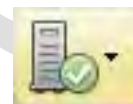


WILEY

Online Proofing System Instructions

The Wiley Online Proofing System allows authors and proof reviewers to review PDF proofs, mark corrections, respond to queries, upload replacement figures, and submit these changes directly from the PDF proof from the locally saved file or while viewing it in your web browser.

1. For the best experience reviewing your proof in the Wiley Online Proofing System please ensure you are connected to the internet. This will allow the PDF proof to connect to the central Wiley Online Proofing System server. If you are connected to the Wiley Online Proofing System server you should see the icon with a green check mark above in the yellow banner.
2. Please review the article proof on the following pages and mark any corrections, changes, and query responses using the Annotation Tools outlined on the next 2 pages.

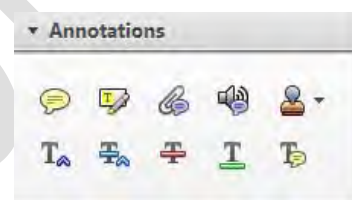


Connected



Disconnected

3. To save your proof corrections, click the “Publish Comments” button appearing above in the yellow banner. Publishing your comments saves your corrections to the Wiley Online Proofing System server. Corrections don’t have to be marked in one sitting, you can publish corrections and log back in at a later time to add more before you click the “Complete Proof Review” button below.

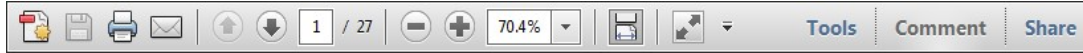


4. If you need to supply additional or replacement files bigger than 5 Megabytes (MB) do not attach them directly to the PDF Proof, please click the “Upload Files” button to upload files:
5. When your proof review is complete and you are ready to submit corrections to the publisher, please click the “Complete Proof Review” button below:

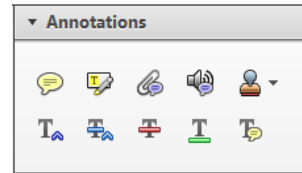
IMPORTANT: Do not click the “Complete Proof Review” button without replying to all author queries found on the last page of your proof. Incomplete proof reviews will cause a delay in publication.

IMPORTANT: Once you click “Complete Proof Review” you will not be able to publish further corrections.

Once you have Acrobat Reader open on your computer, click on the [Comment](#) tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the [Annotations](#) section, pictured opposite. We've picked out some of these tools below:



1. Replace (Ins) Tool – for replacing text.

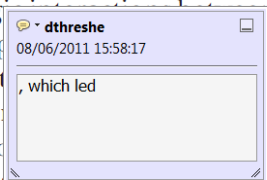


Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the [Replace \(Ins\)](#) icon in the Annotations section.
- Type the replacement text into the blue box that appears.

standard framework for the analysis of microeconomic activity. Nevertheless, it also led to the development of a number of strategic approaches to the analysis of the number of competitors in an industry. This is that the strategic approaches to the analysis of the main components of an industry, which led to the development of the level, are explained in the following section. The important words on this page by Shiraz (M henceforth) we open the 'black b



2. Strikethrough (Del) Tool – for deleting text.



Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the [Strikethrough \(Del\)](#) icon in the Annotations section.

there is no room for extra profits as mark ups are zero and the number of firms (net) values are not determined by market structure. Blanchard ~~and Kiyotaki~~ (1987), perfect competition in general equilibrium. The effects of aggregate demand and supply shocks in the classical framework assuming monopoly. An exogenous number of firms

3. Add note to text Tool – for highlighting a section to be changed to bold or italic.



Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the [Add note to text](#) icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

dynamic responses of mark ups consistent with the VAR evidence

sation by Markov. The VAR model is used to estimate the dynamic responses of mark ups consistent with the VAR evidence. The VAR model is used to estimate the dynamic responses of mark ups consistent with the VAR evidence.



4. Add sticky note Tool – for making notes at specific points in the text.



Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the [Add sticky note](#) icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.

and supply shocks. Most of the literature on the effects of aggregate demand and supply shocks in the classical framework assuming monopoly. An exogenous number of firms and the impact of aggregate demand and supply shocks in the classical framework assuming monopoly. An exogenous number of firms and the impact of aggregate demand and supply shocks in the classical framework assuming monopoly.



