding of the molecular mechanisms involved in this process, mainly due to experiments performed in the yeast *Saccharomyces cerevisiae* in which at least 32 autophagy related genes (Atg) are known. Autophagy is a conserved process characteristic for fungus, plant and animal cells, connected with both pathological and non-pathological conditions [16]. Cellular processes associated with autophagy are development and cell differentiation, viral and bacterial infections including development of malignant diseases, and neurodegenerative disorders such as Alzheimer's or Parkinson's disease [45].

Autophagy relies on the degradation of cellular components (organelles and macromolecules) with participation of lysosomes in response to different stimuli. In regard to the way substrates are delivered to the lysosome, three types of autophagy have been defined: (i) macroautophagy, (ii) chaperone-mediated autophagy and (iii) microautophagy [16,45]. Autophagy can be called non-selective when partial degradation of the cytoplasm occurs in order to maintain the balance of cytoplasm size and composition, and selective when specific structures are digested, for instance: mitochondria – mitophagy; ribosomes – ribophagy; viruses and bacteria – xenophagy. As compared to degradation in proteasomes, autophagy is not limited to protein digestion only, it includes other substrates such as lipids, DNA and RNA. Thus autophagy provides a new pool of amino acids, fatty acids and nucleosides that are crucial in all anabolic processes [63].

Macroautophagy was observed first and is the best known type of autophagy; hence the term macroautophagy is generally referred to as autophagy. During this process part of the cytoplasm is surrounded by a two-membrane structure phagophore, followed by elongation leading to enclosure of organelles and macromolecules forming what is called an autophagosome. Subsequently, the autophagosome matures and fuses with the lysosome in order to digest the engulfed cargo. The whole autophagy process can be divided into several stages: (i) induction of phagophore formation, (ii) elongation, (iii) autophagosome maturation, (iv) fusion of autophagosome and lysosome, (v) digestion within autophagolysosome [63].

Role and induction of autophagy during viral infections

Viral infections are one of many factors that provoke autophagy in host cells. Autophagy during viral infections participates in the degradation of infectious virus particles within an autophagolysosome in order to restrict viral replication and spread as well as providing ligands for endosomal Toll-like receptors (TLR) (viral components) important for induction of innate immunity [7,28]. Autophagy takes part in adaptive immunity through provision of peptides for antigen presentation by MHC I and MHC II [7,14].

There is evidence that autophagy may be utilized by certain viruses to support their replication or facilitate viral particle regress. For instance, occurrence of autophagy increases the yields of viruses such as hepatitis C virus, poliovirus and Dengue virus. It has been proposed that polio virus uses autophagy to exit the cell in the late stage of infection. Hepatitis C virus induces autophagosome formation, but prevents the fusion with lysosomes [7,54].

Many DNA and RNA viruses have the ability to interfere with pathways that regulate autophagy, stimulating or inhibiting this process and thus providing optimal conditions for their replication [28,54]. Over the past 10 years it has been demonstrated that *Herpesviruses* have various strategies to control the host autophagic machinery [7]. In analyzing autophagy within *Herpesviridae* consideration should be given to the fact that the process may accompany productive infection as well as latent infection. So far the influence of latency on autophagy has been well documented in γ-herpesviruses that encode homologues of Bcl-2 protein which allow them to evade not only apoptosis, but also autophagy [41].

The induction of autophagy during viral infections has not been fully elucidated. Recent experimental data show that autophagy induction may occur in many ways, during different stages of infection. The first step of infection- adsorption of virus to receptors located on permissive cells can

