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Campbell, Grant R
Spector, Stephen A

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Inhibition of Human Immunodeficiency Virus Type-1 through Autophagy

Grant R. Campbell¹ and Stephen A. Spector^{1,2}

¹Department of Pediatrics, Division of Infectious Diseases, University of California San Diego, La Jolla, California 92093-0672, USA

²Rady Children's Hospital, San Diego, California, 92123, USA

Abstract

As an obligatory intracellular pathogen, human immunodeficiency virus type-1 (HIV) is dependent upon its ability to exploit host cell machinery for replication and dissemination, and to circumvent cellular processes that prevent its growth. One such intracellular process is autophagy, a component of the host defense against HIV with roles in innate immune signaling, adaptive immunity and intracellular degradation of HIV. During permissive infection, HIV down-modulates autophagy, promoting its own replication. Inducers of autophagy can overcome this suppression and inhibit HIV. This review summarizes recent advances in understanding the antiviral and replicative roles of autophagy during HIV infection. Dissecting the molecular mechanisms by which HIV utilizes autophagy may lead to the identification of novel drug candidates to treat and potentially eradicate HIV infection.

INTRODUCTION

Following an acute primary infection, human immunodeficiency virus type-1 (HIV) establishes a reservoir of infected long-lived cells while CD4⁺ T cells are progressively lost leading to AIDS. Whether HIV establishes true latency or a persistent infection with continued low-level viral replication during periods of prolonged viral suppression remains controversial. However, increasing data examining occult reservoirs support some continued viral replication in most persons. As an obligatory intracellular parasite, HIV survival is dependent upon its ability to exploit host cell machinery for replication and dissemination and to evade intrinsic cellular processes and defenses that may limit viral replication and pathogenesis including autophagy – a cell-autonomous innate immune defense whereby cells eliminate intracellular pathogens by capture into autophagosomes with subsequent killing through macroautophagy (a process sometimes referred to as xenophagy) [1].

Macroautophagy (hereafter referred to as autophagy) is a degradation pathway whereby cytosolic double membrane-bound compartments termed autophagosomes engulf cytoplasmic constituents such as sub-cellular organelles and microbial pathogens, including viruses, and target them for lysosomal degradation. During autophagy, the physical

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Address correspondence to: Stephen A. Spector, MD, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0672, USA, **Phone:** 858-534-7055, **Fax:** 858-534-7411, saspector@ucsd.edu.

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interaction of the phosphatidylinositol 3-kinase, catalytic subunit type 3 (PIK3C3) with Beclin-1 (BECN1) forms the PIK3C3 kinase complex producing phosphatidylinositol-3-phosphate (PI3P), which is essential for the induction of the nascent phagophore by recruiting PI3P binding proteins such as RAB5A and ATG14. To form the autophagosome, two intertwined conjugation systems play a role. In the first, ATG12 is activated by ATG7, an E1-like ubiquitin activating enzyme, transferred to ATG10, an E2-like ubiquitin conjugating enzyme which covalently conjugates it to ATG12. ATG12–ATG5 non-covalently binds ATG16L1 and further elongates the phagophore membrane. In the second, the cytosolic microtubule-associated protein 1 light chain 3 beta (LC3B)-I, encoded by *MAP1LC3B*, is converted to LC3B-II by a ubiquitin-like system that involves ATG7 and ATG3. The ATG12–ATG5–ATG16L1 complex functions as an E3-like ubiquitin ligase, ligating LC3B-II to the nascent autophagosome membrane through phosphatidylethanolamine with the LC3B-II associated with the inner membrane degraded after fusion of the autophagosome with lysosomes.

Although the first reports of autophagy during viral infections appeared in the 1960s [2], the potential significance of this is only recently being appreciated. An expanding literature indicates that autophagy, in a virus-specific manner, may function to both degrade viruses and promote viral replication. Moreover, autophagy is not only an important component of the innate immune system, but also helps to modulate the adaptive immune system through MHC class I and class II antigen presentation (Figure 1).

The role of autophagy in HIV pathogenesis has, until recently, been under appreciated. However, over the past five years an increasing number of laboratories have described the interaction of HIV and autophagy. As often occurs in new areas of investigation, there are contradictory findings. Of note, it is important to differentiate studies that are performed in continuous transformed cell lines or with pseudotyped virus from those performed using primary cells and infectious HIV isolates. Often, transformed cells are under different autophagic homeostasis than primary cells and may lead to findings that are not corroborated when evaluated in primary cells. Similarly, single-round replication deficient HIV may not provide a complete picture of cells that are infected with multiple-round replication competent virus, although they can help to dissect molecular processes. In this review, we have summarized many of the key studies that have been performed, to date, regarding HIV and autophagy.