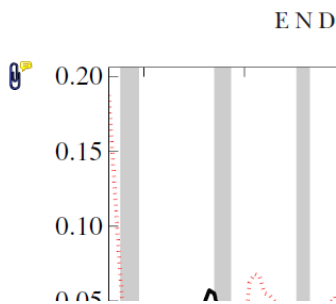
5. Attach File Tool – for inserting large amounts of text or replacement figures.



Inserts an icon linking to the attached file in the appropriate place in the text.

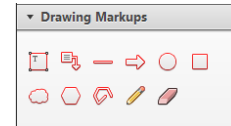
How to use it

- Click on the **Attach File** icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



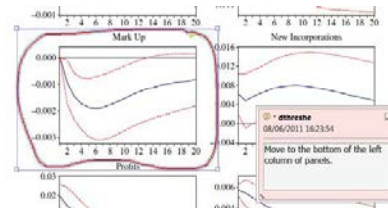
6. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks.



How to use it

- Click on one of the shapes in the Drawing Markups section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



Role of autophagy in HIV infection and pathogenesis

■ R. Nardacci¹, F. Ciccocanti¹, C. Marsella¹, G. Ippolito¹, M. Piacentini^{1,2} & G. M. Fimia^{1,3}

From the ¹National Institute for Infectious Diseases 'L. Spallanzani', IRCCS; ²Department of Biology, University of Rome 'Tor Vergata', Rome; and ³Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Lecce, Italy

Abstract. Nardacci R, Ciccocanti F, Marsella C, Ippolito G, Piacentini M, Fimia GM (National Institute for Infectious Diseases 'L. Spallanzani', IRCCS; University of Rome 'Tor Vergata', Rome; University of Salento, Lecce, Italy) (Review). *J Intern Med* 2017; doi: 10.1111/joim.12596.

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

Dispatch: 2.2.17	CE: Sudha
No. of pages: 11	PE: Anul S.
WILEY	
12596	Manuscript No.
J O I M	Journal Code
	

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 as IL-1 β , 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 autophagosome 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, autophagosome 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

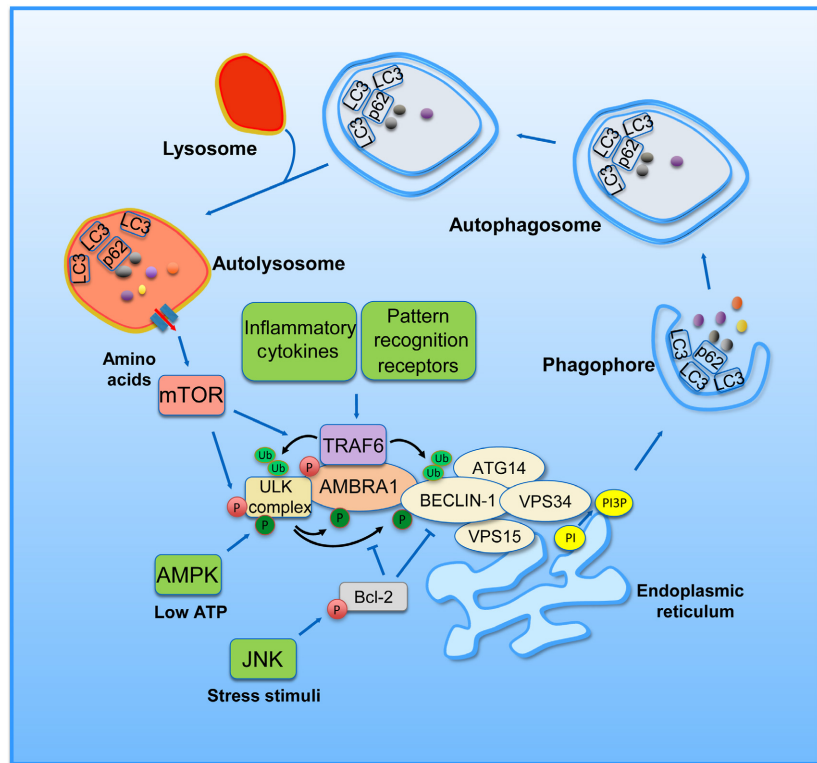


Fig. 1 Regulation of the autophagic process. The major protein complexes involved in the regulation of the different steps of autophagy are shown. The roles of the indicated proteins in the autophagic process are described in the text. PI, phosphatidylinositol; PI3P, phosphatidylinositol 3-phosphate; P, phosphorylation; Ub, ubiquitination.

