Role of autophagy in HIV infection and pathogenesis

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The aim of autophagy is to re-establish homeostasis in response to a variety of stress conditions. By forming double-membrane vesicles, autophagy engulfs damaged or superfluous cytoplasmic material and recycles degradation products for new synthesis or energy production. Of note, the same mechanism is used to capture pathogens and has important implications in both innate and adaptive immunity. To establish a chronic infection, pathogens have therefore evolved multiple mechanisms to evade autophagy-mediated degradation. HIV infection represents one of the best characterized systems in which autophagy is disarmed by a virus using multiple strategies to prevent the sequestration and degradation of its proteins and to establish a chronic infection. HIV alters autophagy at various stages of the process in both infected and bystander cells. In particular, the HIV proteins TAT, NEF and ENV are involved in this regulation by either blocking or stimulating autophagy through direct interaction with autophagy proteins and/or modulation of the mTOR pathway. Although the roles of autophagy during HIV infection are multiple and vary amongst the different cell types, several lines of evidence point to a potential beneficial effect of stimulating autophagy-mediated lysosomal degradation to potentiate the immune response to HIV. Characterization of the molecular mechanisms regulating selective autophagy is expected to be valuable for developing new drugs able to specifically enhance the anti-HIV response.

Keywords: autophagy, cell death, HIV, inflammation.

Introduction

Autophagy is the main catabolic process by which intracellular components are delivered to the lysosome for degradation [1]. Autophagy takes part in a large variety of cellular activities, by ensuring the rapid degradation of damaged or unnecessary components, as well as by replenishing the cellular stores of energy and building blocks when extracellular nutrients are limited [2].

Cellular components targeted by autophagy are transported to the lysosome through different mechanisms. Macroautophagy is characterized by the formation of double-membrane vesicles, called autophagosomes, which enwrap the target materials and fuse with lysosomes to allow their degradation [1]. Microautophagy captures cytosolic components through the invagination of membranes of the endolysosomal compartment [3]. Chaperone-mediated autophagy consists of the translocation inside the lysosome of single proteins containing the KFERQ motif, assisted by the lysosomal membrane protein LAMP2A and the HSC70 chaperone [4].

Inactivation of autophagy genes in different animal models has revealed the essential role of autophagy in maintaining homeostasis in normal and stress conditions. Autophagy inhibition results in the degeneration of several organ systems, as shown for muscle, liver, pancreas and the nervous system, due to the accumulation of unfolded protein aggregates and damaged organelles which sensitize cells to death [1]. Equally, excessive autophagy is also associated with increased susceptibility to cell death due to uncontrolled self-digestion [5]. In line with these observations, dysfunctional autophagy is associated with several pathological conditions, including cancer, neurodegenerative diseases and metabolic disorders. Importantly, autophagy gene variants have been linked to human diseases,
thus confirming the pathogenic potential of autophagy dysregulation [2].

A key contribution of autophagy to both innate and adaptive immunity has emerged in recent years [6]. Autophagy contributes to the innate immune response through multiple mechanisms. Intracellular pathogens can be captured by autophagosome receptors and delivered to the lysosome for degradation [7, 8]. In addition, autophagosomes engulfing pathogenic components can fuse to the endosomal compartment to make these components accessible to endosomal toll-like receptors, such as TLR7 and TLR9 [9]. Autophagy also contributes to the unconventional secretion of proinflammatory agents, such IL-1β, IL-18 and HMGB1 [10]. Conversely, autophagy is essential for limiting the inflammatory response by degrading either pathogens or activated inflammasome components, such as AIM2, CASPASE 1, NLRP1 and NLRP3 [11, 12]. Moreover, inflammatory pathways may be inhibited by autophagy proteins as in the case of the cytosolic DNA sensors cGAS and STING that are repressed by BECLIN-1 and ULK1, respectively [13, 14]. The relevance of autophagy proteins in regulating the inflammatory process has been highlighted by the identification of polymorphisms of the Atg16L1 gene which are associated with the development of Crohn’s disease, a severe form of inflammatory bowel disease [15, 16].

