



## Review

## Contribution of autophagy to antiviral immunity



Emma Rey-Jurado<sup>a</sup>, Claudia A. Riedel<sup>c</sup>, Pablo A. González<sup>a</sup>, Susan M. Bueno<sup>a</sup>, Alexis M. Kalergis<sup>a,b,d,\*</sup>

<sup>a</sup> Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>b</sup> Departamento de Inmunología Clínica y Reumatología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

<sup>c</sup> Millennium Institute on Immunology and Immunotherapy, Departamento de Ciencias Biológicas, Facultad de Ciencias Biológicas y Facultad de Medicina, Universidad Andrés Bello, Santiago, Chile

<sup>d</sup> INSERM U1064, Nantes, France

## ARTICLE INFO

## Article history:

Received 3 May 2015

Revised 20 July 2015

Accepted 29 July 2015

Available online 20 August 2015

Edited by Wilhelm Just

## Keywords:

Autophagy

Macroautophagy

Virus

Innate immunity

Adaptive immunity

## ABSTRACT

**Although identified in the 1960's, interest in autophagy has significantly increased in the past decade with notable research efforts oriented at understanding as to how this multi-protein complex operates and is regulated. Autophagy is commonly defined as a "self-eating" process evolved by eukaryotic cells to recycle senescent organelles and expired proteins, which is significantly increased during cellular stress responses. In addition, autophagy can also play important roles during human diseases, such as cancer, neurodegenerative and autoimmune disorders. Furthermore, novel findings suggest that autophagy contributes to the host defense against microbial infections. In this article, we review the role of macroautophagy in antiviral immune responses and discuss molecular mechanisms evolved by viral pathogens to evade this process. A role for autophagy as an effector mechanism used both, by innate and adaptive immunity is also discussed.**

© 2015 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

### 1. Introduction

Autophagy is a major cellular pathway involved in the degradation of decaying organelles and expired proteins inside eukaryotic cells [1,2]. To date, three major autophagy pathways have been described: macroautophagy, microautophagy and chaperone-mediated autophagy [3]. In this review we will focus on macroautophagy and refer to it as autophagy hereafter. Although autophagy was initially described as a process carried out for the maintenance of cellular homeostasis [1,2], accumulating studies have also provided data that associate alterations in autophagy components with important diseases, such as neurodegenerative disorders, cancer, chronic autoimmune-inflammatory syndromes and infectious diseases [4,5]. Because a role for autophagy in pathogen control has also been suggested by various studies, important research efforts are currently being invested on better characterizing the contribution of this process to antiviral immunity. Indeed, autophagy has been proposed to work as an intrinsic cell immune defense mechanism against viral infections by targeting intracellular pathogens,

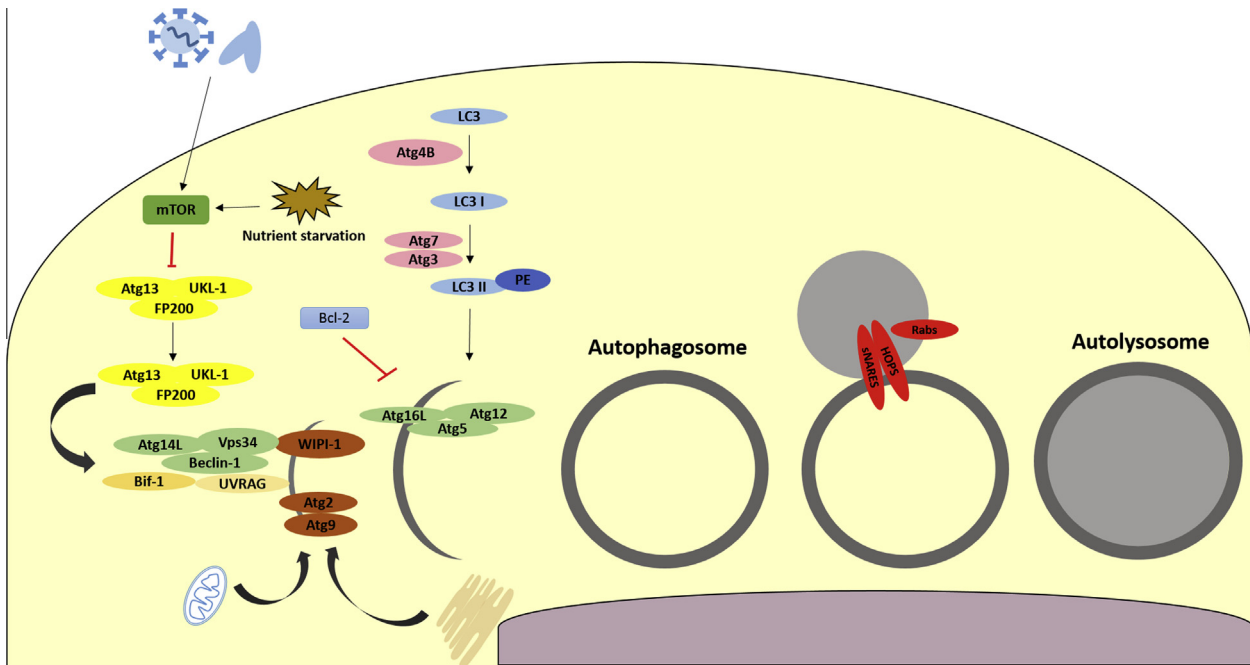
such as viruses, for degradation [6]. Paradoxically, certain viruses and other pathogens seem to rely on autophagy elements to replicate and persist inside the host. Thus, viruses have evolved molecular strategies to exploit and modulate the function of autophagy components to use them for their benefit [7–11]. Some of these findings are discussed below in this article.

### 2. The autophagy pathway

Autophagy is a process that requires the concerted assembly of multiple proteins into molecular complexes that carry out sequential steps with defined functions [2]. The identification of autophagy components was significantly advanced due to the use of yeast genetic screening assays. Indeed, multiple autophagy-related proteins (Atgs) have been identified using the yeast genetic screening assay with further mammalian counterparts being defined in other systems [12]. The major common physiological activator of autophagy is starvation and growth factor deprivation [2]. However, immune-inflammation signals can also lead to an increase in autophagy [13], as well as the expression of several proteins involved in this process (Fig. 1). mTOR, the mammalian target of rapamycin, has been shown to be involved in the negative modulation of autophagy [14]. It is thought that mTOR keeps both, Unc51-like kinase 1 (ULK1) and Atg13 in a hyperphosphorylated

\* Corresponding author at: Millennium Institute on Immunology and Immunotherapy, Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago-Chile, Av. Portugal 49, Santiago E-8330025, Chile.

E-mail address: [akalergis@bio.puc.cl](mailto:akalergis@bio.puc.cl) (A.M. Kalergis).



**Fig. 1.** The autophagy pathway. In response to stimuli that promote autophagy, such as nutrient starvation or pathogen infection, mammalian target of rapamycin (mTOR) dephosphorylates Unc51-like kinase 1 (ULK-1) and autophagy related protein 13 (Atg13), leading to the dissociation of mTOR from the ULK-1-Atg13-FAK family-interacting protein of 200kD (FIP200) complex that together with the vacuolar protein sorting 34 (Vps34)-Atg14L-Beclin-1 complex favor nucleation of the membrane that will become the autophagosome. Afterwards, autophagosome elongation and completion follows with the help of WD repeat domain phosphoinositide-interacting protein 1 (WIPI-1)-Atg2, Atg9, Vps34 and the ubiquitin-like conjugations of Atg12-Atg5-ATG16L and Atg4B-Atg3-Atg7 complexes. Further, light chain 3 (LC3) precursor is cleaved by Atg4B to produce the cytosolic form (LC3 I) and thereafter convert to membrane bound (LC3 II) – phosphatidylethanolamine (PE) conjugated through the activity of Atg7 and Atg3. The Atg12-Atg5-ATG16L complex determines the sites for LC3 lipidation. Once the autophagosome is formed, it is fused with the lysosome to become autolysosome through recruitment of the soluble N-ethylmaleimide-sensitive factor attachment (NSF) protein receptor (SNARE) proteins and its interaction with the homotypic fusion and vacuole protein sorting (HOPS) and vesicle trafficking by GTPases. At the end, the components are trapped inside the autophagolysosome and degraded.

state within the ULK1-Atg13-FAK family-interacting protein of 200 kD (FIP200) complex [15]. Stimuli that promote autophagy, such as nutrient starvation and pathogen infections, initiate mTOR-mediated dephosphorylation of ULK-1 and Atg13, leading to the dissociation of mTOR from the FIP200 complex [15]. Avibirnavirus and human cytomegalovirus (hCMV) have been described to induce autophagy in a mTOR-dependent manner [16,17]. mTOR dephosphorylation activates autophagy, initiating the nucleation of a cellular membrane (lipid bilayer) that will ultimately become the autophagosome. This enveloping membrane has been named as the “isolation membrane” or “phagophore” [18]. Importantly, it has been proposed that the endoplasmic reticulum, the Golgi apparatus and mitochondria may be the suppliers of lipid bilayers that form the phagophore [19–21]. To promote the formation of this membrane, the class III phosphoinositide-3-kinase (PIK3) complex, which is composed of vacuolar protein sorting 34 (Vps34), Atg14 and Beclin-1, is recruited to the phagophore [18]. Indeed, Beclin-1 is a scaffolding protein for the regulation of the PIK3 pathway [22]. Alternatively, the UV irradiation resistance-associated gene (UVRAG) may induce this PI3K complex [23] and promotes Atg9 trafficking [24]. Although Atg9 has been shown to be essential for starvation-induced autophagy [25], the specific function of this transmembrane protein remains somewhat unknown. During the first steps of phagophore generation, Vps34 is recognized by the beta-transducin repeat domain (WD) phosphoinositide-interacting protein 1 (WIPI-1), which cooperates with Atg2 and Atg9 to form of this structure [26]. These events trigger a downstream Atg conjugation system, which regulates autophagosome elongation [18].