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-I 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

1 viral protease into the mature GAG proteins
2 responsible for the generation of the HIV capsid.
3 The polymerase (POL) polyprotein is produced
4 together with GAG, as GAG–POL protein precursor.
5 *POL* gene is encoded by using a different reading
6 frame following a -1 frameshifting event, which
7 occurs with a frequency of 5–10%. The POL
8 precursor is processed by the viral protease into
9 the following enzymes: protease, integrase and the
10 reverse transcriptase. The ENV precursor gp160 is
11 processed in the two components of the viral
12 envelope: GP120 and GP41. The remaining group
13 of genes encodes transcription activators (TAT,
14 REV and VPR) and other regulatory proteins (VIF,
15 NEF and VPU).

17 HIV replication occurs in two distinct phases: the
18 early phase in which the HIV RNA is released in the
19 host cell cytoplasm, retro-transcribed and inte-
20 grated into the host genome and the late phase that
21 culminates in the production of new viral particles
22 [40].

24 The first step of the HIV life cycle is entry of the
25 virus into the cell. HIV recognizes the CD4 receptor
26 expressed on the surface of the target cell through
27 the viral glycoprotein GP120 [41]. This interaction
28 causes conformational changes in the GP120 pro-
29 tein that permits its interaction with the HIV
30 coreceptors chemokine CC receptor 5 (CCR5) or
31 chemokine CXC receptor 4 (CXCR4). The interac-
32 tion between GP120 and the coreceptors activates
33 the glycoprotein GP41, allowing the insertion of its
34 N-terminal fusion peptide into the target mem-
35 brane. This last step causes fusion between the
36 viral envelope and the host cellular membrane [41].
37 Once inside the cell, the HIV core is uncoated from
38 the genome to initiate viral replication. The genome
39 consists of two copies of single-stranded RNA held
40 together by two small proteins: P6 and P7; it is
41 released into a complex with the reverse transcrip-
42 tase, the integrase, the viral protease and the
43 accessory proteins NEF, VPR and VIF. HIV reverse
44 transcriptase transcribes single-stranded copies of
45 viral RNA into double-stranded DNA (dsDNA) [42].
46 The dsDNA is then transported into the nucleus by
47 the preintegration complex, composed of VPR and
48 the integrase. In the nucleus, the integrase catal-
49 yses the insertion of the dsDNA into the host
50 chromosome [43, 44].

52 HIV accessory proteins are not essential for viral
53 replication, but are important for *in vivo* infection
54 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 cooper-
ate 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 pro-
teins, 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 mul-
tiple strategies to avoid the recognition and degra-
dation of the newly synthesized viral particles
(Table 1).

The requirement of autophagy genes for HIV repli-
cation first emerged from unbiased RNA interfer-
ence (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 autophago-
some may provide membrane support for viral
replication [49].

Different pathways contribute to autophagy induc-
tion during HIV infection. IRGM, a member of a
large family of GTPases activated in response to
several types of infection, is required for stimulat-
ing 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

Table 1 Crosstalk between autophagy and HIV proteins

Host system	Cell type	HIV protein	Host protein	Role of the interaction	
Central nervous system	Glial cells	TAT	BAG3	Autophagy induction [86]	
	Astrocytes	NEF	?	Inhibition of autophagosome maturation [87]	
	Neurons	TAT	?	Autophagy inhibition [84]	
				LAMP2A	Autophagosome/lysosome fusion inhibition [80]
		ENV	mTOR signalling	Autophagy inhibition [79]	
Immune system	CD4+ T cells	ENV	CXCR4	Accumulation of BECLIN-1 and apoptosis induction [66–68, 93]	
		TAT	P62	Substrate of autophagy [60]	
	Dendritic cells	ENV	mTOR activation	Autophagy inhibition [75]	
		Macrophages	NEF	IRGM	Autophagy induction [50, 51]
				BECLIN-1	Inhibition of the maturation steps of autophagy [49, 54, 55]
				P62	Inhibition of TFEB nuclear translocation [55]
		GAG	LC3	GAG processing [49]	
		VIF	LC3	Autophagy inhibition [56]	
		HDAC6	VIF degradation [59]		
	P24	TRIM5a	Viral particle degradation [58]		

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 TRIM5 α 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 interaction between TRIM5 α and LC3 [58]. Moreover, the autophagy regulator HDAC6 directly interacts with VIF 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).