Autophagy also plays an essential role in adaptive immunity, by contributing to the survival and function of B and T cells and lymphoid progenitors [7]. For example, the expansion of T cells after antigen stimulation requires autophagy to sustain proliferation and neutralize cell death pathways [17]. Interestingly, a feedback loop activated by effectors of death receptor pathways limits the autophagy response to prevent self-digestion [17]. Moreover, autophagy allows MHC class II presentation of intracellular antigens and also stimulates presentation of extracellular antigens as well as MHC class I [18].

Pathogens have evolved a series of strategies to inhibit the immunity-supporting roles of autophagy and to hijack autophagy protein activities for their own benefit [19, 20]. In this review, we discuss the complex relationship between HIV and autophagy, highlighting how this process may have both pro- and antiviral functions, which must be controlled by HIV to establish chronic infection and trigger pathogenesis.

Regulation of autophagy

Macroautophagy, hereafter referred to as autophagy, is the best characterized form of autophagy. This process is regulated by a set of evolutionarily conserved genes, ATGs, first identified in yeast as a result of the pioneering work of Yoshinori Ohsumi, who was awarded the Nobel Prize in Physiology or Medicine in 2016.

Specific ATG proteins regulate different steps of autophagy (Fig. 1). BECLIN-1 (the ortholog of the yeast ATG6) initiates autophagosome formation by stimulating class III PI3K VPS34 to generate phosphatidylinositol 3-phosphate (PI3P) [21, 22]. PI3P represents the signal to recruit the autophagy machinery required for assembling the autophagosomal membrane precursor, the phagophore [23]. BECLIN-1 acts in complex with ATG14 and VPS15 proteins, its activity being modulated by a plethora of positive and negative regulators, such as AMBRA1 and BCL2, respectively [5, 24].

The expansion and closure of the nascent autophagosome is under the control of several ATG proteins, including the transmembrane protein ATG9, which shuttles from the trans-Golgi network and endosomes probably to deliver membrane to the phagophore, and LC3 (the ortholog of the yeast ATG8), which has tethering and hemifusion activity [25, 26]. The positioning of LC3 on the autophagosomal membrane requires two ubiquitin-like conjugation systems formed by several ATG proteins, including ATG3, ATG4, ATG5, ATG7, ATG12 and ATG16, leading to the covalent binding of LC3 to the membrane lipid phosphatidylethanolamine [27]. In addition, LC3 also plays a key role in the selection of cargos to be degraded by interacting with a series of autophagy receptor proteins, such as p62, NBR1, NDP52 and OPTINEURIN, which bind ubiquitinated or glycosylated proteins [28].

Finally, autophagosomal fusion to the endo-lysosomal compartment is regulated by a different BECLIN-1 complex with UVRAG replacing ATG14, which acts in concert with RAB proteins, such as RAB7, SNARE proteins, such as Syntaxin 17, and the HOPS-tethering complex [29].

The induction of autophagy is regulated by different upstream signals depending on the...
stress stimuli. Most converge directly on the BECLIN-1 complex or indirectly through the upstream kinase ULK1 (the ortholog of yeast ATG1) [5]. Nutrient starvation activates autophagy by inhibiting the activity of mTOR, an amino acid- and growth factor-stimulated kinase which, when active, represses autophagy by phosphorylating ULK1 and AMBRA1 [30, 31]. Energy-limiting conditions induce autophagy via the AMP-activated protein kinase, which mediates activating phosphorylation on both ULK1 and BECLIN-1 [30]. Other types of damaging stress induce autophagy by activating a large series of stress-activated kinases, including JNK and DAPK, which act on the BECLIN-1 complex by disrupting the inhibitory interaction between BECLIN-1 and antiapoptotic members of the BCL-2 family [32, 33].

During infection, autophagy is stimulated by immune-related signalling pathways activated by inflammatory cytokines and pattern recognition receptors (PRRs), including toll-like proteins, nucleotide oligomerization domain (NOD)-like proteins, C-type lectin receptors, RIG-1 like proteins and cGAS/STING, which recognize conserved molecular structures amongst pathogens [34]. Signal transduction proteins that mediate autophagy induction by these receptors include the kinase TAK1, which interacts with BECLIN-1 and also regulates the mTOR–AMPK axis [35], and the E3 ubiquitin ligase TRAF6, which carries out an activating, nondegradative ubiquitination of both ULK1 and BECLIN-1 [31, 36].