Subsequently, autophagosome formation is catalyzed by two ubiquitin-like conjugation systems: the Atg12-Atg5-ATG16L and

Atg4B-Atg3-Atg7 complexes [27,28]. As part of this process, light chain 3 (LC3) precursor is cleaved by Atg4B to produce a cytosolic form (LC3 I) and later converted into a membrane-bound form (LC3 II), which is conjugated with phosphatidylethanolamine (PE) through the activity of Atg7 and Atg3 [29–31]. The Atg12-Atg5-ATG16L complex in turn acts as an E3-like enzyme and determine particular sites for LC3 lipidation [32]. Gamma-aminobutyric acid receptor-associated protein (GABARAP) and Golgi-associated ATPase enhancer of 16kDa (GATE-16) are homologs of LC3 and are involved in the late stages of autophagosome maturation [33]. P62 (also known as the sequestosome, SQSTM1) and the curvature-driving protein (Bif-1) are ubiquitination-binding proteins that contribute also to this maturation process [34]. Bif-1 also interacts with Beclin-1 through UVRAG as a positive regulator, aiding to autophagosome formation [35]. Importantly, conversion from LC3-I to LC3-II has been extensively used as a marker of autophagy [36–40]. Concomitantly, because p62 accumulates when autophagy is inhibited, this protein has also been used as an autophagic flux marker [41]. Once the autophagosome is formed, it fuse with the lysosome to form the autolysosome through the recruitment of the soluble N-ethylmaleimide-sensitive factor attachment (NSF) protein receptor (SNARE), protein syntaxin 17 (Stx17) and interact with the homotypic fusion and vacuole protein sorting (HOPS) complex [42,43]. HOPS is a class-C Vps complex which is composed by Vps11, Vps16, Vps18, Vps33, Vps39 and Vps41. The vesicle trafficking process that occurs during this fusion is regulated by GTPases such as Ras superfamily of monomeric G protein (Rab) 7, Rab8b and Rab24 [44]. At the end of the process, specific components, such as decaying organelles and expired proteins are trapped inside the autophagolysosome and degraded [1].

### 3. Autophagy and antiviral innate immunity

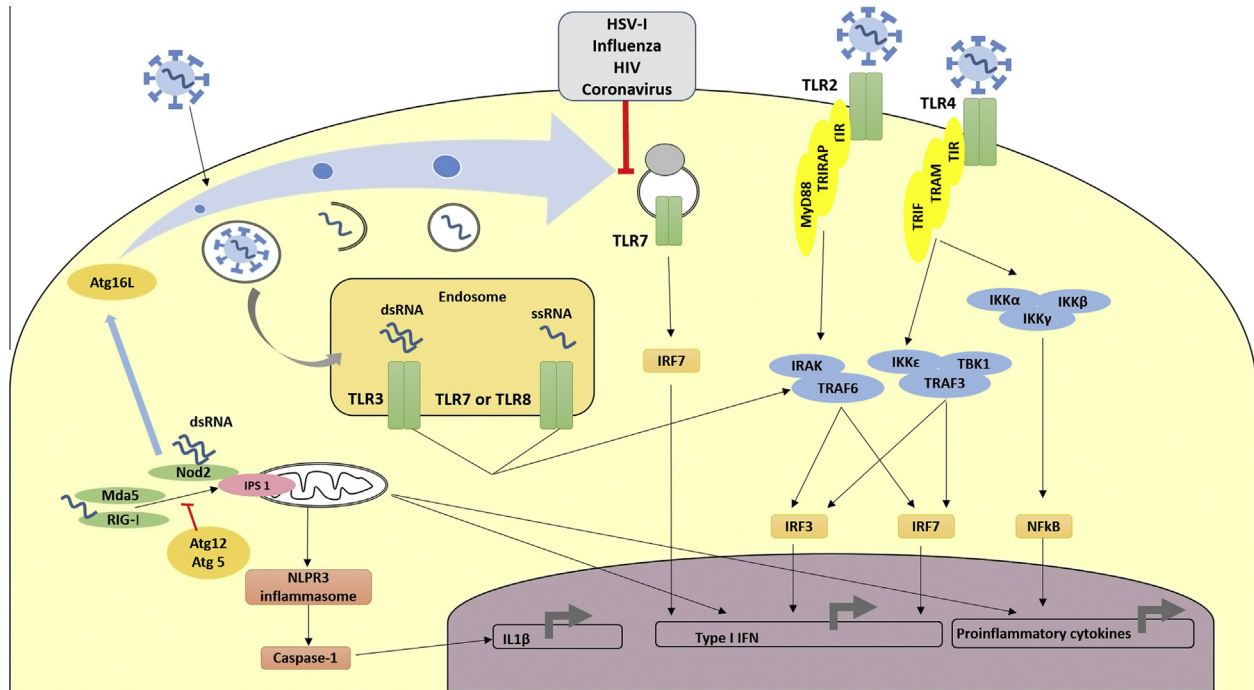
#### 3.1. Virus recognition

Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and Retinoic acid-inducible gene (RIG-1)-I-like receptors (RLRs) are the pattern recognition receptors (PRR) involved in detecting pathogen components, such as single- and double-stranded viral RNAs [45,46]. Several TLRs are known to activate innate responses after virus infection, including TLR2, TLR3, TLR4, TLR7 and TLR8 [45,46]. TLR2 and TLR4 are expressed on the cell surface whereas TLR3, TLR7 and TLR8 are localized within cytoplasmic compartments, such as endosomes [47–49]. TLR activation within endosomes requires endocytosis of pathogen associated molecular patterns (PAMPs), such as viral RNA molecules, damage associated molecular patterns (DAMPs), apoptotic cells or the induction of autophagy [50,51]. Several viruses can evade immediate recognition by TLRs after entering cells through endosome-associated compartments, thereby delaying the onset of an immune response [50]. TLR signaling results in the activation of nuclear factor kappa B (NF- $\kappa$ B), interferon regulatory transcription factor (IRF)-3 and IRF-7 and the assembly of inflammasome to activate caspase-1 and further process pro-interleukin (IL)-1 $\beta$  and IL-18 into their active forms [52]. Upon viral RNA detection, cytosolic PRRs, for instance Nod domain 2 (Nod2), RNA helicases such as melanoma differentiation-association gene-5 (MDA5), and RIG-I are activated [53]. RIG-I interacts with the adaptor interferon (IFN)- $\beta$  promoter stimulator (IPS-1; also known as MAVS, VISA or CARDIF) on the mitochondrial membrane, and consequently activates the transcription factors IRF-3 and NF- $\kappa$ B, leading to the production of

IFN- $\beta$  and other cytokines (Fig. 2). IPS-1 has been shown to contribute to the type-I IFN-mediated mechanisms of antiviral host defense [50]. This notion is supported by the observation that IPS-1-knockout mice show an increased recruitment of inflammatory neutrophils, monocytes and dendritic cells (DCs) in the lungs after human respiratory syncytial virus (hRSV) infection [50], evidencing the importance of this adaptor in antiviral responses for the clearance of hRSV. The papain-like protease 2 (PLP2) protein from coronaviruses promotes Beclin-1 association with STING [10]. Another protein that has been shown to regulate autophagy and the antiviral defense is Tripartite motif protein family (TRIM) [54], which blocks IRF-3/7 via serine/threonine protein kinase (TBK1) and NF- $\kappa$ B [55]. In addition, TRIMs associate with autophagy factors. Indeed, TRIM5 was found to recognize and target human immunodeficiency virus 1 (HIV-1) for destruction [56].

#### 3.2. Innate immunity components and autophagy-related proteins

Molecular components related to autophagy have been frequently described to contribute to the replication of certain viruses [57–59]. Indeed, RIG-1 has been described to be modulated by Atgs activated by viruses, such as vesicular stomatitis virus (VSV) [57]. For instance, embryonic fibroblasts from Atg5-deficient mouse were shown to be resistant to VSV replication, suggesting that autophagy components can contribute to viral replication in host cells. The constitutive overexpression of a mutant of RIG-1 induced the activation of NF- $\kappa$ B and IFN- $\beta$ , which was suppressed by the co-expression of the Atg5-Atg12 complex [57]. This suppression seemed to be dependent on infection with VSV and was reported to induce a conformational change in RIG-1 [57]. Nucleotide-binding domain and leucine-rich-repeats containing proteins



**Fig. 2.** Autophagy and antiviral innate immunity. Viruses may enter cells by endocytosis resulting in the delivery of viral RNA in the endosome. Once in the endosome, dsRNA can be recognized by Toll-like receptor 3 (TLR3) and ssRNA by TLR7 or TLR8, triggering interleukin 1 (IL-1) receptor associated kinase (IRAK) and tumor necrosis factor (TNF) receptor associated receptor 6 (TRAF6) followed by Type I interferon (IFN) production. Cytosolic RNA can also trigger autophagosome formation followed by fusion with lysosomes. Herpes simplex virus 1 (HSV-1), influenza virus, human immunodeficiency virus and viruses from *Coronaviridae* have been shown to inhibit autophagy by directly blocking Beclin-1. The phagosome-lysosome fusion induces activation of interferon regulatory factor 7 (IRF7) via TLR7. Autophagy can be triggered by the cytosolic presence of retinoic acid-inducible gene-1 (RIG-I), nucleotide-binding oligomerization domain (NOD) or melanoma differentiation-association gene 5 (MDA5). RIG-I interacts with the adaptor IFN promoter stimulator (IPS-1) on the mitochondrial membrane triggering activation of IRF3/7 and nuclear factor kappa B (NF- $\kappa$ B). This interaction can be blocked by Atg12-Atg5 complex. The phagosome-lysosome fusion triggers induction of NOD-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome, which triggers caspase-1-mediated IL-1 $\beta$  production. IRF3 and IRF7 are transcription factors of type-I IFN and NF- $\kappa$ B of proinflammatory cytokines.

(NLRX1), as well as the mitochondrial Tu translation elongation factor (TUFM) in turn have been shown to modulate autophagy via RIG-1 signaling and activation of IFN-I, as well as the association with the Atg5-Atg12 complex and ATG16L [60]. The induction of autophagy by NLRX1 was specifically shown in VSV-infected peritoneal macrophages from *nrlx1* knockout mice [60]. Similarly, it was shown that IFN- $\beta$ -induced phagosomes were directed to lysosomes for degradation in hepatitis B virus (HPV) infection in a non-structural serine protease 3(NS3)/4A transgenic mouse model [61].