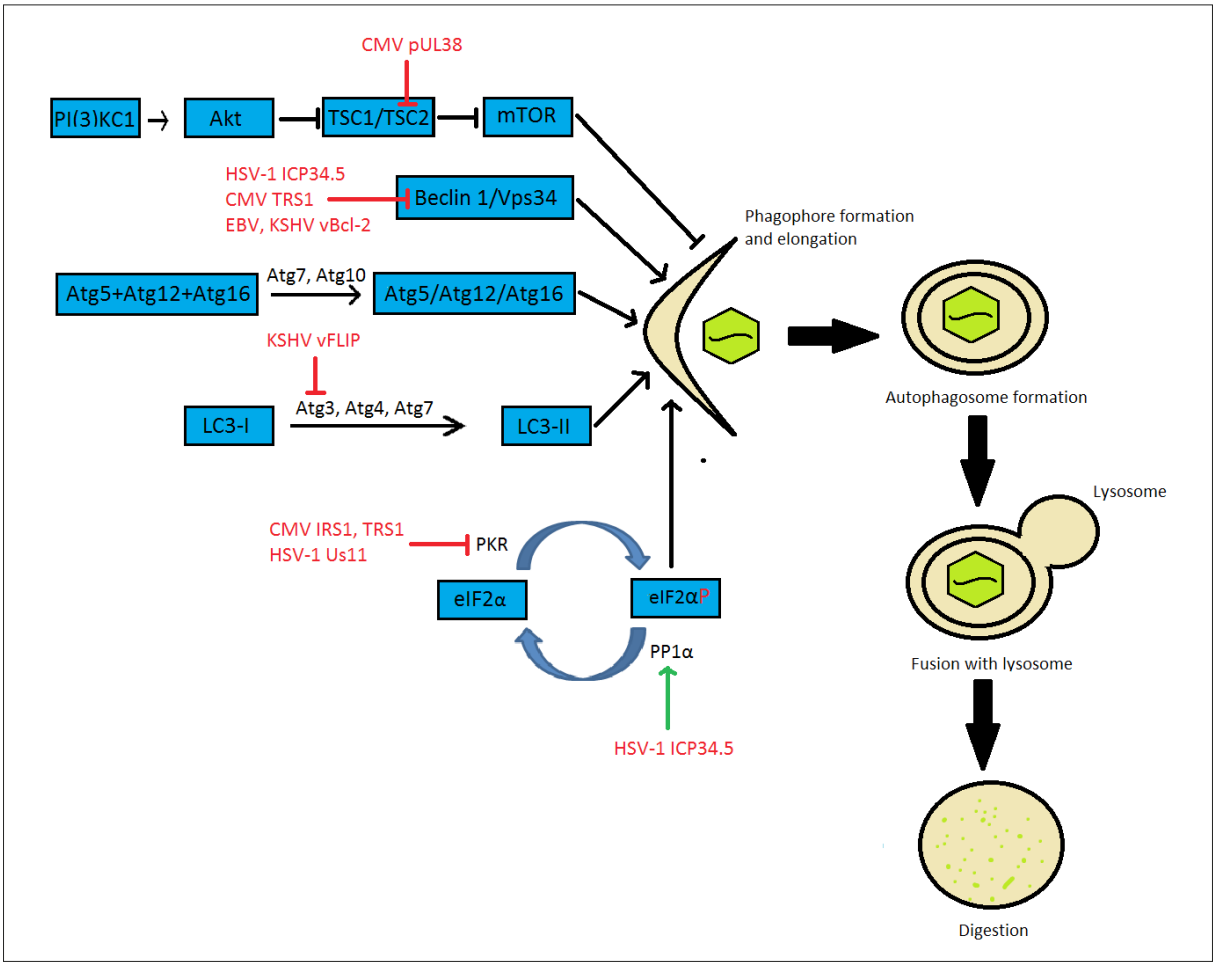

Fig.1. Autophagy induction during viral infections. The process may be provoked at different stages of virus replication cycle, including virion adsorption, genome release and expression of viral genes. Besides, a crucial role in autophagy induction belongs also to pattern recognition receptors (PRRs) and interferon-induced antiviral mechanism.