HIV subverts autophagy to promote its own replication

Of the more than 35 human autophagy-associated genes currently known to be involved in autophagy, ten have been linked with HIV replication. Using a large-scale small RNAi screen to identify host factors required by replication competent HIV in a HeLa-derived cell line, Brass *et al.* [3] identified more than 250 HIV-dependency factors. These included four genes involved in the nucleation and elongation of autophagosomes (*ATG7*, *MAP1LC3B*, *ATG12*, *ATG16L2*) and two involved in lysosomal function (*CLN3* and *LAPTM5*) that were essential for HIV replication. In another cell-line model, Eekels *et al.* [4] demonstrated that RNAi of *PIK3R4*, *ATG4A*, *ATG5* or *ATG16* resulted in the inhibition of replication competent CXCR4-tropic HIV in SupT1 cells. An additional two groups have demonstrated that HIV replication in primary cells is inhibited upon autophagy-associated gene silencing: *BECN1* and *ATG5* in macrophages [5–7], and *BECN1* and *ATG7* in monocytes [8].

In agreement with these RNAi studies, the current data suggest that the early steps of autophagy are important in HIV replication in cells of the monocyte/macrophage lineage. Espert *et al.* [9] demonstrated that autophagosome accumulation occurs in productively infected primary macrophages infected through exposure to chronically infected MOLT-4

effector cells. They found that virions were only detectable in cells with moderate, but not high levels of accumulated autophagosomes suggesting that HIV elicits the induction of autophagy but blocks its late proteolytic stage. Consistent with these findings, HIV assembly is thought to occur on endocytic membranes that intersect with recycling endosomes [10,11]. HIV Gag-derived proteins colocalize and interact with LC3B in macrophages, and are present at LC3B-II enriched membranes suggesting that autophagy may be involved in Gag processing and the production of nascent virions [8]. Moreover, the HIV accessory protein Nef interacts with immunity-associated GTPase family M (IRGM), which itself interacts with ATG5, ATG10, LC3B and SH3GLB1, inducing autophagosome formation and enhancing HIV replication [12]. Finally, in HIV-infected macrophages where the nucleation and formation of autophagosomes was inhibited using small-molecule chemical inhibitors or RNAi, the production of infectious virions was significantly decreased [7–9] (Figure 1).

Although HIV may upregulate autophagy during the initial stages of primary infection, HIV must control the antiviral proteolytic and degradative late stages of autophagy to avoid its degradation (Figure 2). The current data suggest that HIV has developed mechanisms to inhibit autophagic degradation in CD4⁺ T cells [13] and cells of the monocyte/macrophage lineage [8]. Kyei *et al.* [8] demonstrated that in HIV-infected macrophages, HIV Nef plays a major role in the inhibition of the proteolytic stages of autophagy by binding BECN1. Interestingly, the authors demonstrated that the Nef¹⁷⁴DD motif previously shown to be required for CD4 downregulation and interactions with the V₁ domain of the vacuolar H⁺-ATPase [14] was required for Nef to act as an anti-autophagic maturation factor. Moreover, Nef-deficient HIV was unable to overcome autophagic degradation and thus replicated less efficiently. It is unlikely that Nef influences H⁺-ATPase assembly or activity thereby inhibiting autophagosome acidification or autophago-lysosome fusion as the inhibition of endosome acidification was previously shown to be independent of Nef [15]. Thus, the protein complex containing Nef and BECN1 may act through a mechanism other than acidification, possibly through the redistribution of membrane-associated PIK3C3 [8]. A recent study demonstrated that Nef binds to amino acids 267–284 in the BECN1 evolutionarily conserved domain [16]. This is the same region that is necessary and sufficient for BECN1 to bind GLIPR2 (GAPR-1) [16], a protein that associates with lipid rafts at the cytosolic leaflet of the Golgi membrane [17] and that negatively regulates autophagy by sequestering BECN1 to the Golgi complex (where it is inactive in autophagy) [16]. Whereas Nef may not alter autophagosome acidification, recent findings from our laboratory suggest that during permissive infection HIV prevents the fusion of autophagosomes with lysosomes through the inhibition of the hepatocyte growth factor required tyrosine kinase substrate (HGS) by a yet unidentified mechanism.