Inhibition of Nod-dependent response by ATG16L has also been described in mouse embryonic fibroblasts and epithelial cells [62]. Nod1, Nod2 have been shown to colocalize with ATG16L and Nod2 to trigger autophagy by recruiting ATG16L at the site of pathogen entry [63]. TLR4 recognition via tumor necrosis factor (TNF) receptor-associated factor (TRAF) also induces the K63-linked ubiquitination of Beclin-1, triggering autophagy as well as blocking the inhibitor of autophagy anti-apoptotic B-cell lymphoma 2 (Bcl-2) [64]. Thus, the Atg5-Atg12 complex, ATG16L and Beclin-1 all autophagy-related proteins that have been reported to interact with components of the innate immune system.

### 3.3. Autophagy and inflammatory cytokines

Viruses have evolved several molecular strategies to evade antiviral host responses, such as modulation of inflammatory cytokines. Recently, it was shown that a lack of Atg5 in dendritic cells (DCs) infected with herpes simplex virus (HSV), resulted in reduced IFN- $\beta$  production [65]. Autophagy has also been shown to be important for the production of cytokines produced from innate immune cells. Indeed, blockade of autophagy by the 3-methyladenine (3-MA) inhibitor in DCs infected with hRSV has been shown to prevent the expression of interferons, chemokines and cytokines, such as IFN- $\beta$ , TNF- $\alpha$ , C-C motif ligands 5 (CCL5), IL-6, and IL-12p35, but not IL-1b [66]. Furthermore, knock-out of Beclin-1 resulted in increased lung pathology in hRSV-infected mice [67].

An association between autophagy and lung inflammation with other viruses, different than hRSV has also been described. Consistent with this notion, autophagy has been shown to be involved in avian influenza A virus (IAV)-induced lung inflammation [68]. Influenza A virus subtype H5N1 infection induces IL-1 $\beta$ , TNF- $\alpha$ , IL-6, IL-8, and CCL2, CCL5 and C-X-C motif ligand 2 (CXCL2) chemokines. Treatment with the autophagy inhibitor 3-MA or either Atg5 or Beclin-1 siRNA, significantly reduced the production of cytokines and chemokines as well as the nuclear translocation of NF- $\kappa$ B in human lung epithelial cells [68]. Interestingly, the administration of the autophagy inhibitor 3-MA to H5N1-infected mice resulted in an attenuation of lung inflammation, as well as the activation of NF- $\kappa$ B and p38 mitogen-activated protein kinases (MAPK) [68].

## 4. Autophagy and antiviral adaptive immunity

### 4.1. Autophagy in antigen presentation

In addition to playing a role during innate immune responses to virus infections, autophagy also contributes to the regulation of adaptive immune responses against these and other pathogens. For instance, autophagy contributes to antigen presentation to T cells by antigen presenting cells and is required for an effective initiation of the adaptive immune response [11,67,69]. Indeed, presentation of pathogen-derived peptides on major histocompatibility complex (MHC) class II molecules to CD4<sup>+</sup> T cells has been reported to be facilitated by autophagy [70] (Fig. 3). Antigen acquired by phagocytosis will be degraded into peptides by the

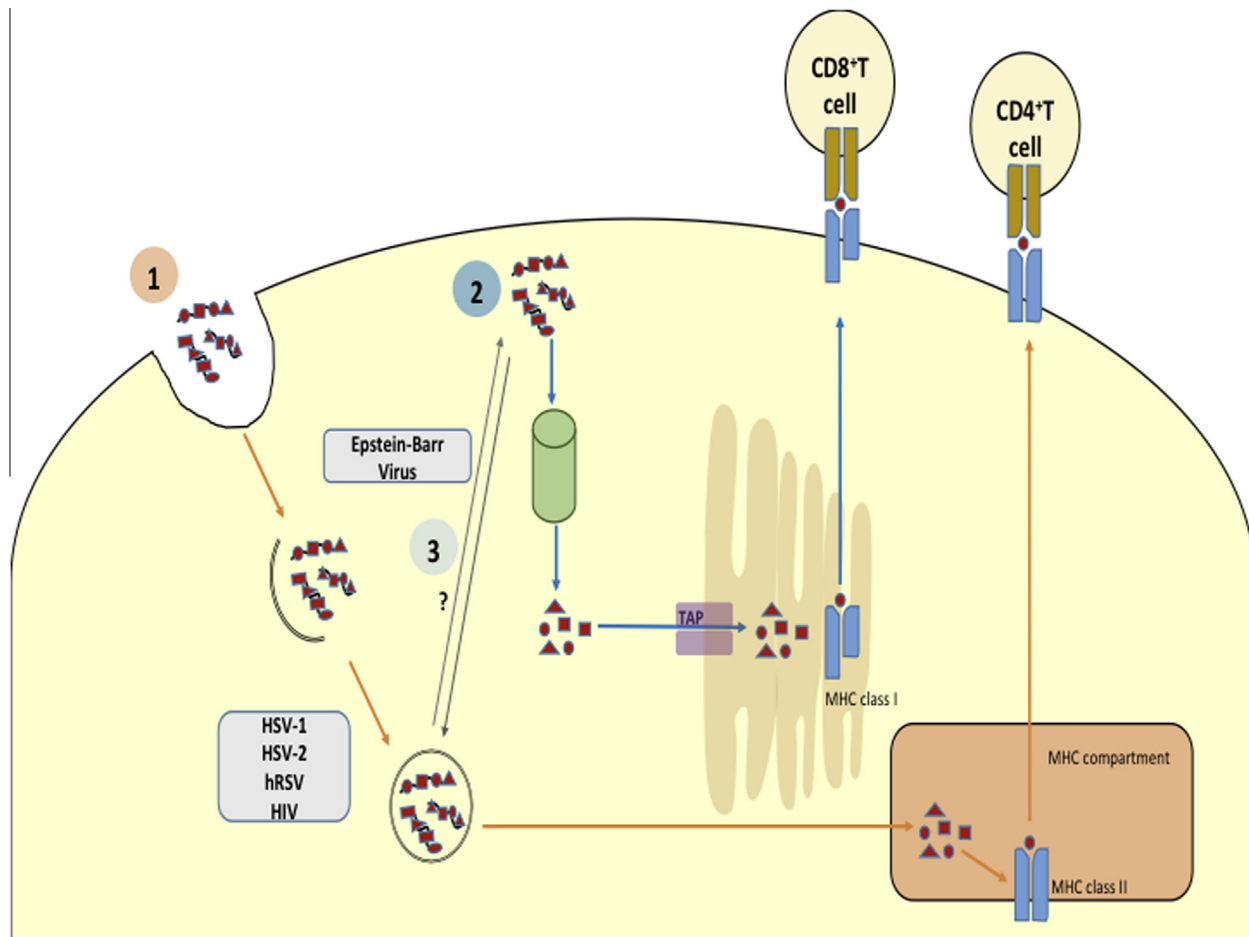
autophagosome and eventually loaded onto MHC molecules [71]. The peptide-MHC (pMHC) complexes generated are then transported to the cell surface and presented to CD4<sup>+</sup> T cells. In contrast to antigen presentation on MHC-II molecules, antigen presentation on MHC-I molecules can be mediated by two pathways in which only one involves autophagy. During conventional MHC-I antigen presentation, endogenous protein antigens are degraded into peptidic fragments by the proteasome and then transported into the endoplasmic reticulum, where they are translocated to the lumen through the transporter associated with antigen processing (TAP) [71]. Once in the endoplasmic reticulum, peptides are loaded onto MHC-I molecules and then transported to the surface for presentation to CD8<sup>+</sup> T cells [71]. It has been suggested that the alternative pathway for antigen presentation on MHC-I molecules, called cross-presentation, may require autophagy components [72–74]. Cross-presentation consists on the presentation of exogenous antigens on MHC I molecules to CD8<sup>+</sup> T cells [71]. This pathway has been reported practically in every type of immune response, such as tumor immunity [74], autoimmunity [75] and microbial infections, such as in macrophage infection by HSV-1 [72], as well as DCs stimulated with the yellow fever vaccine [73]. Furthermore, it has been shown that viral proteins undergo vacuolar processing in autophagosomes, which is followed by proteasome degradation and peptide loading on MHC-I molecules in the endoplasmic reticulum [72]. However, the molecular mechanisms involved in vacuolar processing of viral proteins remains unknown.

### 4.2. Evasion of autophagy mediated antigen presentation by viral pathogens

Because of the importance of antigen presentation on MHC molecules for virus clearance by the host immune response, pathogens have evolved molecular mechanisms to evade this process. Along these lines, it has been shown that human hepatitis virus C (HVC) reduces cathepsin S through IRF-1 and upstream stimulatory factor (UPS-1), thereby altering the degradation of the invariant chain and consequently antigen presentation [76]. Similarly, LC3-deficient DCs show a significant impairment in their capacity to present HIV-1-derived antigens to CD4<sup>+</sup> T cells [69]. Epstein-Barr virus nuclear antigen 1 (EBNA-1) has been shown to inhibit autophagy, thereby decreasing recognition by EBNA-1-specific CD4<sup>+</sup> T cell clones [77]. An association between autophagy and adaptive immunity is suggested by observation that Atg5-deficient DCs fail at presenting antigens on MHC class II molecules, as well as at priming and promoting the proliferation of T cells in vivo after HSV infection [65]. However, abrogation of Atg5 in DCs had no effect on antigen cross-presentation on MHC class I molecules, suggesting that Atg5 is not essential for this pathway [65]. Interestingly, phagosomes coated with LC3 protein maintain antigens for prolonged periods of time, favoring antigen presentation on MHC class II molecules [78]. Recently, a Beclin-1<sup>+/-</sup> mouse showed that loss of autophagy function could lead to decreased MHC-II expression after hRSV infection and failure to produce IFN- $\gamma$  and IL-17, thereby impairing both DC maturation, as well as the onset of an effective antiviral adaptive immune response against this virus [67]. Furthermore, the expression of granzyme B in CD8<sup>+</sup> T cells has been shown to be decreased in Beclin-1<sup>+/-</sup> mice [67].