provoke the signal initiating autophagy. A splicing variant of the CD46 receptor is able to induce autophagy via the Vps34/Beclin 1 complex by interaction with the Golgi-associated PDZ domain and coiled-coil motif-containing protein (GOPC) [23]. CD46 is a complement regulator and it is used by several viruses to enter a host cell, for example human herpes virus type 6, measles virus, bovine viral diarrhea virus or different serotypes of adenoviruses [6]. Another receptor known for its role in the induction of apoptosis via the extrinsic pathway is Fas, which initiates autophagy in HeLa cells. Moreover, autophagy induced in these cells by anti-Fas antibody is dependent on c-Jun N-terminal kinase (JNK) and its role is to prevent apoptosis [67]. The next step in viral infection, release of the viral genome, may also induce signals leading to autophagy. Some viruses can induce autophagy by the mere presence of viral DNA in a cell, independent of viral protein synthesis [38,48].

Viruses as pathogens are identified by pathogen-associated molecular patterns (PAMPs) that activate pattern recognition receptors (PRRs) present in innate immune cells. The PRRs are divided into several groups: (i) Toll-like receptors in plasma membrane and endosomes, (ii) retinoic acid-inducible gene I-like receptors (RLRs), (iii) nucleotide oligomerization domain (NOD)-like receptors (NLRs) and (iv) cytosolic DNA sensors such as AIM2 (absent in melanoma 2) and NLRP3 (NOD-like receptor family, pyrin domain containing 3). The activation of PRRs leads to the induction of innate immunity mechanisms [14]. It has been discovered lately that PRRs may affect autophagy, as well. The NLRX1 (nucleotide-binding oligomerization domain, leucine rich repeat containing X1) and TUFM (mitochondrial Tu translation elongation factor) proteins act together to increase the autophagy level during viral infection [34].

Cell protection against viral infections includes interferon-induced mechanisms that are based on the activity of viral proteins such as protein kinase R (PKR), myxovirus resistance 1 protein (MX1), 2'-5'-oligoadenylate synthetase 1 (OAS1), apolipoprotein B mRNA-editing, enzyme-catalytic, polypeptide-like 3G protein (APOBEC3G), adenosine deaminase (ADAR) and guanylate-binding proteins (GBPs) [50]. Some of these proteins are also involved in autophagy. PKR activated by dsDNA plays a crucial role in the inhibition of

virus replication by phosphorylation of eukaryotic initiation factor 2α (eIF2α); thus it inhibits protein biosynthesis [12] and regulates autophagy induced by viral infections [56]. PKR-dependent autophagy is regulated by cytoplasmic Signal Transducer and Activator of Transcription 3 protein (STAT3) that inhibits PKR activity by interacting with the SH2 domain of STAT3 and the catalytic domain of PKR [52].

RNase L is an antiviral protein activated by adenylate oligonucleotides synthesized by OAS1. RNase L degrades viral and cellular RNA during viral replication [2], but the latest studies show that it also induces autophagy, affecting the viral yield [53,8]. This kind of autophagy is dependent on JNK and PKR [53]. Bacterial infections also influence autophagy activity of guanylate-binding proteins, e.g., GBP1 interacts with p62/SQSTM1 and GBP7 acts together with Atg4 [25].

Moreover, autophagy is connected with the so-called unfolded protein response (UPR), which is induced as a protective mechanism against unfolded proteins overloading the endoplasmic reticulum (ER). Consequently, protein synthesis is inhibited and chaperones are produced to support the folding of newly synthesized proteins. During productive infection viruses such as human herpesvirus type 1 (HHV-1), and cytomegalovirus (CMV) use UPR to increase endoplasmic reticulum capacity whereas Epstein-Barr virus uses UPR during latency to regulate B cell proliferation through alteration in levels of latent membrane protein 1 (LMP1) [31]. The participation of ER was also observed in rotavirus-induced autophagy. The virus uses its viroporin (pore-forming protein) to permeabilize the ER membrane, thus releasing calcium into the cytoplasm. The increased level of calcium in the cytoplasm initiates a cascade of events mediated by calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2) and 5' adenosine monophosphate-activated protein kinase (AMPK) in order to elicit autophagy [11].