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

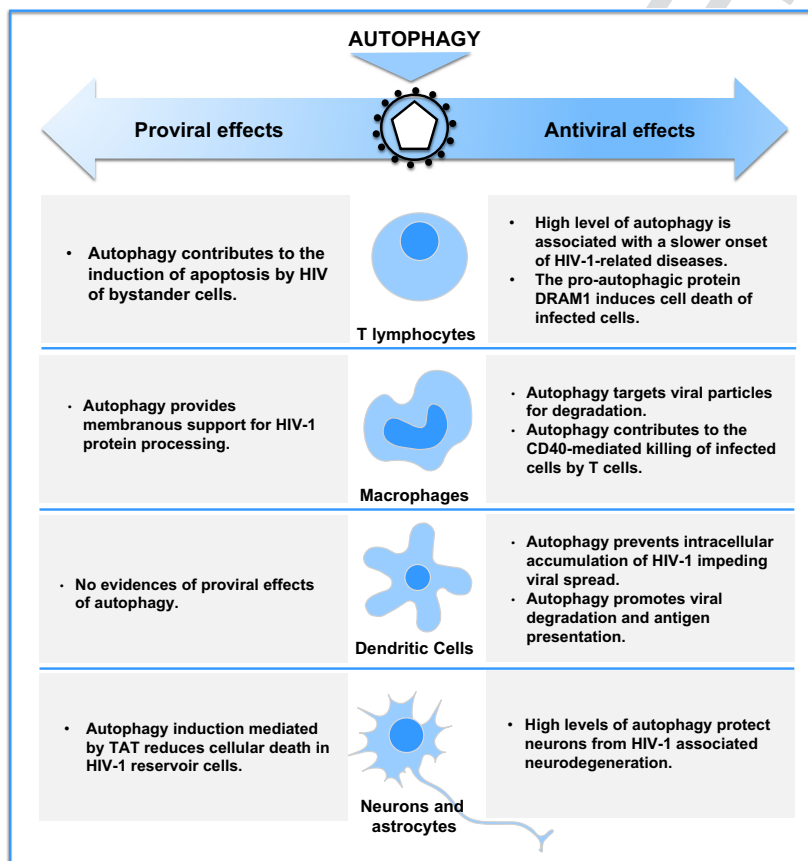


Fig. 2 Role of autophagy in HIV infection in different cell types. The different roles played by autophagy during HIV infection in different cellular systems are described. Further details are provided in the text.

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 induces 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 TRIM5 α , is expected to be valuable in paving the way for the development of new drugs able to specifically enhance the anti-HIV response.

Conflict of interest statement

No conflicts of interest to declare.

Acknowledgements

This work was supported in part by grants from AIRC (IG2015 no. 17404 to GMF and IG2014 no. 15244 to MP), the Italian Ministry of University and Research (FIRB Accordi di Programma 2011, PRIN2015 20152CB22L), the Italian Ministry of Health (Ricerca Corrente and Ricerca Finalizzata

RF2010 2305199) and Fondazione Fibrosi Cistica (Progetto FFC#8/2015).