### 4.3. Therapeutic strategies to improve antigen presentation

Due to the fact that weak CD4<sup>+</sup> T cell-mediated responses have been observed in more than one HIV vaccine clinical trial [79,80], Jin and coworkers [81] designed a recombinant simian immunodeficiency virus expressing Gag fused to LC3b. Such a construction caused that Gag localizes to the autophagosome and lysosomes,



**Fig. 3.** Autophagy and antiviral adaptive immunity. (1) Major histocompatibility complex (MHC) II antigen presentation. Antigen is captured by phagocytosis, degraded by the autophagosome and then loaded onto MHC molecules in the late endosomal MHC compartment and eventually peptide-MHC complexes trafficked to be presented to CD4<sup>+</sup> T cells. (2) Conventional MHC I antigen presentation. Endogenous antigen is degraded into peptide fragments in proteasomes, transported to the endoplasmic reticulum via antigen processing (TAP), loaded into MHC class I molecule and then transported to the surface to be presented to CD8<sup>+</sup> T cells. (3) Antigen cross presentation on MHC-I. Autophagy is suggested to be involved in exogenous antigen processing but its mechanism is unknown. In cells infected with respiratory syncytial virus (RSV), herpes simplex virus (HSV)-1, HSV-2 and human immunodeficiency virus, autophagy have been shown to be important for antigen presentation on MHC class II molecules. Vacuolar protein processing has been reported for Epstein-Barr virus nuclear antigen 1 (EBNA1) and HSV-1.

which promotes presentation in MHC-II molecules. This strategy was shown to significantly improve the immunogenicity of HIV antigens and the induction of an adaptive immune response upon infection [81]. Along these lines, other novel strategies have been undertaken to enhance antigen presentation and immune response induction. For instance, a peptide derived from HSV that induces autophagosomal degradation of antigens was implemented to protect from tumor challenge [82]. Such a peptide could be attached to antigenic viral proteins for vaccine design, as a manner to enhance immunogenicity. However, as noted above not all autophagy proteins are essential for antigen presentation, as Atg7-deficient thymocytes and DCs showed no alterations in any antigen presentation pathway for exogenous or endogenous antigens [83]. Taken together, these studies provide an important link between antigen presentation of virus-derived proteins and the autophagosome function in antigen presenting cells. Ultimately, modulating autophagy during infection could improve the overall immune response to pathogens.

## 5. Autophagy exploitation by viruses to evade host immunity

Autophagy is critical for limiting infection by pathogens [84,85]. However, some microorganisms, such as coronavirus, HIV and cytomegalovirus have developed mechanisms to evade this

microbicide system [13,86,87]. Numerous evasion mechanisms evolved by these microbes have been associated with the inhibition of autophagosome-lysosome fusion, escape from phagocytic vacuoles in the cytoplasm and resistance to lysosome degradation components. Indeed for antiviral responses, an important role for autophagy has been shown in several virus infections (Table 1).

### 5.1. Autophagosome-lysosome fusion modulation by viruses

HSV-1, influenza virus, HIV, HCV and coronavirus are all able to induce autophagy. However, at the same time these viruses have been shown to inhibit the last step of autophagosome-lysosome fusion, which promotes viral replication and reduces cell apoptosis or death [7–11]. HSV-1 induces autophagy in such a way that it antagonizes autophagosome maturation by the interaction of HSV  $\gamma$ 34.5 protein with Beclin-1 [7]. It has been suggested that an inefficient fusion between autophagosomes and lysosomes in HSV-infected cells is induced by oxidative stress and has been associated with neurodegeneration [88]. In addition,  $\gamma$ 34.5 has been shown to be important for HSV virulence in the brain of adult and neonatal animals and dependent on type-I IFN only in adults [89]. HCV has also been reported to induce autophagy and endoplasmic reticulum stress, leading to an accumulation of autophagosomes that require the activation of the unfolded protein response

**Table 1**  
Interactions of various viruses with the autophagy pathway.

Virus	Interactions viruses with autophagy machinery	Viral proteins involved	Autophagy related proteins	References
Herpes Simplex Virus 1 (HSV-1)	HSV-1 triggers autophagy but inhibit autophagosome-lysosome fusion, autophagosomes are accumulated	$\gamma$ 34.5 protein	Beclin-1, Atg5	[7,65]
Influenza virus	Influenza induces autophagy but inhibit autophagosome-lysosome fusion, autophagosomes are accumulated	Matrix protein 2 (MP2)	LC3, Atg5	[8,39]
Human immunodeficiency virus (HIV)	HIV triggers autophagy but inhibit autophagosome-lysosome fusion, autophagosomes are accumulated in CD4 <sup>+</sup> T cells while HIV interferes in autophagosome-mediated degradation	Nef protein	Beclin-1	[9,101,102]
Coronaviridae family	Viruses from <i>Coronaviridae</i> family trigger autophagy but inhibit autophagosome-lysosome fusion, autophagosomes are accumulated. Autophagy is critical for virus replication	Membrane-associated papain-like protease PLP2 (PLP2-TM)	Beclin-1, LC3	[10,103]
Vesicular stomatitis virus (VSV)	Autophagy in plasmacytoid dendritic cells are required for VSV recognition and autophagy is required for virus replication	Unknown	Atg5	[65]
Sindbis virus	Sindbis virus induces autophagy and overexpression autophagy proteins protects from infection	Capsid protein	p62, Atg7, Atg5	[58,59]
Human respiratory syncytial virus (hRSV)	Beclin $-/+$ mice show higher levels Th2, lower IFN and IL17, decrease expression granzyme B in CD8 <sup>+</sup> T cells and increased viral replication	Unknown	Beclin-1	[50,67]
Human hepatitis virus C (HVC)	HVC induces autophagy and ER stress, and autophagosomes are accumulated. HVC core and NS5A proteins reduce expression cathepsin S which is involved in the degradation of the autophagy cargo	HVC core and NS5A	UPR	[11,76,90,91]
Human hepatitis virus B (HVB)	HVB triggers autophagy favoring replication of DNA HVB, the production of viral envelopment and release of naked capsids	Unknown	Unknown	[115–117]
Cytomegalovirus	Cytomegalovirus induces autophagy in early stages but inhibits it in late stages	TRS protein	Beclin-1	[36–38]
Varizella-zoster (VZV)	VZV triggers complete autophagy which facilitate VZV glycoproteins biosynthesis and processing	Unknown	Unknown	[92]
Measles (MeV)	MeV increases autophagy flux	Unknown	LC3	[93]
Adenovirus	Adenovirus is more efficiently replicated in cells with induced autophagy	Unknown	Unknown	[97]
Simian immunodeficiency virus type 1 (SIV)	SIV inhibits neuronal autophagy	Unknown	Unknown	[109]

(UPR) [90,91]. Interestingly, serum from HCV-infected patients has been reported to block autophagy in differentiating monocytes suggesting the participation of soluble components in sera with this particular capacity [11]. In contrast, varicella-zoster virus (VZV) promotes a complete autophagy process, which has been evidenced by the degradation of some of its long-lived proteins [92]. Autophagy in this latter case seems to be used by the virus to facilitate VZV glycoprotein biosynthesis and processing [92]. Similar to VZV, measles virus (MeV) increases autophagy but prevents the degradation of viral proteins [93]. Interestingly, MeV-induced autophagy is required for the replication of the virus. In addition, enteroviruses, such as coxsackievirus and rotavirus have also been reported to interact with autophagy elements to evade immunity by increasing respectively protein- and calcium-activated signaling in infected cells [94–96]. Another example of virus exploitation of the autophagy machinery is provided by adenoviruses [97]. These pathogens replicate more efficiently in the airways when epithelial cells undergo nutrient deprivation-induced autophagy [97]. The mechanisms used by other viruses that induce autophagy, such as dengue and parvovirus B19 remain to be undetermined [98,99].

### 5.2. Viral proteins interacting with autophagy-related proteins

An extended two-hybrid array, with 35 associated autophagy proteins and 80 different viral proteins from 5 different families of RNA viruses (*Paramyxoviridae*, *Flaviviridae*, *Orthomyxoviridae*, *Togaviridae* and *Retroviridae*), showed that these viruses targeted several autophagy proteins [100]. Among these proteins, immunity-associated GTPase family M (IRGM) was a common target for all the viruses studied [100]. Importantly, autophagosome accumulation due to the inhibition of autophagosome-lysosome fusion in MeV, HCV and HIV was shown to be IRGM-dependent by confocal microscopy [100]. In addition, it is likely that IRGM can modulate initial autophagy steps because this molecule

targeted Atg5, Atg10 and LC3, all components of the initial phase of autophagy [100].

Viral  $\gamma$ 34.5 protein from HSV-1 [7], Nef protein from HIV [9,101,102], non-structural protein 3 from coronavirus [10] and matrix protein 2 (M2) protein from influenza viruses [8] have been shown to interact with Beclin-1, thereby modulating the function of autophagy. PLP2 from coronaviruses induces an incomplete autophagy process by interacting with LC3 and Beclin-1 [10]. In addition, the deletion of Beclin-1 has been shown to activate IFN- $\beta$  promoter and increase viral replication in infected coronavirus cells [10]. Alternatively, non-structural protein 6 (Nsp6) from viruses belonging to the *Coronaviridae* family induces LC3-positive vesicles.

### 5.3. Autophagy in different cell types infected with viruses

On the other hand, the mouse hepatitis coronavirus (hCMV), induces autophagy in murine embryonic stem cells, which is critical for the replication of the virus [103]. In contrast, Atg5 was not required for mHPV replication in both primary bone marrow macrophages and primary low-passage mouse embryonic fibroblasts [104]. Therefore, it is likely that this virus may be interacting differently with the autophagy components depending on the cell type encountered. Similar to mHPV, infectious bronchitis virus induces the formation of autophagosomes when inhibited through the silencing of Atg5 [105]. However, this phenomenon did not alter the replication of the virus, which suggests that this protein is not essential for autophagosome induction by this virus [105].