HIV
Understanding how the human immunodeficiency virus (HIV) counteracts the host immune response remains a major challenge, but is important for developing novel therapeutic approaches to eradicate infection and prevent pathogenesis. HIV weakens the host defence system by eliminating CD4+ T cells [37]. The most advanced stage of HIV infection is the acquired immunodeficiency syndrome (AIDS), which can take from 2 to 15 years to develop in different individuals [38]. AIDS is characterized by the development of severe clinical manifestations, such as opportunistic infections or cancers [38]. No cure for HIV infection is yet available; however, antiretroviral drugs can efficiently repress viral replication and help to prevent transmission [38].
The HIV life cycle

The HIV genome contains nine genes that encode 15 different viral proteins [39, 40]. Three of these genes generate polyprotein precursors (GAG, POL and ENV). The GAG precursor is cleaved by the viral protease into the mature GAG proteins responsible for the generation of the HIV capsid. The polymerase (POL) polyprotein is produced together with GAG, as GAG–POL protein precursor. POL gene is encoded by using a different reading frame following a -1 frameshifting event, which occurs with a frequency of 5–10%. The POL precursor is processed by the viral protease into the following enzymes: protease, integrase and the reverse transcriptase. The ENV precursor gp160 is processed in the two components of the viral envelope: GP120 and GP41. The remaining group of genes encodes transcription activators (TAT, REV and VPR) and other regulatory proteins (VIF, NEF and VPU).

HIV replication occurs in two distinct phases: the early phase in which the HIV RNA is released in the host cell cytoplasm, retro-transcribed and integrated into the host genome and the late phase that culminates in the production of new viral particles [40].

The first step of the HIV life cycle is entry of the virus into the cell. HIV recognizes the CD4 receptor expressed on the surface of the target cell through the viral glycoprotein GP120 [41]. This interaction causes conformational changes in the GP120 protein that permits its interaction with the HIV coreceptors chemokine CC receptor 5 (CCR5) or chemokine CXC receptor 4 (CXCR4). The interaction between GP120 and the coreceptors activates the glycoprotein GP41, allowing the insertion of its N-terminal fusion peptide into the target membrane. This last step causes fusion between the viral envelope and the host cellular membrane [41]. Once inside the cell, the HIV core is uncoated from the genome to initiate viral replication. The genome consists of two copies of single-stranded RNA held together by two small proteins: P6 and P7; it is released into a complex with the reverse transcriptase, the integrase, the viral protease and the accessory proteins NEF, VPR and VIF. HIV reverse transcriptase transcribes single-stranded copies of viral RNA into double-stranded DNA (dsDNA) [42]. The dsDNA is then transported into the nucleus by the preintegration complex, composed of VPR and the integrase. In the nucleus, the integrase catalyses the insertion of the dsDNA into the host chromosome [43, 44].

HIV accessory proteins are not essential for viral replication, but are important for in vivo infection because of their ability to counteract the defensive mechanisms activated by the host cell [45]. In particular, most HIV accessory proteins stimulate proteasome-mediated degradation of antiviral proteins by hijacking different members of the CULLIN E3 ubiquitin ligase family. VIF and VPR arrest HIV genome mutagenesis activated by the host proteins APOBEC3G and UNG2, a cytidine deaminase and the uracil glycosylase, respectively, which cooperate to generate abasic sites in the HIV genome [45]. By contrast, VPU induces degradation of the transmembrane protein BST2, which has an essential role in retaining viral particles on the plasma membrane, whilst NEF reduces plasma membrane levels of many receptors involved in adaptive immunity, including CD4 and MHC proteins, by stimulating their lysosomal turnover [46].