References

- Mizushima N. Autophagy in protein and organelle turnover. *Cold Spring Harb Symp Quant Biol* 2011; **76**: 397–402.
- Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. *N Engl J Med* 2013; **368**: 651–62.
- Sahu R, Kaushik S, Clement CC *et al.* Microautophagy of cytosolic proteins by late endosomes. *Dev Cell* 2011; **20**: 131–9.
- Cuervo AM, Wong E. Chaperone-mediated autophagy: roles in disease and aging. *Cell Res* 2014; **24**: 92–104.
- Antonoli M, Di Rienzo M, Piacentini M, Fimia GM. Emerging Mechanisms in Initiating and Terminating Autophagy. *Trends Biochem Sci* 2016; **???**: ???–???
- Deretic V, Kimura T, Timmins G, Moseley P, Chauhan S, Mandell M. Immunologic manifestations of autophagy. *J Clin Invest* 2015; **125**: 75–84.
- Deretic V, Saitoh T, Akira S. Autophagy in infection, inflammation and immunity. *Nat Rev Immunol* 2013; **13**: 722–37.
- Randow F, Munz C. Autophagy in the regulation of pathogen replication and adaptive immunity. *Trends Immunol* 2012; **33**: 475–87.
- Lee HK, Lund JM, Ramanathan B, Mizushima N, Iwasaki A. Autophagy-dependent viral recognition by plasmacytoid dendritic cells. *Science* 2007; **315**: 1398–401.
- Ponpuak M, Mandell MA, Kimura T, Chauhan S, Cleyrat C, Deretic V. Secretory autophagy. *Curr Opin Cell Biol* 2015; **35**: 106–16.
- Liu T, Tang Q, Liu K *et al.* TRIM11 suppresses AIM2 inflammasome by degrading AIM2 via p62-dependent selective autophagy. *Cell Rep* 2016; **16**: 1988–2002.
- Kimura T, Jain A, Choi SW *et al.* TRIM-mediated precision autophagy targets cytoplasmic regulators of innate immunity. *J Cell Biol* 2015; **210**: 973–89.
- Liang Q, Seo GJ, Choi YJ *et al.* Crosstalk between the cGAS DNA sensor and Beclin-1 autophagy protein shapes innate antimicrobial immune responses. *Cell Host Microbe* 2014; **15**: 228–38.
- Konno H, Konno K, Barber GN. Cyclic dinucleotides trigger ULK1 (ATG1) phosphorylation of STING to prevent sustained innate immune signaling. *Cell* 2013; **155**: 688–98.
- Hampe J, Franke A, Rosenstiel P *et al.* A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1. *Nat Genet* 2007; **39**: 207–11.
- Rioux JD, Xavier RJ, Taylor KD *et al.* Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596–604.
- Walsh CM, Bell BD. T cell intrinsic roles of autophagy in promoting adaptive immunity. *Curr Opin Immunol* 2010; **22**: 321–5.
- Munz C. Autophagy beyond intracellular MHC class II antigen presentation. *Trends Immunol* 2016; **???**: ???–???
- Baxt LA, Garza-Mayers AC, Goldberg MB. Bacterial subversion of host innate immune pathways. *Science* 2013; **340**: 697–701.