It has been shown that autophagy can be inhibited in epithelial cells during influenza infection [8]. Autophagosome fusion with acidified proteolytic lysosomes in influenza-infected human epithelial cell lines is significantly inhibited [8], thereby influenza may exploit autophagy as a mechanism to avoid degradation by lysosomes. The M2 influenza protein, which plays important roles in several steps of influenza virus infection, which works as a

proton channel and has been shown to be key for the modulation of autophagy in infected cells [8,39]. While M2-transfected cells showed autophagosome accumulation, cells transfected with other influenza A virus proteins did not show alterations of the autophagy pathways [39]. In addition, M2 has been shown to interact with LC3 and promotes its LC3 redistribution to the plasma membrane [39]. Along these lines, different compounds with anti-influenza activity have been shown to either block or modulate autophagy, by altering host-virus protein interactions [40,106]. Indeed, the replication of influenza within the autophagosome may be reduced by blocking the induction of autophagy. Baicalin, a flavonoid compound with antiviral properties, shows inhibitory activity over autophagy by inhibiting mTOR signaling and Atg5-Atg12 during influenza infection [106]. Interestingly, the same group showed that simvastatins can also promote the accumulation of LC3-II levels that could favor autophagosome building up [40].

HIV has also received significant attention because of its capacity to modulate autophagy in a cell type-dependent manner [80]. HIV modulates autophagy in adipocytes, CD4<sup>+</sup> T cells, macrophages and DCs, by different mechanisms depending on the cell type [69,107,108]. HIV has been shown to trigger autophagy in CD4<sup>+</sup> T cells, leading to higher LC3-II, Atg5 and Beclin-1 [9,108]. In contrast, HIV-1 infection in DCs leads to the loss of LC3-II accumulation, which interferes with autophagosome-mediated degradation of the virus [69].

Autophagy in neurons is also of particular interest. Overexpression of Beclin-1 in neurons from neonatal mice showed protection against a lethal Sindbis virus infection [58]. Atg5 and Atg7 have also been shown to trigger autophagy in Sindbis virus-infected neurons and to prevent virus-induced cell death *in vivo* and *in vitro* when performing studies using knockout techniques [59]. Interestingly, Atg5 disruption in neurons did not vary viral titers in the central nervous system, suggesting that Atg5 does not play an essential role in the clearance of Sindbis virus, at least in these cells [59]. On the other hand, simian immunodeficiency virus type I-infected microglia inhibits neuronal autophagy, thereby decreasing neuron survival [109]. An association between autophagy and apoptosis and cell death derives from the interaction of Beclin-1 with the major anti-apoptotic B-cell lymphoma (Bcl) family protein [110]. These observations suggest that autophagy has been shown to be critical for blocking cell death in virus-infected neurons. Because autophagy can be considered as a process opposite to cell death or apoptosis in cells, the promotion of this process may provide an advantageous environment for viruses to replicate and spread onto neighboring cells.

A role for autophagy components in immune cells, such as invariant natural killer T (NKT) cells has also been evidenced. Indeed, deletion of Atg7 has been reported to impair NKT cell development in a T cell-intrinsic manner [83]. These findings suggest that autophagy may play a role in the development and function of these cells during antiviral responses. Similar to DCs, different populations of NKT cells have been shown to contribute to the immune response against HSV-1 [111], HSV-2 [112], encephalomyocarditis virus [113] and hRSV [114]. Thus, it is likely that autophagy could modulate the function of NKT cells during anti-viral immune responses against viral infections. Based on reports that support a role for autophagy during the replication and life cycle of certain viruses, the use of specific autophagy-inducing agents has been proposed as a strategy for treating or preventing several infectious diseases [102].

#### 5.4. Autophagy and virus components needed for replication

A role for autophagy in viral infection with HPV has also acquired particular attention. HPV induces autophagy and exploits

the components of this cellular process for genome replication and viral envelope formation. However, the mechanisms involved in the interactions between autophagy components and virus determinants remains controversial [115,116]. For instance, HPV utilizes some of the host's autophagy machinery molecules to release its naked viral capsids, requiring for example Rab33B, a GTPase that participates in autophagosome formation via the Atg5-Atg12-ATG16L complex [117]. Nevertheless, the suppression of conventional autophagy processes using chemical inhibitors, such as 3-MA did not produce any effect on capsid release, indicating that the virus does not require an intact autophagosome to release capsids [117].

## 6. Concluding remarks

Autophagy components, which include autophagy-related proteins and adaptors have been demonstrated to interact with numerous components belonging to innate and adaptive immune responses. However, whether autophagy is positive or negative for viral infection and pathology remains to be elucidated. Indeed, in the context of an infection, autophagy may act as a double edge sword. While autophagy may help to eliminate pathogens, it can also could work as a reservoir for pathogen growth and escape from apoptosis or cell death. However, consistent with an important role for autophagy during viral infections, viruses have evolved different mechanisms to counteract this cellular process. Because of its involvement during viral infections, autophagy is actively being explored as a cellular target for the development of vaccines and therapies for preventing virus spread or dissemination [81,82,118]. Undoubtedly, further research is required to understand the interactions occurring between components of autophagy and the immune system. This knowledge will contribute explaining as to how viruses counteract this defensive host system for their own benefit and open new possibilities to improve antiviral therapeutics and vaccines.

## Acknowledgments

This work was supported by the Millennium Institute on Immunology and Immunotherapy from Chile (P09/016-F for C.R., P.G., S.B. and A.M.K.), FONDECYT Grants number: 1150862, 1070352, 1050979, 1040349, 1100926, 1110397, 1131012, 1140010, 1140011, 3140455. Biomedical Research Consortium (BMRC 13CTI-21526 for A.M.K. and S.B.), FONDEF Grant D1111080.

## References

- [1] Levine, B. and Klionsky, D. (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. *Dev. Cell* 6 (4), 463–477.
- [2] Codogno, P., Mehrpour, M. and Proikas-Cezanne, T. (2011) Canonical and non-canonical autophagy: variations on a common theme of self-eating? *Nat. Rev. Mol. Cell Biol.* 13 (1), 7–12.
- [3] Saitoh, T. and Akira, S. (2010) Regulation of innate immune responses by autophagy-related proteins. *J. Cell Biol.* 189 (6), 925–935.
- [4] Komatsu, M., Waguri, S., Chiba, T., Murata, S., Iwata, J., Tanida, I., Ueno, T., Koike, M., Uchiyama, Y., Kominami, E. and Tanaka, K. (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* 441 (7095), 880–884.
- [5] Chakradeo, S., Sharma, K., Alhaddad, A., Bakhshwin, D., Le, N., Harada, H., Nakajima, W., Yeudall, A., Torti, S., Torti, F. and Gewirtz, D. (2015) Yet another function of p53: the switch that determines whether radiation-induced autophagy will be cytoprotective or nonprotective. Implications for autophagy inhibition as a therapeutic strategy. *Mol. Pharmacol.* 114.095273.
- [6] Chiramel, A., Brady, N.R. and Bartenschlager, R. (2013) Divergent roles of autophagy in virus infection. *Cells* 2 (1), 83–104.
- [7] Gobeil, P. and Leib, D. (2012) Herpes simplex virus  $\gamma$ 34.5 interferes with autophagosome maturation and antigen presentation in dendritic cells. *in: MBio* 3 (5), e00267-12.
- [8] Gannage, M., Schmid, D., Albrecht, R., Dengjel, J., Torossi, T., Ramer, P.C., Lee, M., Strowig, T., Arrey, F., Conenello, G., Pypaert, M., Andersen, J., Garda-Sastre, A. and Munz, C. (2009) Matrix protein 2 of influenza A virus blocks autophagosome fusion with lysosomes. *Cell Host Microbe* 6 (4), 367–380.