Influence of herpesvirus infections on autophagy

Human herpesvirus type 1

Human herpesvirus type 1 (HHV-1), also referred to as herpes simplex virus 1 (HSV-1) is a member of the α-*herpesvirinae* subfamily and is well known for its interference with the autophagic machinery. The virus is responsible for oral herpes resulting from productive infection of oral epithelium and it establishes latency in neurons of the trigeminal ganglia. Research using many types of cells – human fibroblasts [38,56], mice fibroblasts [1], primary murine neurons [57], astrocytes [27], macrophages [13] and dendritic cells [17] – has demonstrated that HHV-1 evolved an ability to affect autophagy. In the early stage of infection, the virus induces autophagy in human fibroblasts, independent of viral protein synthesis, as demonstrated by inhibition of viral gene expression [38]. In contrast to fibroblasts, autophagosomes in infected macrophages are observed in the late stage of infection [13] and in the case of dendritic cells autophagosomes and p62 accumulation have been observed [17]. Induction of autophagy in myeloid cells (macrophages and dendritic cells) occurs in response to viral DNA, independent of viral gene expression, but in a manner dependent on stimulator of interferon genes (STING) that participate in the initiation of IFN type I and proinflammatory cytokine synthesis [48]. Therefore, induction of autophagy during HHV-1 infection may depend on the cell type or stage of infection. Interestingly, in neuroblastoma cells HHV-1 causes accumulation of amyloid α inside autophagosomes, preventing the fusion with lysosomes. It suggests that the virus may have an influence on the development of Alzheimer's disease [49]. On the other hand, it has been recently reported that HHV-1-induced autophagy in astrocytes is positively regulated by cellular prion protein [27].

The first discovery referring to modulation of autophagy by HHV-1 describes the interference of neurovirulence factor infected cell protein 34.5 (ICP34.5) with autophagy dependent on PKR and eIF2α [56]. ICP34.5 is encoded by the γ34.5 gene of HHV-1 and its role relies on the binding with Beclin 1[42] and the recruitment of protein phosphatase 1α (PP1α) that dephosphorylates eIF2α despite PKR activity, switching on protein synthesis [20]. ICP34.5 activity not only includes autophagy inhibition, but it is critical to virus replication cycle, too. However, it interacts with proliferating cell nuclear antigen (PCNA) [4], switching from repair to support of replication activity in non-dividing cells crucial for the initiation of viral replication [19]. Furthermore, it disrupts the interaction between TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), preventing the expression of interferon and interferon stimulated genes [61]. Additionally, it participates in virion egress [3,22]. Activity of the ICP34.5 protein is dispensable in macrophages and dendritic cells, because virus-induced autophagy in these cells is not dependent on PKR activity and does not correlate with the phosphorylation of eIF2α [48]. Another aspect of the ICP34.5 protein is the impact on oncolytic properties of HHV-1. The replication of γ34.5-null HSV-1 mutant is attenuated in cancer cells compared with wild type. This problem has been solved by creating a mutant that only lacks the Beclin 1 binding domain (BBD). This mutant replicates at the appropriate level in human glioblastoma cell lines and human glioma cells, efficiently killing malignant cells *in vitro* and extending survival of mice suffering from orthotopic brain tumors [24]. Recently, a new HHV-1 gene that inhibits virus-induced and PKR-dependent autophagy was discovered. The gene, Us11, is a late gene which directly interacts with PKR, inhibiting autophagosome formation and in turn autophagy in fibroblasts and HeLa cells. Interestingly, immediate early expression of Us11 is sufficient for HHV-1 (a mutant without the γ34.5 gene) to escape from the host autophagic machinery [37].