HIV infection of CD4⁺ T cells is associated with the initial induction of autophagy associated with the fusion activity of the HIV gp41 protein [18] followed by the inhibition of autophagy and reduced autophagosome accumulation during productive infection [9,13,19]. It is important to note that during permissive infection of primary cells, HIV down modulates autophagy. Nevertheless, autophagy is never completely abrogated since it is required for cell survival. However, inducers of autophagy, including amino acid starvation and rapamycin, overcome the Nef-imposed phagosome maturation block leading to inhibition of viral replication [13] (Figure 1).

HIV-induced autophagy in bystander cells

HIV infection and the progression to AIDS are characterized by the depletion of CD4⁺ T cells through apoptosis of the uninfected bystander cells and the direct killing of HIV-infected cells. More recently, HIV has been shown to influence autophagy in HIV-

uninfected bystander cells. HIV Tat inhibits interferon- γ -induced autophagy induction in uninfected macrophages through the inhibition of STAT1 phosphorylation [20]. Tat has also been shown to inhibit autophagy in uninfected macrophages through binding CXCR4, VEGFR1 and β -integrins, and through the induction of AKT1, SRC and IL10 production [21]. As autophagy is an immune effector mechanism for the targeted destruction of several intracellular pathogens, the inhibition of autophagy in bystander macrophages may contribute to the susceptibility of HIV-1-infected patients to tuberculosis and toxoplasmosis amongst others. Moreover, as autophagy promotes antigen presentation, the inhibition of autophagy may promote pathogen immune evasion.

In contrast to the effect of Tat on uninfected macrophages, HIV Env protein induces apoptosis in uninfected CD4⁺ T cells [9,22] and neurons [23] through a mechanism involving the accumulation of Beclin-1 and the induction of autophagy. Within CD4⁺ T cells, this is dependent upon the fusion activity of the HIV gp41 protein [18] regardless of the co-receptor used for HIV entry [9]. How the fusogenic function of gp41 induces autophagy in CD4⁺ T cells and why gp41 induces autophagy in CD4⁺ T cells but not in macrophages is unknown [9]. Within the central nervous system, HIV gp120 induces autophagy in neuroblastoma cells [23]. Postmortem brains from HIV encephalitis (HIVE) patients exhibit increased markers of autophagy compared with brains from HIV infected individuals without HIVE or HIV uninfected controls [23,24]. However, it remains unknown whether autophagy proceeds to completion in these brains or if there is an accumulation of autophagic proteins without autophagy-related degradation. These findings, however, provide support for the hypothesis that the dysregulation of autophagy during HIV infection is important in the pathogenesis of neuroAIDS [25].

Utilizing autophagy to control HIV pathogenesis

Cells can use autophagy in a number of different antiviral roles. These include xenophagy, the presentation of endogenous viral antigens to the major histocompatibility complexes and subsequent activation of the adaptive immune system, and as a mechanism to detect intracellular pathogens through delivery of viral products to endosomal pattern-recognition receptors (PRRs), which recognize signature molecules of pathogens termed pathogen-associated molecular patterns (PAMPs).

Recent research has focused on the role of autophagy in the innate and adaptive immune systems with pharmacological inducers, enhancers and inhibitors of autophagy currently being examined for the treatment of cancer [26], lymphangiomyomatosis, [27], proteinopathies such as Huntington's disease [28], and infectious diseases including *Mycobacterium tuberculosis* [5,29] with some currently in clinical trials. In contrast, despite the strong need for the development of alternative antiretrovirals due to the emergence of resistant viruses, the complexity of current treatment regimens and the toxicity of currently used antiretrovirals, few studies have examined the use of pharmacologic enhancers of autophagy in the treatment of HIV. The induction and modulation of autophagy through pharmacological means to enhance HIV treatment is attractive. Since autophagy works at the host cellular level to improve intracellular killing of both replicating and non-replicating HIV within endosomes, it has the potential to be used in combination with antiretrovirals to treat and potentially eradicate HIV. Moreover, as autophagy is a cellular process, viral resistance is less likely to develop.

Rapamycin, a specific MTOR inhibitor and inducer of autophagy inhibits HIV replication through both the downregulation of CCR5 [30] and the induction of autophagy [7]. Rapamycin has also been found to inhibit HIV infection in human peripheral blood leucocytes-SCID reconstituted mice [31]. Of interest, rapamycin also works synergistically

to enhance the activity of entry inhibitors such as vicriviroc, aplaviroc and enfuvirtide [31]. However, rapamycin has immunosuppressive and other adverse effects that limit its potential usefulness in the treatment of HIV.