- [9] Zhou, D. and Spector, S.A. (2008) Human immunodeficiency virus type-1 infection inhibits autophagy. *AIDS* 22 (6), 22.
- [10] Chen, X., Wang, K., Xing, Y., Tu, J., Yang, X., Zhao, Q., Li, K. and Chen, Z. (2014) Coronavirus membrane-associated papain-like proteases induce autophagy through interacting with Beclin1 to negatively regulate antiviral innate immunity. *Protein Cell* 5 (12), 912–927.
- [11] Granato, M., Laccioni, V., Peddis, M., Renzo, L.D., Valia, S., Rivanera, D., Antonelli, G., Frati, L., Faggioni, A. and Cirone, M. (2014) Hepatitis C virus present in the sera of infected patients interferes with the autophagic process of monocytes impairing their in-vitro differentiation into dendritic cells. *Biochim. Biophys. Acta* 1843 (7), 1348–1355.
- [12] Yang, Z. and Klionsky, D.J. (2010) Mammalian autophagy: core molecular machinery and signaling regulation. *Curr. Opin. Cell Biol.* 22 (2), 124–131.
- [13] Deretic, V. (2012) Autophagy as an innate immunity paradigm: expanding the scope and repertoire of pattern recognition receptors. *Curr. Opin. Immunol.* 24 (1), 21–31.
- [14] Laplante, M. and Sabatini, D.M. (2012) mTOR signaling in growth control and disease. *Cell* 149 (2), 274–293.
- [15] Hosokawa, N., Hara, T., Kaizuka, T., Kishi, C., Takamura, A., Miura, Y., Iemura, S., Natsume, T., Takehana, K., Yamada, N., Guan, J.L., Oshiro, N. and Mizushima, N. (2009) Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol. Biol. Cell* 20 (7), 20.
- [16] Hu, B., Zhang, Y., Jia, L., Wu, H., Fan, C., Sun, Y., Ye, C., Liao, M. and Zhou, J. (2015) Binding of the pathogen receptor HSP90AA1 to avibirnavirus VP2 induces autophagy by inactivating the AKT-mTOR pathway. *Autophagy*.
- [17] Zhao, J., Li, Z., Wang, M., Zhang, Z., Ma, H., Chang, J., Gao, D. and Wang, S. (2013) Manipulation of autophagy by HCMV infection is involved in mTOR and influences the replication of virus. *Acta Biochim. Biophys. Sin. (Shanghai)* 45 (11), 979–981.
- [18] Ohsumi, Y. and Mizushima, N. (2004) Two ubiquitin-like conjugation systems essential for autophagy. *Semin. Cell Dev. Biol.* 15, 231–236.
- [19] Hayashi-Nishino, M., Fujita, N., Noda, T., Yamaguchi, A., Yoshimori, T. and Yamamoto, A. (2009) A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. *Nat. Cell Biol.* 11, 1433–1437.
- [20] Hailey, D.W., Rambold, A.S., Satpute-Krishnan, P., Mitra, K., Sougrat, R., Kim, P. K. and Lippincott-Schwartz, J. (2010) Mitochondria supply membranes for autophagosome biogenesis during starvation. *Cell* 141, 656–667.
- [21] Geng, J., Nair, U., Yasumura-Yorimitsu, K. and Klionsky, D.J. (2010) Post-Golgi Sec proteins are required for autophagy in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 21, 2257–2269.
- [22] McKnight, N.C. and Zhenyu, Y. (2013) Beclin 1, an essential component and master regulator of PI3K-III in health and disease. *Curr. Pathobiol. Rep.* 1 (4), 231–238.
- [23] Liang, C., Feng, P., Ku, B., Dotan, I., Canaani, D., Oh, B.H. and Jung, J.U. (2006) Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat. Cell Biol.* 8 (7), 688–699.
- [24] He, S., Ni, D., Ma, B., Lee, J.H., Zhang, T., Ghazalli, I., Pirooz, S.D., Zhao, Z., Bharatham, N., Li, B., Oh, S., Lee, L.H., Takahashi, Y., Wang, H.G., Minassian, A., Feng, P., Deretic, V., Pepperkok, R., Tagaya, M., Yoon, H.S. and Liang, C. (2013) PtdIns(3)P-bound UVRAG coordinates Golgi-ER retrograde and Atg9 transport by differential interactions with the ER tether and the beclin 1 complex. *Nat. Cell Biol.* 15 (10), 1206–1219.
- [25] Jin, M. and Klionsky, D.J. (2014) Transcriptional regulation of ATG9 by the Pho23-Rpd3 complex modulates the frequency of autophagosome formation. *Autophagy* 10 (9), 1681–1682.
- [26] Proikas-Cezanne, T., Takacs, Z., Donnes, P. and Kohlbacher, O. (2015) WIPI proteins: essential PtdIns3P effectors at the nascent autophagosome. *J. Cell Sci.* 128 (2), 207–217.
- [27] Hanada, T., Noda, N.N., Satomi, Y., Ichimura, Y., Fujioka, Y., Takao, T., Inagaki, F. and Ohsumi, Y. (2007) The Atg12-Atg5 conjugate has a novel E3-like activity for protein lipidation in autophagy. *J. Biol. Chem.* 282, 37298–37302.
- [28] Walczak, M. and Martens, S. (2013) Dissecting the role of the Atg12-Atg5-Atg16 complex during autophagosome formation. *Autophagy* 9 (3), 424–425.
- [29] Kabeya, Y., Mizushima, N., Yamamoto, A., Oshitani-Okamoto, S., Ohsumi, Y. and Yoshimori, T. (2004) LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J. Cell Sci.* 117, 2805–2812.
- [30] Martinez-Lopez, N., Athonvarangkul, D., Mishall, P., Sahu, S. and Singh, R. (2013) Autophagy proteins regulate ERK phosphorylation. *Nat. Commun.* 4, 2799.
- [31] Ichimura, Y., Kirisako, T., Takao, T., Satomi, Y., Shimonishi, Y., Ishihara, N., Mizushima, N., Tanida, I., Komai, E., Ohsumi, M., Noda, T. and Ohsumi, Y. (2000) A ubiquitin-like system mediates protein lipidation. *Nature* 408, 488–492.
- [32] Fujita, N., Itoh, T., Omori, H., Fukuda, M., Noda, T. and Yoshimori, T. (2008) The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. *Mol. Biol. Cell* 19 (5), 2092–2100.
- [33] Shpilka, T., Weidberg, H., Pietrokovski, S. and Elazar, Z. (2011) Atg8: an autophagy-related ubiquitin-like protein family. *Genome Biol.* 12, 226.
- [34] Bjørkøy, G., Lamark, T., Brech, A., Outezn, H., Perander, M., Overvatn, A., Stenmark, H. and Johansen, T. (2005) P62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. *J. Cell Biol.* 171 (4), 603–614.
- [35] Takahashi, Y., Coppola, D., Matsushita, N., Cialing, H.D., Sun, M., Sato, Y., Liang, C., Jung, J.U., Cheng, J.Q., Mule, J.J., Pledger, W.J. and Wang, H.G. (2007) Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis. *Nat. Cell Biol.* 9 (10), 1142–1151.
- [36] Chaumontel, M., Lussignol, M., Mouna, L., Cavignac, Y., Fahie, K., Cotte-Laffitte, J., Geballe, A., Brune, W., Beau, I., Codogno, P. and Esclatine, A. (2012) The human cytomegalovirus protein TRS1 inhibits autophagy via its interaction with Beclin 1. *J. Virol.* 86 (5), 2571–2584.
- [37] McFarlane, S., Aitken, J., Sutherland, J.S., Nicholl, M.J., Preston, V.G. and Preston, C.M. (2011) Early induction of autophagy in human fibroblasts after infection with human cytomegalovirus or herpes simplex virus 1. *J. Virol.* 85 (9), 4212–4221.
- [38] Zhao, J., Li, Z., Wang, M., Zhang, Z., Ma, H., Chang, J., Gao, D. and Wang, S. (2013) Manipulation of autophagy by HCMV infection is involved in mTOR and influences the replication of virus. *Acta Biochim. Biophys. Sin.* 45 (11), 979–981.
- [39] Beale, R., Wise, H., Stuart, A., Ravenhill, B.J., Digard, P. and Randow, F. (2014) A LC3-interacting motif in the influenza A virus M2 protein is required to subvert autophagy and maintain virion stability. *Cell Host Microbe* 15 (2), 239–247.
- [40] Mehrbod, P., Hair-Bejo, M., Ibrahim, T.A.T., Omar, A.R., Zowalaty, M.E., Ajdari, Z. and Ideris, A. (2014) Simvastatin modulates cellular components in influenza A virus-infected cells. *Int. J. Mol. Med.* 34 (1), 61–73.
- [41] Lee, H.S., Daniels, B.H., Salas, E., Bollen, A.W., Debnath, J. and Margeta, M. (2012) Clinical utility of LC3 and p62 immunohistochemistry in diagnosis of drug-induced autophagic vacuolar myopathies: a case-control study. *PLoS One* 7 (4), e36221.
- [42] Hegedus, K., Takats, S., Kovacs, A.L. and Juhasz, G. (2013) Evolutionarily conserved role and physiological relevance of a STX17/Syx17 (syntaxin 17)-containing SNARE complex in autophagosome fusion with endosomes and lysosomes. *Autophagy* 9 (10), 1642–1646.
- [43] Jiang, P., Nishimura, T., Sakamaki, Y., Itakura, E., Hattai, T., Natsume, T. and Mizushima, N. (2014) The HOPS complex mediates autophagosome-lysosome fusion through interaction with syntaxin 17. *Mol. Biol. Cell* 25 (8), 1327–1337.
- [44] Ao, X., Zou, L. and Wu, Y. (2014) Regulation of autophagy by the Rab GTPase network. *Cell Death Differ.* 21 (3), 348–358.
- [45] Liu, H., Cao, M., Wang, Y., Li, L., Zhu, L., Xie, G. and Li, Y. (2014) Endoplasmic reticulum stress is involved in the connection between inflammation and autophagy in type 2 diabetes. *Gen. Comp. Endocrinol.* 210, 124–129.
- [46] Zeng, R., Cui, Y., Hai, Y. and Liu, Y. (2011) Pattern recognition receptors for respiratory syncytial virus infection and design of vaccines. *Virus Res.* 167, 138–145.
- [47] Heil, F., Ahmad-Nejad, P., Hemmi, H., Hochrein, H., Ampenberger, F., Gellert, T., Dietrich, H., Lipford, G., Takeda, K., Akira, S., Wagner, H. and Bauer, S. (2003) The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. *Eur. J. Immunol.* 33 (11), 2987–2997.
- [48] Matsumoto, M., Funami, K., Tanabe, M., Oshiumi, H., Shingai, M., Seto, Y., Yamamoto, A. and Seya, T. (2003) Subcellular localization of Toll-like receptor 3 in human dendritic cells. *J. Immunol.* 171 (6), 3154–3162.
- [49] Brandt, K.J., Fickentscher, C., Kruihof, E.K. and Moerlose, P.D. (2013) TLR2 ligands induce NF- $\kappa$ B activation from endosomal compartments of human monocytes. *PLoS One* 8 (12), e80743.
- [50] Demoor, T., Petersen, B.C., Morris, S., Mukherjee, S., Ptaschinski, C., Nagata, D. A., Kawai, T., Ito, T., Akira, S., Kunkel, S.L., Schaller, M.A. and Lukacs, N.W. (2012) IPS-1 signaling has a nonredundant role in mediating antiviral responses and the clearance of respiratory syncytial virus. *J. Immunol.* 189 (12), 5942–5953.
- [51] Sanjuan, M.A., Dillon, C.P., Tait, S.W., Moshiah, S., Dorsey, F., Connell, S., Komatsu, M., Tanaka, K., Cleveland, J.L., Withoff, S. and Green, D.R. (2007) Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 450, 1253–1257.
- [52] Wu, M.-F., Chen, S.-T. and Hsieh, S.-L. (2013) Distinct regulation of dengue virus-induced inflammasome activation in human macrophage subsets. *J. Biomed. Sci.* 20 (36).
- [53] Szabo, A. and Rajnavolgyi, E. (2013) Collaboration of Toll-like and RIG-I-like receptors in human dendritic cells: rTRIGGERING antiviral innate immune responses. *Am. J. Clin. Exp. Immunol.* 2 (3), 195–207.
- [54] Mandell, M.A., Jain, A., Arko-Mensah, J., Chauhan, S., Kimura, T., Dinkins, C., Silvestri, G., Munch, J., Kirchhoff, F., Simonsen, A., Wei, Y., Levine, B., Johansen, T. and Deretic, V. (2014) TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition. *Dev. Cell* 30 (4), 394–409.
- [55] Yang, K., Shi, H.X., Liu, X.Y., Shan, Y.F., Wei, B., Chen, S. and Wang, C. (2009) TRIM21 is essential to sustain IFN regulatory factor 3 activation during antiviral response. *J. Immunol.* 182 (6), 3782–3792.
- [56] Mandell, M., Kimura, T., Jain, A., Johansen, T. and Deretic, V. (2014) TRIM proteins regulate autophagy: TRIM5 is a selective autophagy receptor mediating HIV-1 restriction. *Autophagy* 10 (12), 2387–2388.
- [57] Jounai, N., Takeshita, F., Kobiyama, K., Sawano, A., Miyawaki, A., Xin, K., Ishii, K., Kawai, T., Akira, S., Suzuki, K. and Okuda, K. (2007) The Atg5-Atg12 conjugate associates with innate antiviral immune responses. *Proc. Natl. Acad. Sci. USA* 104 (35), 14050–14055.
- [58] Liang, X.H., Kleeman, L.K., Jiang, H.H., Gordon, G., Goldman, J.E., Berry, G., Herman, B. and Levine, B. (1998) Protection against fatal Sindbis virus encephalitis by beclin, a novel Bcl-2-interacting protein. *J. Virol.* 72 (11), 8586–8596.