Role of autophagy in the HIV life cycle

There is much evidence to suggest a complex role of autophagy during HIV infection, which depends on the different phases of infection. It is now clear that HIV requires autophagy to accomplish the early replication steps, whilst it has evolved multiple strategies to avoid the recognition and degradation of the newly synthesized viral particles (Table 1).

The requirement of autophagy genes for HIV replication first emerged from unbiased RNA interference (RNAi) screening showing that ATG7, GABARAPL2, ATG12 and ATG16L2 are required for productive HIV infection [47]. Targeted RNAi and pharmacological approaches confirmed the pro-viral role of autophagy in both T cells and macrophages [48, 49]. A direct link between the autophagy machinery and the HIV proteins was identified when the HIV GAG precursor was found to interact with the autophagosome protein LC3. Notably, this association is required for proper processing of GAG, indicating that the autophagosome may provide membrane support for viral replication [49].

Different pathways contribute to autophagy induction during HIV infection. IRGM, a member of a large family of GTPases activated in response to several types of infection, is required for
stimulating both autophagy and HIV replication [50]. IRGM activation during HIV infection is mediated by interaction with the accessory protein NEF. IRGM is then able to activate autophagy by favouring the assembly of the ULK1/BECLIN-1/ATG16 complex [51]. The interaction between HIV and TLR8 has also been shown to trigger autophagy [52]. Whether TLR8 signalling converges with IRGM via TRAF6 activation remains to be investigated. In addition, the HIV minus-strand-encoded antisense protein (ASP) binds to and colocalizes with LC3 and promotes HIV infection by stimulating autophagy [53].

Although autophagy levels are upregulated during infection, both the antiviral and immune properties of this process are severely inhibited by HIV, through a plethora of direct and indirect mechanisms. An important example is represented by NEF, which interacts with BECLIN-1 and inhibits autophagosome maturation [49, 54]; this step is under the control of the UVRAG-containing BECLIN-1 complex. Interestingly, the interaction between NEF and BECLIN-1 mimics the function of GLIPR2, a host autophagy inhibitor which sequesters BECLIN-1 on the Golgi apparatus. Recently, the NEF/BECLIN-1 interaction has been shown to be responsible for inhibition of autophagy at the transcriptional level, by preventing the nuclear translocation of the pro-autophagic transcription factor TFEB in an mTOR-dependent manner [55].

VIF is required for autophagy inhibition during HIV infection. The antiautophagic properties of VIF reside in its ability to interact with the LC3 protein, independently of its binding to APOBEC3G and CULLIN 5 [56]. In a proteomic screening, VIF was also shown to associate with AMBRA1 and the adaptor protein p62 (SQSTM), suggesting the presence of additional mechanisms by which VIF could inhibit autophagy [57].

Inhibition of autophagy by HIV is required to prevent the sequestration of HIV proteins within autophagosomes and their lysosomal degradation. Indeed, the interaction between the HIV restriction factor TRIM5α and the HIV capsid protein p24 results both in autophagy stimulation, by allowing the recruitment of ULK1 and BECLIN-1 complexes to the viral particles, and in the recognition of HIV capsid as an autophagy cargo, via direct

Table 1  Crosstalk between autophagy and HIV proteins

<table>
<thead>
<tr>
<th>Host system</th>
<th>Cell type</th>
<th>HIV protein</th>
<th>Host protein</th>
<th>Role of the interaction</th>
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<tbody>
<tr>
<td>Central nervous system</td>
<td>Glial cells</td>
<td>TAT</td>
<td>BAG3</td>
<td>Autophagy induction [86]</td>
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<td></td>
<td>Astrocytes</td>
<td>NEF</td>
<td>Unknown</td>
<td>Inhibition of autophagosome maturation [87]</td>
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<td>Neurons</td>
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<td>LAMP2A</td>
<td>Autophagosome/lysosome fusion inhibition [80]</td>
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<td>ENV</td>
<td>mTOR signalling</td>
<td>Autophagy inhibition [79]</td>
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<td>Immune system</td>
<td>CD4+ T cells</td>
<td>ENV</td>
<td>CXCR4</td>
<td>Accumulation of BECLIN-1 and apoptosis induction [66–68, 93]</td>
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<td>TAT</td>
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<td>Dendritic cells</td>
<td>ENV</td>
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<td>Macrophages</td>
<td>NEF</td>
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<td>BECLIN-1</td>
<td>Inhibition of the maturation steps of autophagy [49, 54, 55]</td>
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<td>P62</td>
<td>Inhibition of TFEB nuclear translocation [55]</td>
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<td>HDAC6</td>
<td>VIF degradation [59]</td>
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<td>P24</td>
<td>TRIM5alpha</td>
<td>Viral particle degradation [58]</td>
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interaction between TRIM5α and LC3 [58]. Moreover, the autophagy regulator HDAC6 directly interacts with VIP and promotes its autophagic degradation [59]. Finally, it has been reported that autophagy selectively degrades the HIV transactivator TAT, which depends on its ubiquitin-independent interaction with the autophagy adaptor p62 [60].