Binding of ICP34.5 to Beclin 1 is an important aspect of virulence and pathogenesis of HHV-1. Studies based on mouse models have demonstrated that the Beclin 1 binding domain plays a key role in inhibiting viral antigen presentation through MHC II molecules via autophagy. A mutant of HHV-1 lacking the BBD is not able to take control of adaptive immunity, since it induced a stronger $CD4^+$ T lymphocyte response through increased production of interferon gamma and interleukin 2 [35]. The BBD is important for counter-

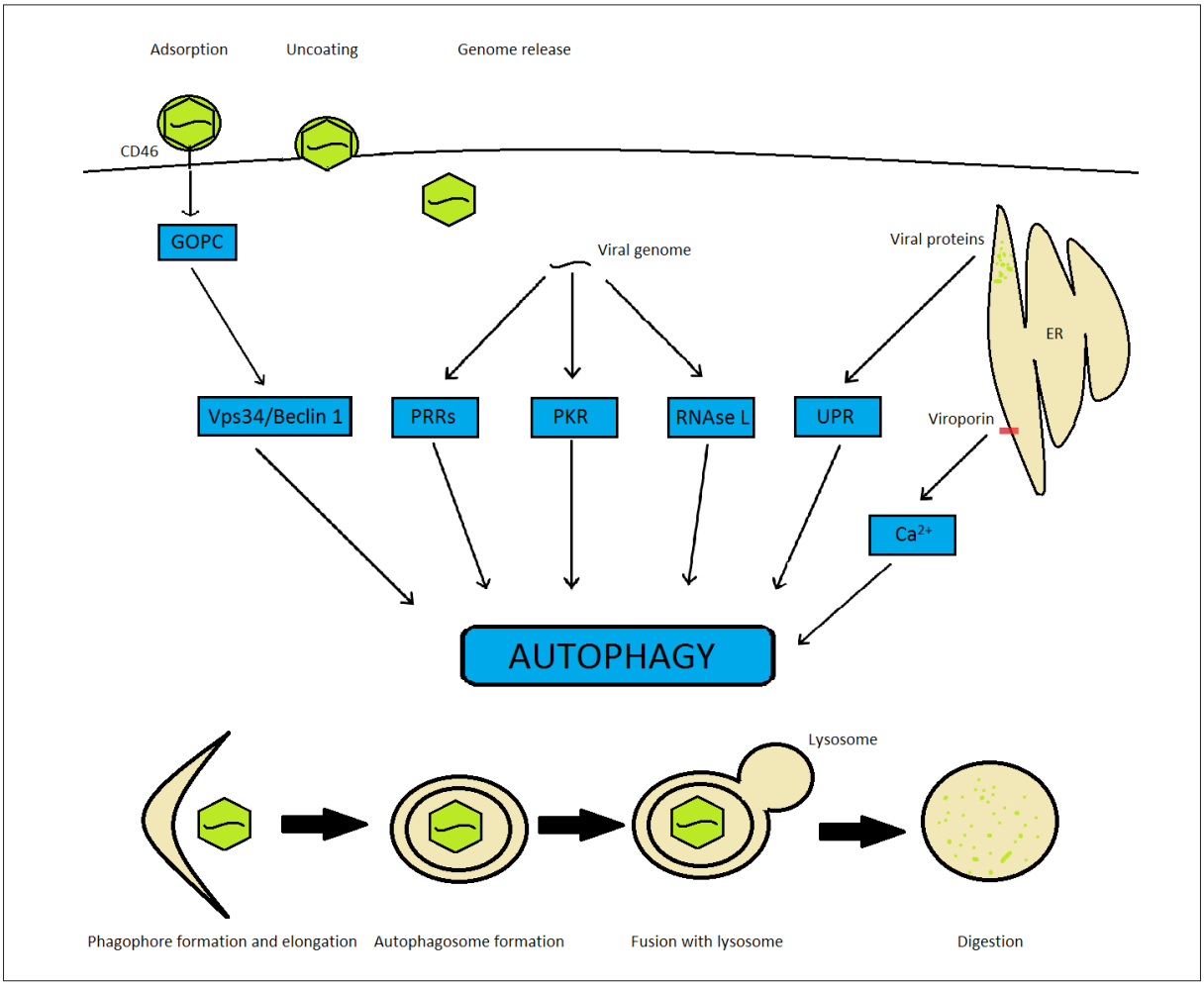

Fig.2. Autophagy manipulation by herpesviruses. The members of the *Herpeviridae* family have various strategies to evoke autophagy during infection. Herpesviral proteins act on diverse signaling pathways that regulate autophagy. Phagophore formation can be prevented by the interaction with TSC2, Beclin 1 and Atg3. Moreover, herpesviral proteins may disrupt PKR-dependent signaling, as well.

acting innate immunity, as well. Infection with a BBD-null mutant enhances autophagy and activation of the NLRP3 inflammasome, enhancing the innate immune response [66].