- [59] Orvedahl, A., MacPherson, S., Sumpter, Rhea, Talloczy, Z., Zou, Z. and Levine, B. (2010) Autophagy protects against Sindbis virus infection of the central nervous system. *Cell Host Microbe* 7 (2), 115–127.
- [60] Lei, Y., Wen, H., Yu, Y., Taxman, D.J., Zhang, L., Widman, D.G., Swanson, K.V., Wen, K.-W., Damania, B., Moore, C.B., Giguere, P.M., Siderovski, D.P., Hiscott, J., Razani, B., Semenkovich, C.F., Chen, X. and Ting, J.P.-Y. (2012) The mitochondrial proteins NLRX1 and TUFM form a complex that regulates type 1 interferon and autophagy. *Immunity* 36 (6), 933–946.
- [61] Desai, M.M., Gong, B., Chan, T., Davey, R.A., Soong, L., Kolokoltsov, A.A. and Sun, J. (2011) Differential, type 1 interferon-mediated autophagic trafficking of hepatitis C virus proteins in mouse liver. *Gastroenterology* 141 (2), 674–685.
- [62] Sorbara, M.T., Ellison, L.K., Ramjeet, M., Travassos, L.H., Jones, N.L., Girardin, S. E. and Philpott, D.J. (2013) The protein ATG16L1 suppresses inflammatory cytokines induced by the intracellular sensors Nod1 and Nod2 in an autophagy-independent manner. *Immunity* 39 (5), 858–873.
- [63] Travassos, L.H., Carneiro, L.A., Ramjeet, M., Hussey, S., Kim, Y.G., Magalhaes, J. G., Yuan, L., Soares, F., Chea, E., Bourhis, L.L., Boneca, I.G., Allaoui, A., Jones, N. L., Nunez, G., Girardin, S.E. and Philpott, D.J. (2010) Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry. *Nat. Immunol.* 11 (1), 55–62.
- [64] Shi, C.S. and Kehrl, J.H. (2010) Traf6 and A20 differentially regulate TLR4-induced autophagy by affecting the ubiquitination of Beclin 1. *Autophagy* 6 (7), 986–987.
- [65] Lee, H.K., Mattei, L.M., Steinberg, B.E., Alberts, P., Lee, Y.H., Chervonsky, A., Mizushima, N., Grinstein, S. and Iwasaki, A. (2013) In vivo requirement for Atg5 in antigen presentation by dendritic cells. *J. Immunol.* 191 (5), 2526–2537.
- [66] Morris, S., Swanson, M.S., Lieberman, A., Reed, M., Yue, Z., Lindell, D. and Lukacs, N.W. (2011) Autophagy-mediated DC activation is essential for innate cytokine production and APC function with respiratory syncytial virus responses. *J. Immunol.* 187 (8), 3953–396.
- [67] Reed, M., Morris, S.H., Jang, S., Mukherjee, S., Yue, Z. and Lukacs, N.W. (2013) Autophagy-inducing protein beclin-1 in dendritic cells regulates CD4 T cell responses and disease severity during respiratory syncytial virus infection. *J. Immunol.* 191 (5), 2526–2537.
- [68] Pan, H., Zhang, Y., Luo, Z., Li, P., Liu, L., Wang, C., Wang, H., Li, H. and Ma, Y. (2014) Autophagy mediates avian influenza H5N1 pseudotyped particle-induced lung inflammation through NF- $\kappa$ B and p38 MAPK signaling pathways. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 306 (2), L183–L195.
- [69] Blanchet, F.P., Moris, A., Nikolic, D.S., Lehmann, M., Cardinaud, S., Stalder, R., Garcia, E., Dinkins, C., Leuba, F., Wu, L., Schwartz, O., Deretic, V. and Pignatelli, V. (2010) Human immunodeficiency virus-1 inhibition of immunosomes in dendritic cells impairs early innate and adaptive immune responses. *Immunity* 32 (5), 654–669.
- [70] Deretic, V., Saitoh, T. and Akira, S. (2013) Autophagy in infection, inflammation and immunity. *Nat. Rev. Immunol.* 13 (10), 722–737.
- [71] Mintern, J.D., Macri, C. and Villadangos, J.A. (2015) Modulation of antigen presentation by intracellular trafficking. *Curr. Opin. Immunol.* 34C, 16–21.
- [72] English, L., Chemali, M., Duron, J., Rondeau, C., Laplante, A., Gingras, D., Alexander, D., Leib, D., Norbury, C., Lippe, R. and Desjardins, M. (2009) Autophagy enhances the presentation of endogenous viral antigens on MHC class I molecules during HSV-1 infection. *Nat. Immunol.* 10, 480–487.
- [73] Ravindran, R., Khan, N., Nakaya, H.I., Li, S., Loebbermann, J., Maddur, M.S., Park, Y., Jones, D.P., Chappert, P., Davoust, J., Weiss, D.S., Virgin, H.W., Ron, D. and Pulendran, B. (2014) Vaccine activation of the nutrient sensor GCN2 in dendritic cells enhances antigen presentation. *Science* 343 (6168), 313–317.
- [74] Li, Y., Wang, L.X., Yang, G., Hao, F., Urba, W.J. and Hu, H.M. (2008) Efficient cross-presentation depends on autophagy in tumor cells. *Cancer Res.* 68, 6889–6895.
- [75] Wiede, F., Ziegler, A., Zehn, D. and Tigani, T. (2014) PTPN2 restrains CD8<sup>+</sup> T cell responses after antigen cross-presentation for the maintenance of peripheral tolerance in mice. *J. Autoimmun.* 53, 105–114.
- [76] Kim, H., Mazumbara, B., Bosea, S.K., Meyera, K., Biscaglia, A.M.D., Hofta, D.F. and Raya, R. (2012) Hepatitis C virus-mediated inhibition of cathepsin S increases invariant-chain expression on hepatocyte surface. *J. Virol.* 86 (18), 9919–9928.
- [77] Paludan, C., Schmid, D., Landthaler, M., Vockerodt, M., Kube, D., Tuschl, T. and Munz, C. (2005) Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science* 307 (5709), 593–596.
- [78] Romao, S., Gasser, N., Becker, A.C., Guhl, B., Bajagic, M., Vanoaica, D., Ziegler, U., Roesler, J., Dengjel, J., Reichenbach, J. and Munz, C. (2013) Autophagy proteins stabilize pathogen-containing phagosomes for prolonged MHC II antigen processing. *J. Cell Biol.* 203 (5), 757–766.
- [79] Barouch, D.H. (2010) Novel adenovirus vector-based vaccines for HIV-1. *Curr. Opin. HIV AIDS* 5, 386–390.
- [80] McElrath, M.J., Rosa, S.C.D., Moodie, Z., Dubey, S., Kierstead, L., Janes, H., Defawe, O.D., Carter, D.K., Hural, J., Akondy, R., Buchbinder, S.P., Robertson, M. N., Mehrotra, D.V., Self, S.G., Corey, L., Shiver, J.W., Casimiro, D.R. and Team, S.P. (2008) HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet* 372 (9653), 1894–1905.
- [81] Jin, Y., Sun, C., Feng, L., Li, P., Xiao, L., Ren, Y., Wang, D., Li, C. and Chen, L. (2014) Regulation of SIV antigen-specific CD4<sup>+</sup> T cellular immunity via autophagosome-mediated MHC II molecule-targeting antigen presentation in mice. *PLoS One* 9 (3), e93143.
- [82] Fu, X., Tao, L. and Zhang, X. (2010) A short polypeptide from the herpes simplex virus type 2 ICP10 gene can induce antigen aggregation and autophagosomal degradation for enhanced immune presentation. *Hum. Gene Ther.* 21 (12), 1687–1696.
- [83] Salio, M., Puleston, D.J., Mathan, T.S., Shepherd, D., Stranks, A.J., Adamopoulou, E., Veerapen, N., Besra, G.S., Hollander, G.A., Simon, A.K. and Cerundolo, V. (2014) Essential role for autophagy during invariant NKT cell development. *Proc. Natl. Acad. Sci. USA* 111 (52), E5678–87.
- [84] Beron, W., Gutierrez, M.G., Rabinovitch, M. and Colombo, M.I. (2002) *Coxiella burnetii* localizes in a Rab7-labeled compartment with autophagic characteristics. *Infect Immunol.* 70, 5816–5821.
- [85] Nakagawa, I., Amano, A., Mizushima, N., Yamamoto, A., Yamaguchi, H., Kamimoto, T., Nara, A., Funao, J., Nakata, M., Tsuda, K., Hamada, S. and Yoshimori, T. (2004) Autophagy defends cells against invading group A *Streptococcus*. *Science* 306 (5698), 1037–1040.
- [86] Cemma, M. and Brumell, J.H. (2012) Interactions of pathogenic bacteria with autophagy systems. *Curr. Biol.* 22 (13), R540–5.
- [87] Corr, S.C. and O'Neill, L.A. (2009) *Listeria monocytogenes* infection in the face of innate immunity. *Cell. Microbiol.* 11 (5), 703–709.
- [88] Santana, S., Sastre, I., Recuero, M., Bullido, M.J. and Aldudo, J. (2013) Oxidative stress enhances neurodegeneration markers induced by herpes simplex virus type 1 infection in human neuroblastoma cells. *PLoS One* 8 (10), e75842.
- [89] Wilcox, D.R., Wadhvani, N.R., Longnecker, R. and Muller, W.J. (2015) Differential reliance on autophagy for protection from HSV encephalitis between newborns and adults. *PLoS Pathog.*
- [90] Ait-Goughoulte, M., Kanda, T., Meyer, K., Rysse, J.S., Ray, R.B. and Ray, R. (2008) Hepatitis C virus genotype 1a growth and induction of autophagy. *J. Virol.* 82 (5), 2241–2249.
- [91] Sir, D., Chen, W.-L., Choi, J., Wakita, T., Yen, B. and Ou, J.-H.J. (2008) Induction of incomplete autophagic response by hepatitis C virus via the unfolded protein response. *Hepatology* 48 (4), 1054–1061.
- [92] Buckingham, E.M., Carpenter, J.E., Jackson, W. and Grose, C. (2014) Autophagy and the effects of its inhibition on varicella-zoster virus glycoprotein biosynthesis and infectivity. *J. Virol.* 88 (2), 890–902.
- [93] Richetta, C., Gregoire, I.P., Verlhac, P., Azocar, O., Baguet, J., Flacher, M., Tangy, F., Rabourdin-Combe, C. and Faure, M. (2013) Sustained autophagy contributes to measles virus infectivity. *PLoS Pathog.* 9 (9), e1003599.
- [94] Delorme-Axford, E., Morosky, S., Bomberger, J., Stolz, D.B., Jackson, W.T. and Coyne, C.B. (2014) BPIFB3 regulates autophagy and coxsackievirus B replication through a noncanonical pathway independent of the core initiation machinery. *MBio* 5 (6), e02147.
- [95] Crawford, S.E., Hyser, J.M., Utama, B. and Estes, M.K. (2012) Autophagy hijacked through viroporin-activated calcium/calmodulin-dependent kinase kinase-P signaling is required for rotavirus replication. *Proc. Natl. Acad. Sci. USA* 109 (50), E3405–13.
- [96] Kemball, C.C., Alirezai, M., Flynn, C.T., Wood, M.R., Harkins, S., Kiosses, W.B. and Whitton, J.L. (2010) Coxsackievirus infection induces autophagy-like vesicles and megaphagosomes in pancreatic acinar cells in vivo. *J. Virol.* 23 (84), 23.
- [97] Zeng, X. and Carlin, C.R. (2013) Host cell autophagy modulates early stages of adenovirus infections in airway epithelial cells. *J. Virol.* 87 (4), 2307–2319.
- [98] Nakashima, A., Tanakaa, N., Tamaia, K., Kyuumaa, M., Ishikawaa, Y., Satoh, H., Yoshimori, T., Saito, S. and Sugamura, K. (2006) Survival of parvovirus B19-infected cells by cellular autophagy. *Virology* 349 (2), 254–263.
- [99] Chu, L.W., Huang, Y.L., Lee, J.H., Huang, L.Y., Chen, W.J., Lin, Y.H., Chen, J.Y., Xiang, R., Lee, C.H. and Ping, Y.H. (2014) Single-virus tracking approach to reveal the interaction of Dengue virus with autophagy during the early stage of infection. *J. Biomed. Opt.* 19 (1), 011018.
- [100] Gregoire, I.P., Richetta, C., Meyniel-Schicklin, L., Borel, S., Pradezynski, F., Diaz, O., Deloire, A., Azocar, O., Baguet, J., Breton, M.L., Mangeot, P.E., Navratil, V., Joubert, P.-E., Flacher, M., Vidalain, P.-O., Andre, P., Lotteau, V., Biard-Piechaczyk, M., Rabourdin-Combe, C. and Faure, M. (2011) IRGM is a common target of RNA viruses that subvert the autophagy network. *PLoS Pathog.* 7 (12), e1002422.
- [101] Kyei, G.B., Dinkins, C., Davis, A.S., Roberts, E., Singh, S.B., Dong, C., Wu, L., Kominami, E., Ueno, T., Yamamoto, A., Federico, M., Pangniban, A., Vergne, I. and Deretic, V. (2009) Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J. Cell Biol.* 86 (2), 255–268.
- [102] Shoji-Kawata, S., Sumpter, R., Leveno, M., Campbell, G., Zou, Z., Kinch, L., Wilkins, A., Sun, Q., Pallauf, K., MacDuff, D., Huerta, C., Virgin, H.W., Helms, J. B., Eerland, R., Tooze, S.A., Xavier, R., Lenschow, D., Yamamoto, A., King, D., Lichtarge, O., Grishin, N., Spector, S., Kalayanova, D. and Levine, B. (2013) Identification of a candidate therapeutic autophagy-inducing peptide. *Nature* 494 (7436), 201–206.
- [103] Prentice, E., Jerome, W.G., Yoshimori, T., Mizushima, N. and Denison, M.R. (2004) Coronavirus replication complex formation utilizes components of cellular autophagy. *J. Biol. Chem.* 279 (11), 10136–10141.
- [104] Zhao, Z., Thackray, L.B., Miller, B.C., Lynn, T.M., Becker, M.M., Ward, E., Mizushima, N., Denison, M.R. and Virgin, H.W. (2007) Coronavirus replication does not require the autophagy gene ATG5. *Autophagy* 3 (6), 581–585.
- [105] Cottam, E.M., Maier, H.J., Manifava, M., Vaux, L.C., Chandra-Schoenfelder, P., Gerner, W., Britton, P., Ktistakis, N.T. and Wileman, T. (2011) Coronavirus nsp6 proteins generate autophagosomes from the endoplasmic reticulum via an omegasome intermediate. *Autophagy* 7 (11), 1335–1347.
- [106] Zhu, H.Y., Han, L., Shi, X.L., Wang, B.L., Huang, H., Wang, X., Chen, D.F., Ju, D.W. and Feng, M.Q. (2015) Baicalin inhibits autophagy induced by influenza A virus H3N2. *Antiviral Res.* 113, 62–70.