- 20 Kirkegaard K. Subversion of the cellular autophagy pathway by viruses. *Curr Top Microbiol Immunol* 2009; **335**: 323–33.
- 21 Ktistakis NT, Tooze SA. Digesting the expanding mechanisms of autophagy. *Trends Cell Biol* 2016; **???:** ???–???.
- 22 Levine B, Liu R, Dong X, Zhong Q. Beclin orthologs: integrative hubs of cell signaling, membrane trafficking, and physiology. *Trends Cell Biol* 2015; **25**: 533–44.
- 23 Lamb CA, Nuhlen S, Judith D *et al.* TBC1D14 regulates autophagy via the TRAPP complex and ATG9 traffic. *EMBO J* 2016; **35**: 281–301.
- 24 Levine B, Sinha S, Kroemer G. Bcl-2 family members: dual regulators of apoptosis and autophagy. *Autophagy* 2008; **4**: 600–6.
- 25 Nakatogawa H, Ichimura Y, Ohsumi Y. Atg8, a ubiquitin-like protein required for autophagosome formation, mediates membrane tethering and hemifusion. *Cell* 2007; **130**: 165–78.
- 26 Lamb CA, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. *Nat Rev Mol Cell Biol* 2013; **14**: 759–74.
- 27 Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. *Annu Rev Cell Dev Biol* 2011; **27**: 107–32.
- 28 Khaminets A, Behl C, Dikic I. Ubiquitin-dependent and independent signals in selective autophagy. *Trends Cell Biol* 2016; **26**: 6–16.
- 29 Shen HM, Mizushima N. At the end of the autophagic road: an emerging understanding of lysosomal functions in autophagy. *Trends Biochem Sci* 2014; **39**: 61–71.
- 30 Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 2011; **13**: 132–41.
- 31 Nazio F, Strappazzon F, Antonioli M *et al.* mTOR inhibits autophagy by controlling ULK1 ubiquitylation, self-association and function through AMBRA1 and TRAF6. *Nat Cell Biol* 2013; **???:** ???–???.
- 32 Wei Y, Pattingre S, Sinha S, Bassik M, Levine B. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol Cell* 2008; **30**: 678–88.
- 33 Zalckvar E, Berissi H, Mizrachy L *et al.* DAP-kinase-mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. *EMBO Rep* 2009; **10**: 285–92.
- 34 Oh JE, Lee HK. Pattern recognition receptors and autophagy. *Front Immunol* 2014; **5**: 300.
- 35 Criollo A, Niso-Santano M, Malik SA *et al.* Inhibition of autophagy by TAB 2 and TAB 3. *EMBO J* 2011; **30**: 4908–20.
- 36 Shi CS, Kehrl JH. TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced autophagy. *Sci Signal* 2010; **3**: ra42.
- 37 Lackner AA, Lederman MM, Rodriguez B. HIV pathogenesis: the host. *Cold Spring Harb Perspect Med* 2012; **???:** ???–???.
- 38 Maartens G, Celum C, Lewin SR. HIV infection: epidemiology, pathogenesis, treatment, and prevention. *Lancet* 2014; **384**: 258–71.
- 39 Frankel AD, Young JA. HIV-1: fifteen proteins and an RNA. *Annu Rev Biochem* 1998; **67**: 1–25.
- 40 Freed EO. HIV-1 assembly, release and maturation. *Nat Rev Microbiol* 2015; **13**: 484–96.
- 41 Wilen CB, Tilton JC, Doms RW. HIV: cell binding and entry. *Cold Spring Harb Perspect Med* 2012; **2**: ???–???. doi: 10.1101/cshperspect.a006866.
- 42 Barre-Sinoussi F, Ross AL, Delfraissy JF. Past, present and future: 30 years of HIV research. *Nat Rev Microbiol* 2013; **11**: 877–83.
- 43 Krishnan L, Engelman A. Retroviral integrase proteins and HIV-1 DNA integration. *J Biol Chem* 2012; **287**: 40858–66.
- 44 Ambrose Z, Aiken C. HIV-1 uncoating: connection to nuclear entry and regulation by host proteins. *Virology* 2014; **454–455**: 371–9.
- 45 Simon V, Bloch N, Landau NR. Intrinsic host restrictions to HIV-1 and mechanisms of viral escape. *Nat Immunol* 2015; **16**: 546–53.
- 46 Sugden SM, Bego MG, Pham TN, Cohen EA. Remodeling of the host cell plasma membrane by HIV-1 Nef and Vpu: a strategy to ensure viral fitness and persistence. *Viruses* 2016; **8**: 67.
- 47 Brass AL, Dykxhoorn DM, Benita Y *et al.* Identification of host proteins required for HIV infection through a functional genomic screen. *Science* 2008; **319**: 921–6.
- 48 Eekels JJ, Sagnier S, Geerts D, Jeeninga RE, Biard-Piechaczyk M, Berkhout B. Inhibition of HIV-1 replication with stable RNAi-mediated knockdown of autophagy factors. *Virol J* 2012; **9**: 9–69.
- 49 Kyei GB, Dinkins C, Davis AS *et al.* Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages. *J Cell Biol* 2009; **186**: 255–68.
- 50 Gregoire IP, Richetta C, Meyniel-Schicklin L *et al.* IRGM is a common target of RNA viruses that subvert the autophagy network. *PLoS Pathog* 2011; **7**: e1002422.
- 51 Chauhan S, Mandell MA, Deretic V. IRGM governs the core autophagy machinery to conduct antimicrobial defense. *Mol Cell* 2015; **58**: 507–21.
- 52 Campbell GR, Spector SA. Toll-like receptor 8 ligands activate a vitamin D mediated autophagic response that inhibits human immunodeficiency virus type 1. *PLoS Pathog* 2012; **8**: e1003017.
- 53 Torresilla C, Larocque E, Landry S *et al.* Detection of the HIV-1 minus-strand-encoded antisense protein and its association with autophagy. *J Virol* 2013; **87**: 5089–105.
- 54 Shoji-Kawata S, Sumpter R, Leveno M *et al.* Identification of a candidate therapeutic autophagy-inducing peptide. *Nature* 2013; **494**: 201–6.
- 55 Campbell GR, Rawat P, Bruckman RS, Spector SA. Human immunodeficiency virus type 1 nef inhibits autophagy through transcription factor EB sequestration. *PLoS Pathog* 2015; **11**: e1005018.
- 56 Borel S, Robert-Hebmann V, Alfaisal J *et al.* HIV-1 viral infectivity factor interacts with microtubule-associated protein light chain 3 and inhibits autophagy. *AIDS* 2015; **29**: 275–86.
- 57 Jager S, Cimermanic P, Gulbahce N *et al.* Global landscape of HIV-human protein complexes. *Nature* 2011; **481**: 365–70.
- 58 Mandell MA, Jain A, Arko-Mensah J *et al.* TRIM proteins regulate autophagy and can target autophagic substrates by direct recognition. *Dev Cell* 2014; **30**: 394–409.
- 59 Valera MS, de Armas-Rillo L, Barroso-Gonzalez J *et al.* The HDAC6/APOBEC3G complex regulates HIV-1 infectiveness by inducing Vif autophagic degradation. *Retrovirology* 2015; **12**: 53.
- 60 Sagnier S, Daussy CF, Borel S *et al.* Autophagy restricts HIV-1 infection by selectively degrading Tat in CD4+ T lymphocytes. *J Virol* 2015; **89**: 615–25.