HHV-1 antigens are also presented by MHC class I molecules via autophagy and processed peptides are further directed for degradation in proteasomes. Interestingly, it has been reported that infection of macrophages induces formation of autophagosomes originating from the nuclear membrane [13]. Presentation of antigens by dendritic cells is disrupted by HHV-1 as a result of interference with the autophagosome maturation process, thereby stimulating a weaker T lymphocyte response [17].

A characteristic of viral infections is development of nuclear envelope-derived autophagy (NEDA), which has been observed in macrophages and other cell types. NEDA occurs simultaneously with macroautophagy in infected cells, but it is regulated in a different manner. Experimental data demonstrate that NEDA is provoked by the interaction of ICP34.5 with PP1α during production of late proteins. It was proposed that NEDA is a cellular stress response elicited in the late stage of HHV-1 infection and its role may be compensation for macroautophagy manipulation [47].

The impact of HHV-1 on autophagy during latent infection is not well examined and further studies are required. It has been demonstrated that HHV-2, which is closely related to HHV-1, encodes microRNA (miRNA) responsible for the inhibition of γ34.5 expression. The miRNA, mi-R1, is encoded by the latency associated transcript (LAT) region of the HHV-2 genome and is expressed both *in vitro* and *in vivo* in guinea pig ganglia (during productive and latent infection). Further, it is also detected in human sacral dorsal root ganglia, where it is expressed under the control of the LAT promoter. A similar miRNA molecule was also found in the HHV-1 genome in a location similar to mi-R1, indicating a conserved strategy used by these viruses. It was hypothesized that mi-R1 might affect the result of viral infection in the peripheral nervous system, changing the expression of the ICP34.5 protein [46]. HHV-1 latency is regulated by mammalian target of

rapamycin kinase (mTOR), a main regulator of autophagy. Suppression of the kinase contributes to reactivation from the latency state and it is enough to inhibit mTOR activity in axons in order to cause virus reactivation [26].

The influence of autophagy on HHV-1 replication is controversial and not clear. Talloczy et al. [57] reported that autophagy effectively degrades virus particles whereas Alexander et al. [1] contradicted those findings. In another study by Pei et al. [44] the antiviral polyphenol compound pentagalloyl glucose induced autophagy by the mTOR pathway suppression, leading to the formation of autophagosomes containing HHV-1 virions (application of transmission electron microscopy). The virions were also found in autophagosome-like structures in Gro29 cells, which are chemically induced mutants of the mouse L cell line. Gro29 cells are known for their high basal autophagy level compared to primary cell lines and because of that they are probably able to survive HHV-1 infection [30]. Recent findings imply that the role of autophagy in control of HHV-1 replication depends on the cell type; in neurons, autophagy is essential for limiting virus replication, but it is not required for epithelial cells and other mitotic cells. There are three main differences between neurons and dividing cells regarding the anti-HHV-1 cell response: (i) neurons produce small amounts of interferon type I and antiviral mechanisms induced by this cytokine are not as efficient as in dividing cells, (ii) IFN type I-induced death in neuronal cells is not as effective as in mitotic cells, (iii) neurons, but not dividing cells, use autophagy for defense against HHV-1 infection [64,65].