In this scenario, it is evident that stimulation of autophagy may have either a positive or a negative effect on HIV infection. The final output may depend on the levels of induced autophagy, as productive infection has also been observed in cells with a moderate number of autophagosomes, whilst exceeding a certain threshold overcomes autophagy flux impairment and inhibits HIV replication [61]. In line with this observation, strong stimulation of TLR8 by specific agonists results in autophagy-dependent inhibition of HIV infection, which is mediated by the cathelicidin microbial peptide and the vitamin D receptor [62, 63]. Of note, peripheral blood mononuclear cells (PBMCs) from long-term non-progressor patients (LTNPs), who are able to control HIV replication without antiretroviral therapy, show higher levels of autophagy proteins compared to normal progressors. Moreover, autophagy is induced to a greater extent when PBMCs of LTNPs are treated ex vivo with the mTOR inhibitor rapamycin [64].

Role of autophagy impairment in the dysregulated immune response and pathogenesis during HIV infection

The consequences of autophagy alteration caused by HIV are not limited to preventing the degradation of viral particles but are directly linked to the ability of the virus to dysregulate the immune system and to promote pathogenesis. How autophagy dysfunction impacts on host functions depends on the characteristics of the target cells (Fig. 2).

Fig. 2 Role of autophagy in HIV infection in different cell types. The different roles played by autophagy during HIV infection in different cellular systems are described. Further details are provided in the text.
Autophagy alterations in CD4+ T cells and macrophages during HIV infection

The main HIV targets are the CD4+ T cells, where the virus actively replicates eluding the innate immune response [42]. Moreover, HIV causes profound depletion of CD4+ T cells, including infected, abortively infected and bystander lymphocytes. This leads to serious impairment of the host immune defence, and eventually death of infected individuals due to their inability to control opportunistic infections and cancer [37].

It has been proposed that abnormal autophagy activation contributes to the reduction in the number of CD4+ T cells. Different mechanisms are employed by HIV to induce cell death of bystander CD4+ T cells. One example is represented by the engagement of the protein ENV, present on the plasma membrane of infected T cells, with the CD4 receptor and associated coreceptors of the noninfected cells which triggers apoptosis through a pathway involving p53 and BAX and activation of CASPASES [65, 66]. Importantly, ENV also stimulates autophagy in bystander cells, which in this context is required for the induction of apoptosis by HIV. Indeed, inhibition of the expression of autophagy genes, such as BECLIN-1 and ATG7, results in apoptosis inhibition due to inefficient CASPASE 3 activation [67, 68]. In addition, autophagy contributes to the induction of cell death in HIV-infected cells. In particular, the pro-autophagy protein DRAM1, the expression of which is upregulated by p53, favours cell death by causing lysosomal membrane permeabilization and the consequent release of the proteolytic enzyme cathepsin D [69].

Recent evidence shows that abortively infected cells undergo a different type of cell death, pyroptosis, which is associated with elevated inflammation activated by CASPASE 1 [70, 71]. Although autophagy is known to selectively control the CASPASE 1 pathways [12], whether it plays a role in this type of cell death during HIV infection remains unexplored.