- 61 Espert L, Varbanov M, Robert-Hebmann V *et al.* Differential role of autophagy in CD4 T cells and macrophages during X4 and R5 HIV-1 infection. *PLoS One* 2009; **4**: e5787.
- 62 Campbell GR, Spector SA. Autophagy induction by vitamin D inhibits both *Mycobacterium tuberculosis* and human immunodeficiency virus type 1. *Autophagy* 2012; **8**: 1523–5.
- 63 Campbell GR, Spector SA. Vitamin D inhibits human immunodeficiency virus type 1 and *Mycobacterium tuberculosis* infection in macrophages through the induction of autophagy. *PLoS Pathog* 2012; **8**: e1002689.
- 64 Nardacci R, Amendola A, Ciccocanti F *et al.* Autophagy plays an important role in the containment of HIV-1 in nonprogressor-infected patients. *Autophagy* 2014; **10**: 1167–78.
- 65 Perfettini JL, Castedo M, Nardacci R *et al.* Essential role of p53 phosphorylation by p38 MAPK in apoptosis induction by the HIV-1 envelope. *J Exp Med* 2005; **201**: 279–89.
- 66 Perfettini JL, Castedo M, Roumier T *et al.* Mechanisms of apoptosis induction by the HIV-1 envelope. *Cell Death Differ* 2005; **12**(Suppl 1): 916–23.
- 67 Espert L, Denizot M, Grimaldi M *et al.* Autophagy is involved in T cell death after binding of HIV-1 envelope proteins to CXCR4. *J Clin Invest* 2006; **116**: 2161–72.
- 68 Denizot M, Varbanov M, Espert L *et al.* HIV-1 gp41 fusogenic function triggers autophagy in uninfected cells. *Autophagy* 2008; **4**: 998–1008.
- 69 Laforge M, Limou S, Harper F *et al.* DRAM triggers lysosomal membrane permeabilization and cell death in CD4(+) T cells infected with HIV. *PLoS Pathog* 2013; **9**: e1003328.
- 70 Doitsh G, Galloway NL, Geng X *et al.* Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature* 2014; **505**: 509–14.
- 71 Monroe KM, Yang Z, Johnson JR *et al.* IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. *Science* 2014; **343**: 428–32.
- 72 Alexaki A, Liu Y, Wigdahl B. Cellular reservoirs of HIV-1 and their role in viral persistence. *Curr HIV Res* 2008; **6**: 388–400.
- 73 Van Grol J, Subauste C, Andrade RM, Fujinaga K, Nelson J, Subauste CS. HIV-1 inhibits autophagy in bystander macrophage/monocytic cells through Src-Akt and STAT3. *PLoS One* 2010; **5**: e11733.
- 74 Manches O, Frleta D, Bhardwaj N. Dendritic cells in progression and pathology of HIV infection. *Trends Immunol* 2014; **35**: 114–22.
- 75 Blanchet FP, Moris A, Nikolic DS *et al.* Human immunodeficiency virus-1 inhibition of immunoamphisomes in dendritic cells impairs early innate and adaptive immune responses. *Immunity* 2010; **32**: 654–69.
- 76 Harman AN, Kraus M, Bye CR *et al.* HIV-1-infected dendritic cells show 2 phases of gene expression changes, with lysosomal enzyme activity decreased during the second phase. *Blood* 2009; **114**: 85–94.
- 77 Zhou D, Kang KH, Spector SA. Production of interferon alpha by human immunodeficiency virus type 1 in human plasmacytoid dendritic cells is dependent on induction of autophagy. *J Infect Dis* 2012; **205**: 1258–67.
- 78 Saylor D, Dickens AM, Sacktor N *et al.* HIV-associated neurocognitive disorder—pathogenesis and prospects for treatment. *Nat Rev Neurol* 2016; **12**: 234–48.
- 79 Zhou D, Masliah E, Spector SA. Autophagy is increased in postmortem brains of persons with HIV-1-associated encephalitis. *J Infect Dis* 2011; **203**: 1647–57.
- 80 Fields J, Dumaop W, Adame A *et al.* Alterations in the levels of vesicular trafficking proteins involved in HIV replication in the brains and CSF of patients with HIV-associated neurocognitive disorders. *J Neuroimmune Pharmacol* 2013; **8**: 1197–209.
- 81 Alirezaei M, Kiosses WB, Flynn CT, Brady NR, Fox HS. Disruption of neuronal autophagy by infected microglia results in neurodegeneration. *PLoS One* 2008; **3**: e2906.
- 82 Bagashev A, Sawaya BE. Roles and functions of HIV-1 Tat protein in the CNS: an overview. *Virology* 2013; **10**: 358.
- 83 Debaisieux S, Rayne F, Yezid H, Beaumelle B. The ins and outs of HIV-1 Tat. *Traffic* 2012; **13**: 355–63.
- 84 Hui L, Chen X, Haughey NJ, Geiger JD. Role of endolysosomes in HIV-1 Tat-induced neurotoxicity. *ASN Neuro* 2012; **4**: 243–52.
- 85 Zhou L, Saksena NK. HIV associated neurocognitive disorders. *Infect Dis Rep* 2013; **5**: e8.
- 86 Bruno AP, De Simone FI, Iorio V *et al.* HIV-1 Tat protein induces glial cell autophagy through enhancement of BAG3 protein levels. *Cell Cycle* 2014; **13**: 3640–4.
- 87 Saribas AS, Khalili K, Sariyer IK. Dysregulation of autophagy by HIV-1 Nef in human astrocytes. *Cell Cycle* 2015; **14**: 2899–904.
- 88 Di Benedetto F, Di Sandro S, De Ruvo N *et al.* First report on a series of HIV patients undergoing rapamycin monotherapy after liver transplantation. *Transplantation* 2010; **89**: 733–8.
- 89 Gangcuangco LM, Kohorn LB, Chow DC *et al.* High 25-hydroxyvitamin D is associated with unexpectedly high plasma inflammatory markers in HIV patients on antiretroviral therapy. *Medicine (Baltimore)* 2016; **95**: e5270.
- 90 Campbell GR, Bruckman RS, Chu YL, Spector SA. Autophagy induction by histone deacetylase inhibitors inhibits HIV type 1. *J Biol Chem* 2015; **290**: 5028–40.
- 91 Demonte D, Quivy V, Colette Y, Van Lint C. Administration of HDAC inhibitors to reactivate HIV-1 expression in latent cellular reservoirs: implications for the development of therapeutic strategies. *Biochem Pharmacol* 2004; **68**: 1231–8.
- 92 Chauhan S, Ahmed Z, Bradfute SB *et al.* Pharmaceutical screen identifies novel target processes for activation of autophagy with a broad translational potential. *Nat Commun* 2015; **6**: 8620.
- 93 Castedo M, Perfettini JL, Roumier T, Kroemer G. Cyclin-dependent kinase-1: linking apoptosis to cell cycle and mitotic catastrophe. *Cell Death Differ* 2002; **9**: 1287–93.