Bovine herpesvirus

Bovine herpesvirus type I (BoHV-1) belongs to the α-*herpesvirinae* subfamily and is a major causative agent of respiratory disorders, abortions, genital infections and conjunctivitis in cattle. The implications of BoHV-1 and autophagy are well understood but it has been reported that the virus-encoded protein bICP0 is able to effectively suppress autophagy so the virus can evade the host defense machinery. Bovine kidney cells infected with a mutant virus void of bICP0 had more autophagosomes compared with the wild type virus, suggesting interference of the protein in autophagy [15].

Bovine herpesvirus type 4 (BoHV-4) is classified as a member of the γ-*herpesvirinae* subfamily. It has been reported that BoHV-4 influences autophagy, too. The virus induces autophagy in the late stage of infection of Madin Darby Bovine Kidney cells (MDBK), increasing the level of LC3II (microtubule-associated protein light chain 3), Beclin 1 PI3 kinase, but reducing the p62 protein level. The level of mTOR was surprisingly higher, since the kinase regulates autophagy negatively [39].

Human herpesvirus type 3

Human herpesvirus type 3 (HHV-3), i.e., varicella zoster virus (VZV) in contrast to other members of the α-*herpesvirinae* subfamily is not able to inhibit autophagy. Infected fibroblast and melanoma cells had features of autophagy during early stages of infection. The results were confirmed by *in vivo* studies using skin zoster vesicles. Moreover, no gene orthologues of γ34.5 or Bcl-2 homologues have been found in the HHV-3 genome [55]. HHV-3 induced autophagy is triggered at least by endoplasmic reticulum stress due to high production of HHV-3 glycoproteins. Unfolded protein response is a mechanism responsible for maintaining cellular homeostasis [5].

Human herpesvirus type 5

Another example of a member of the *Herpesviridae* family is human herpesvirus type 5 (HHV-5), which belongs to the β-*herpesvirinae* subfamily and commonly is called cytomegalovirus (CMV). Several autophagy detection methods including GFP-LC3, LC3-II, p62 accumulation and transmission electron microscopy have demonstrated that the virus strongly inhibits autophagosome formation in primary human fibroblasts. Furthermore, the decrease of autophagosome number was observed only in infected cells where viral gene expression occurred whereas the level of autophagy in other cells in cell culture was similar to control cells. These results indicate that autophagy suppression is caused by viral proteins [10]. Although autophagy inhibition has been reported, this state is established at late stages of infection when the viral gene expression occurs. In the case of cytomegalovirus autophagosome formation was induced 2 hours after infection of fibroblasts. *De novo* synthesis of viral proteins was not necessary, since incubating the cells with virus inactivated by UV or cycloheximide, a protein synthesis inhibitor, still revealed autophagosome formation. These studies for the first time showed a quick cell reaction to the presence of virus and further imply that autophagy may be provoked by foreign DNA within a cell [38].

Although the mechanisms of autophagy suppression are not precisely known, experimental data show that during infection, cytomegalovirus activates the mTOR signaling pathway [29]. Two HHV-5 proteins, TRS1 and IRS1, interact with protein kinase R, keeping it away from its activator, i.e., dsDNA, and its substrate, i.e., eIF2α [10]. HHV-5-infected cells are resistant to induction of autophagy by inhibitors of mTOR kinase, rapamycin and lithium chloride, inducers of autophagy that act independently of the mTOR pathway, indicating that HHV-5 manipulates autophagy by interacting with different signaling pathways [10]. The mechanism of TRS1 protein activity was described recently. The N-terminal fragment of TRS1 protein interacts with Beclin 1, leading to autophagy suppression [9]. Another HHV-5 protein, pUL38, is potentially an anti-autophagy protein and is also known for its anti-apoptotic activity. pUL28 acts as a blocker of the normal cell response to stress. The mechanism is based on the interaction with tuberous sclerosis protein 2 (TSC2), which regulates mTORC1, limiting cell growth [40]. Furthermore, the protein inhibits cell death associated with endoplasmic reticulum stress, independently of mTORC1 activation [46].