A cell-permeable autophagy-inducing peptide, termed Tat–Beclin-1 derived from the region of BECN1 that interacts with HIV Nef and conjugated to the basic region of HIV Tat has recently been found to markedly inhibit HIV replication in primary human macrophages through the induction of autophagy through the canonical pathway with the same magnitude as rapamycin [16]. This peptide was also able to restrict the *in vitro* replication of Sindbis virus, chikungunya virus and West Nile Virus, and reduced the mortality of mice infected with West Nile Virus and chikungunya virus [16].

The antimicrobial effects of vitamin D have been well documented and association studies have linked low levels of 25-hydroxycholecalciferol (25D3) and/or the active metabolite 1 α , 25-dihydroxycholecalciferol (1,25D3) with increased risk of or severity of infection with HIV [32,33]. Physiological concentrations of 1,25D3 induces the dose-dependent inhibition of HIV alone and in *Mycobacterium tuberculosis* co-infected macrophages that is dependent upon both the induction of autophagy and autophagosomal maturation [5,7]. The precise mechanism by which 1,25D3 induces autophagy has yet to be elucidated, but it is known that it is dependent upon the human cathelicidin antimicrobial peptide (CAMP). This conclusion is supported by recent studies demonstrating that CAMP silencing inhibits 1,25D3-induced autophagy and significantly lowers the 1,25D3-mediated restriction of HIV replication and antimycobacterial activities [5,34]. Endogenous CAMP has been implicated in a number of cellular functions including the regulation of inflammatory responses [35] and the formation and maturation of autophagosomes [34]. CAMP has also been shown to play an important role in the activation of mitogen activated protein kinases and CEBPB which contribute to the transcriptional activation of *BECN1* and *ATG5* in response to 1,25D3 [34]. Moreover, during autophagy, autophagosomes recruit CAMP through an AMP kinase, calcium and CAMKK2 dependent mechanism where it is involved in microbial killing [34]. Further work is necessary to determine the precise role of CAMP in autophagy and antiretroviral activity.

Vitamin D has also been implicated in the autophagic response to PAMPs by PRRs. There are several classes of PRRs: Toll like receptors (TLRs), retinoic acid-inducible gene-I-like receptors and nucleotide-binding oligomerization domain-like receptors. The single-stranded RNA genome of HIV contains multiple PAMPs that are recognized by endosomal TLR7 in dendritic cells, and TLR8 in macrophages [36–38]. Upon stimulation, TLR7/8 agonists activate NF κ B1 via MYD88 that leads to the induction of a cascade of antiviral effector functions including the induction of autophagy [6,39] and proinflammatory cytokines [38]; thus significantly impairing the permissivity of peripheral blood mononuclear cells to HIV infection [40]. Interestingly, HIV downregulates IRAK4, which is essential for virtually all TLR signaling [41]. In macrophages, the TLR8-mediated inhibition of HIV is dependent upon both the presence and upregulation of vitamin D metabolism and the vitamin D (1,25D3) receptor which induces autophagy through a CAMP dependent mechanism [6]. In the case of plasmacytoid dendritic cells, the TLR7-mediated expression of the proinflammatory cytokine interferon- α is dependent upon the induction of autophagy [42].

Collectively, these studies demonstrate that inducers of autophagy inhibit HIV replication. Well-controlled clinical trials are needed to determine if autophagy inducers are of value as adjunctive treatment in HIV-infected persons. Dissecting the molecular mechanisms by which HIV utilizes autophagy has the potential to lead to the identification of novel drug candidates to treat and potentially to eradicate HIV infection, as well as preventing HIV-related complications.