In contrast to T cells, macrophages are not depleted during HIV infection and, for this reason, are considered a reservoir for the virus [72]. Consistent with the lack of macrophage reduction during HIV infection, HIV ENV is unable to induce cell death in these cells [61]. As described above, autophagy plays a double role in infected macrophages. HIV infection results both in autophagy induction, which is required for the early steps of viral infection, and in the block of autophagy degradative properties to avoid elimination of the viral particles. Besides regulating HIV replication, autophagy modulation also impacts on the regulation of adaptive immunity by macrophages. Interestingly, it has been shown that HIV infection inhibits the ability of bystander macrophages to degrade Toxoplasma gondii through the induction of autophagy triggered by T cells via the CD40L/CD40 pathway [73]. This autophagy impairment is dependent on Src/Akt and STAT3 pathways, which are triggered directly by the HIV protein TAT or indirectly by stimulating IL-10 production. Of note, defective autophagic killing of T. gondii was confirmed in monocyte-derived macrophages from HIV patients [73].

Autophagy alterations in dendritic cells during HIV infection

Dendritic cells (DCs) are often localized in tissue in proximity to the external environment; for this reason, they are the first cell type to encounter HIV immediately after infection [74]. DCs are only partially permissive to HIV infection. The intracellular pool of virus in DCs is transferred to T cells through cell-to-cell synapses generated by HIV, with 5–10% of initial viral input evading degradation and remaining latent in DCs.

In DCs, HIV ENV inhibits the autophagic process through the activation of mTOR and S6K. These events lead to the inhibition of viral degradation and the accumulation of viral particles in DCs. Moreover, autophagy inhibition by LC3 or ATG5 silencing increases in HIV transfer to CD4+ T cells [75]. To block antigen presentation, HIV also inhibits lysosomal acidification by decreasing the expression of cathepsins, which impairs digestion of viral particles and contributes to the inhibition of antigen processing and presentation to T cells [76]. In addition, autophagy is required for type I interferon production by plasmacytoid DCs exposed to infectious or noninfectious HIV, which is triggered through TLR7 signalling [77].

Autophagy alterations in the central nervous system during HIV infection

Although antiretroviral therapy increases the lifespan of HIV-infected individuals, 50% of these patients develop HIV-associated neurocognitive disorders (HAND) and/or neurodegeneration [78].
In the central nervous system (CNS), microglial cells are a reservoir for HIV infection, with productive infection also detected in astrocytes, whereas neurons are nonpermissive. HIV alters calcium homeostasis and increases oxidative stress, which eventually induces cell death [78].

A contribution of autophagy alteration to HAND pathogenesis has been proposed based on evidence of abnormal accumulation of large autophagosomes and an increase in the autophagic markers ATG5, ATG7 and LC3II in post-mortem brains of HIV patients with encephalitis, compared to infected individuals without encephalitis [79]. Recently, differences in the levels of autophagy markers between young and old HIV patients with encephalitis have been reported, with the former patients displaying an increase in BECLIN-1, CATHEPSIN D and LC3, whilst these proteins are reduced in the latter group compared to age-matched HIV patients without encephalitis [80]. Of note, these differences correlate with the severity of neurodegenerative pathology.

It has been proposed that HIV GP120 contributes to autophagy modulation in the brain. Autophagy is induced in neuroblastoma cells exposed to GP120 [79]. Of interest, when the effect of GP120 on autophagy was analysed in mice, a decrease in autophagy markers and an increase in mTOR levels were observed in aged GP120 transgenic mice, consistent with observations in HIV patients [79]. Moreover, lentivirus-mediated overexpression of BECLIN-1 increased LC3 levels and alleviated the neurological damage caused by GP120 [79].

Besides GP120, viral or cellular factors released from infected glial cells are able to modulate autophagy in neurons. Alirezaei and colleagues reported that the numbers of LC3-positive vesicles were decreased in rat primary neurons exposed to supernatants from simian immunodeficiency virus-infected microglia [81]. Importantly, treatment of neurons with the autophagy inducer rapamycin rescued autophagy inhibition and improved cell survival [81].