- [107] Zha, B.S., Wan, X., Zhang, X., Zha, W., Zhou, J., Wabitsch, M., Wang, G., Lyall, V., Hylemon, P.B. and Zhou, H. (2013) HIV protease inhibitors disrupt lipid metabolism by activating endoplasmic reticulum stress and inhibiting autophagy activity in adipocytes. *PLoS One* 8 (3), e59514.
- [108] Laforge, M., Limou, S., Harper, F., Casartelli, N., Rodrigues, V., Silvestre, R., Haloui, H., Zagury, J.-F., Senik, A. and Estaquier, J. (2013) DRAM triggers lysosomal membrane permeabilization and cell death in CD4<sup>+</sup> T cells infected with HIV. *PLoS Pathog.* 9 (5), e1003328.
- [109] Alirezaei, M., Kiosses, W.B., Flynn, C.T., Brady, N.R. and Fox, H.S. (2008) Disruption of neuronal autophagy by infected microglia results in neurodegeneration. *PLoS One* 3 (8), e2906.
- [110] Liang, X.H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H. and Levine, B. (1999) Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature* 402 (6762), 672–676.
- [111] Raftery, M.J., Wolter, E., Fillatreau, S., Meisel, H., Kaufmann, S.H. and Schonrich, G. (2014) NKT cells determine titer and subtype profile of virus-specific IgG antibodies during herpes simplex virus infection. *J. Immunol.* 192 (9), 4294–4302.
- [112] Ashkar, A.A. and Rosenthal, K.L. (2003) Interleukin-15 and natural killer and NKT cells play a critical role in innate protection against genital herpes simplex virus type 2 infection. *J. Virol.* 77 (18), 10168–10171.
- [113] Ilyinskii, P.O., Wang, R., Balk, S.P. and Exley, M.A. (2006) CD1d mediates T-cell-dependent resistance to secondary infection with encephalomyocarditis virus (EMCV) in vitro and immune response to EMCV infection in vivo. *J. Virol.* 80 (14), 7146–7158.
- [114] Johnson, T.R., Hong, S., Kaer, L.V., Koezuka, Y. and Graham, B.S. (2002) NK T cells contribute to expansion of CD8<sup>+</sup> T cells and amplification of antiviral immune responses to respiratory syncytial virus. *J. Virol.* 76 (9), 4294–4303.
- [115] Li, J., Liu, Y., Wang, Z., Liu, K., Wang, Y., Liu, J., Ding, H. and Yuan, Z. (2011) Subversion of cellular autophagy machinery by hepatitis B virus for viral envelopment. *J. Virol.* 85 (13), 6319–6333.
- [116] Sir, D., Tian, Y., Chen, W.-L., Ann, D.K., Yen, T.-S.B. and Oua, J.-H.J. (2010) The early autophagic pathway is activated by hepatitis B virus and required for viral DNA replication. *Proc. Natl. Acad. Sci. USA* 107 (9), 4383–4388.
- [117] Doring, T. and Prange, R. (2014) Rab33B and its autophagic Atg5/12/16L1 effector assist in hepatitis B virus naked capsid formation and release. *Cell Microbiol.*
- [118] Huang, C.C., Chen, K.L., Cheung, C.H. and Chang, J.Y. (2013) Autophagy induced by cathepsin S inhibition induces early ROS production, oxidative DNA damage, and cell death via xanthine oxidase. *Free Radic. Biol. Med.* 65, 1473–1486.