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Highlights

- HIV inhibits autophagy during permissive infection
- Modulation of autophagy alters HIV pathogenesis and development of CNS impairment
- The induction of autophagy inhibits HIV
- Vitamin D induces autophagy and inhibits HIV at physiologic concentrations
- TLR8 inhibition of HIV requires vitamin D and induction of autophagy and cathelicidin

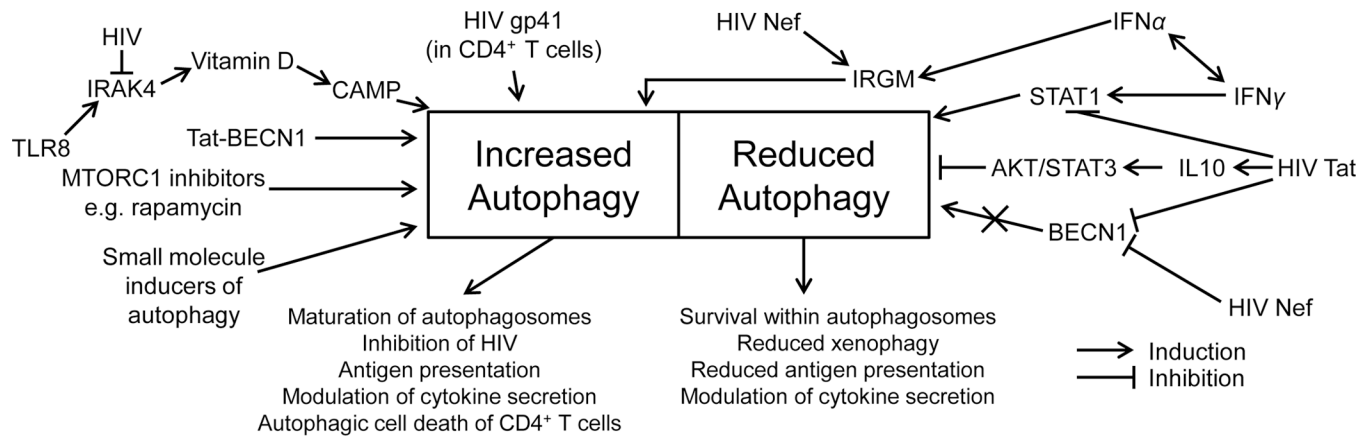


Figure 1. Modulation of autophagy

Autophagy can be pharmacologically induced in macrophages by various inducers of autophagy such as MTORC1 inhibitors, the Tat-Beclin peptide, drugs acting via inositol 1,4,5-triphosphate, by TLR8 agonists through the induction of the vitamin D pathway, by vitamin D directly through the induction of CAMP, and by cytokines such as IFN γ , which is dependent upon IRGM. The HIV protein Tat inhibits autophagy through the inhibition of STAT1 phosphorylation and by increasing the secretion of IL10. HIV Nef both increases autophagy through interactions with IRGM and inhibits autophagy through Beclin-1 sequestration. HIV infection of CD4⁺ T cells is associated with the initial induction of autophagy associated with the fusion activity of the HIV gp41 protein.

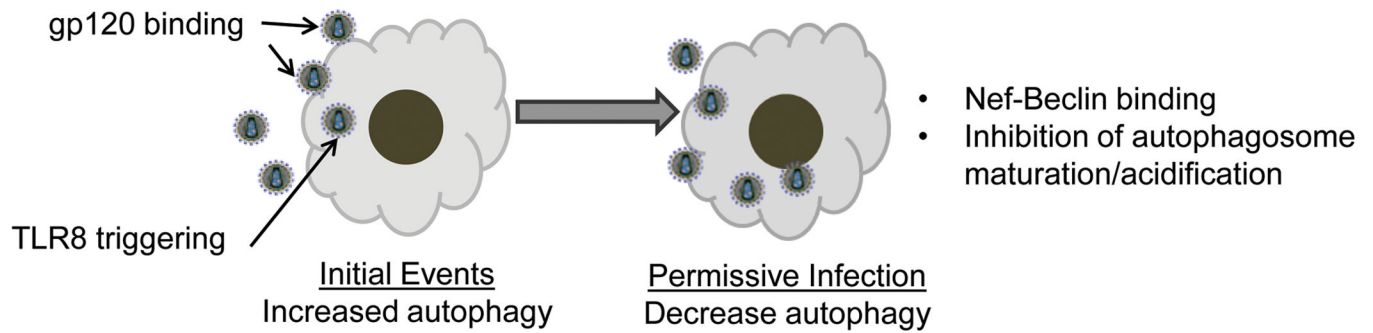


Figure 2. Schematic representation of modulation of autophagy by HIV

During the initial stages of infection, HIV or gp120 binding and TLR8 signaling induce autophagy to promote viral replication. However, during permissive infection autophagy is down-regulated through Nef-Beclin interaction and inhibition of autophagosome acidification promoting viral persistence and cell survival. However, as shown in Figure 1, inducers of autophagy promote increased autolysosome formation with degradation of HIV.