Epstein-Barr virus

Epstein-Barr virus (EBV) belongs to the γ-*herpesvirinae* subfamily. The virus replicates in oral epithelial cells and establishes latency in B lymphocytes. Current knowledge about the implications of EBV in autophagy is limited to two viral antigens: (i) Epstein-Barr nuclear antigen 1 (EBNA1) and (ii) LMP1. Both antigens are expressed during the latency state; hence not much is known about autophagy during EBV acute infection [7]. EBV, like other γ-herpesviruses, encodes a viral Bcl-2 homolog which can counteract autophagy [41]. Autophagy, in the context of EBV infection, has been mainly studied in B lymphocytes [32,33]. But also it has been reported that autophagy participates in viral recognition and consequently the synthesis of IFN type I in plasmacytoid dendritic cells [51]. What is more, the impact of EBV on LC3B level was excluded in human gastric cancer cells [21].

LMP1 is an oncoprotein that modifies B cell physiology due to its ability to control autophagy. Moderate levels of LMP1 induce B cell proliferation, whereas high levels of the protein act cytostatically and contribute to suppression of protein biosynthesis. Autophagy activation depends on the level of LMP1: cells with a low level of LMP1 develop autophagosomes while cells with high levels of LMP1 have autophagolysosomes [31,32]. The inhibition of protein synthesis in cells with LMP1 expression is proceeded by eIF2α phosphorylation by protein kinase RNA-like endoplasmic reticulum kinase (PERK). Activation of PERK is a part of the unfolded protein response and is triggered by high levels of LMP1 [31,33]. Decrease of LMP1 through autophagic degradation induces B cell proliferation [31].

EBNA1 of EBV is an example of an endogenous antigen that is exceptionally presented through MHC class II molecules. Furthermore, the antigen processing is mediated by autophagy. Paludan et al. [43] showed that the inhibition of lysosome acidification was the reason for EBNA1 accumulation in autophagosomes and autophagy suppression led to decreased recognition of EBNA1 by specific clones of T $CD4^+$ lymphocytes. In contrast, two other latency nuclear proteins, EBNA2 and EBNA3C, do not use autophagy as a pathway for processing and presentation through MHC molecules [59].

Human herpesvirus type 8

Human herpesvirus type 8 (HHV-8) is a member of the γ-*herpesvirinae* subfamily. It evolved strategies to counteract both apoptosis and autophagy by expressing two proteins during the latency state: (i) viral homolog of Bcl-2 protein (vBcl-2) that binds beclin 1, thus inhibiting autophagy, and (ii) viral homolog of FLIP (vFLIP) that interacts with Atg3 [7]. The control of autophagy by HHV-8 leads to the disruption of oncogene-induced senescence (OIS). Atg3-binding domain of Atg3 plays the main role in OIS leading to inhibition of autophagy and senescence [36].

Autophagy is an important factor in HHV-8 reactivation from the latency state since autophagy inhibition limits virus reactivation. Autophagy is observed during lytic replication of HHV-8. The process is enhanced by replication and transcription activator (RTA), contributing to an increase in the level of LC3-II, the number of autophagosomes and autolysosome formation. Moreover, the inhibition of autophagy influences lytic gene expression and the replication of viral DNA [62].

Concluding Remarks

In summary, autophagy is a very important process responsible for host cell defense against viral infections in order to reduce virus replication and spread. Induction of autophagy mainly depends on cell type, stage of infection and type of infection (productive or latent) and it is associated with antiviral mechanisms induced by IFN.

Over the past 10 years research has provided valuable information on herpesvirus strategies in modulation of autophagy. Despite close relations in the *Herpesviridae* family, individual viruses have evolved their own mechanisms to manipulate the process. An implication of latency in autophagy induced by herpesviruses deserves special attention because latency is their main strategy of evading the host immune response. Autophagy plays a critical role in the reactivation of HSV-1 and HHV-8. The case of EBV shows that autophagy is also responsible for driving B cell proliferation during the latency state. Herpesviruses, like other viruses, use two strategies to manipulate autophagy: they escape from digestion in autophagolysosomes or they turn autophagy to their advantage. Playing the "autophagy game", the virus wins or it is digested!

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