Correspondence: Mauro Piacentini, Department of Biology, University of Rome 'Tor Vergata', 00173 Rome, Italy. (fax: xxxx; e-mail: mauro.piacentini@uniroma2.it). ■

Author Query Form

Journal: JOIM
Article: 12596

Dear Author,

During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication. Many thanks for your assistance.

Query reference	Query	Remarks
1	AUTHOR: A running head short title was not supplied; please check if this one is suitable and, if not, please supply a short title of up to 40 characters that can be used instead.	
2	AUTHOR: Please confirm that given names (red) and surnames/family names (green) have been identified correctly.	
3	AUTHOR: Please provide fax details for corresponding author.	
4	AUTHOR: Please provide appropriate content for “?” in Table 1.	
5	AUTHOR: Please provide the volume number, page range for reference [5].	
6	AUTHOR: References [12] and [72] are identical. Hence, reference [72] is deleted and rest of the references is renumbered. Please check.	
7	AUTHOR: Please provide the volume number, page range for reference [18].	
8	AUTHOR: Please provide the volume number, page range for reference [21].	
9	AUTHOR: Please provide the volume number, page range for reference [31].	
10	AUTHOR: Please provide the volume number, page range for reference [37].	
11	sAUTHOR: Please provide the page range for reference [41].	
12	AUTHOR: References [67] and [95] are identical. Hence, reference [95] is deleted and rest of the references is renumbered. Please check.	