In this regard, a crucial role in the regulation of autophagy by HIV in neurons is played by the viral protein TAT. TAT, a nonstructural protein essential for viral transcription and replication, is able to enter noninfected cells through receptor-mediated endocytosis and low-density lipoproteins [82, 83]. Neurons may be targeted by both systemic circulating TAT crossing the blood–brain barrier and TAT released from infected glial cells [82]. Consistent with this possibility, TAT has been detected in the brain of patients with HIV-associated dementia [84, 85].

Primary hippocampal neurons exposed to TAT undergo cell death associated with autophagy inhibition as shown by decreases in LC3II and ATG5 levels. It is important to note that some of the effects of TAT on autophagy may be indirect, as it is also able to interfere with the endo-lysosomal compartment, by increasing luminal pH and compromising membrane integrity [84]. A recent detailed study of the effect of TAT on autophagy showed that treatments both in vitro and in GFAP-TAT transgenic mice induce accumulation of abnormal neuronal autophagosomes, suggesting that TAT alters the fusion of autophagosomes with lysosomes [80]. It is interesting that TAT was found in association with the lysosomal protein LAMP2A. It was proposed that this interaction may play a role in the inhibition of autophagosome maturation, because LAMP2A overexpression was able to reverse TAT-induced neurotoxicity [80].

In contrast to neurons, TAT appears to induce autophagy in glioblastoma cells, and this has been proposed as a mechanism by which HIV establishes its cell reservoir. TAT-induced autophagy occurs through the stabilization of BAG3, a chaperone protein that cooperates with various autophagy regulators. Indeed, silencing of BAG3 expression in glioblastoma cells results in the inhibition of the TAT-mediated increase in LC3-II levels [86].

Autophagy in the CNS can also be affected by the viral protein NEF. In fact, the expression of NEF in astrocytes causes inhibition of autophagosomal maturation, as indicated by the accumulation of both LC3 and p62; this resembles what is observed in cells treated with the lysosome inhibitor bafilomycin A1 [87].

Conclusions
The large number of defensive mechanisms evolved by HIV to counteract autophagy in the host underlines the important role of this process in the regulation of the immune response mounted by infected individuals. Although the effects of autophagy during HIV infection are multiple and vary amongst different cell types, evidence from several
studies indicates a potential beneficial effect of stimulating autophagy-mediated lysosomal degradation to enhance the immune response to HIV.

Preclinical studies have indicated that rapamycin, a selective inhibitor of mTOR that induces autophagy, may have positive effects in the control of HIV infection [88]. However, because rapamycin also has immunosuppressive effects, alternative drugs and/or natural compounds able to stimulate autophagy need to be identified to test their therapeutic potential in HIV patients. In this regard, a promising candidate is vitamin D, which has been shown to inhibit HIV replication in primary human macrophages through autophagy induction [62]. However, unexpectedly, a recent study showed that high vitamin D levels correlate with higher concentrations of plasma inflammatory markers in HIV patients, suggesting that excess vitamin D by pharmacological administration may be detrimental for the immune system and worsen the control of HIV infection [89].

Other promising pro-autophagy drugs emerging from in vitro studies are histone deacetylase (HDAC) inhibitors and microtubule-targeting agents. HDAC inhibitors have been shown to promote autophagy-mediated degradation of HIV particles [90]. Moreover, HDAC inhibitors can reverse HIV latency from CD4+ T cells [91]. This dual property of HDAC inhibitors is particularly attractive when attempting to eradicate HIV from reservoir cells. Flubendazole is a drug that increases microtubule acetylation and induces autophagy by activating JNK1 and allowing the release of the autophagy inhibitor Bcl-2 from the Beclin-1 complex [92]. Notably, flubendazole-induced autophagy has been shown to block the transfer of HIV from DCs to T cells [92].

Although several autophagy-promoting drugs are able to inhibit HIV infection in vitro, the side effects of the persistent induction of autophagy in vivo have yet to be assessed in depth. Characterization of the molecular mechanisms regulating selective autophagy, as in the case of the HIV-binding protein TRIM5α, is expected to be valuable in paving the way for the development of new drugs able to specifically enhance the anti-HIV response.

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