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Is Human Immunodeficiency Virus-Mediated Dementia an Autophagic Defect that Leads to Neurodegeneration?

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Abstract

Autophagy is a cellular process that mediates selective degradation of cellular components in lysosomes. Autophagy may protect against neuronal apoptosis, which is induced in a number of neurodegenerative diseases. Thus, compounds that modulate autophagy could be beneficial to treat neurological disorders characterized by apoptosis such as Parkinson's and Alzheimer's diseases, as well as human-immunodeficiency virus-dementia complex. In this paper, we review new and old evidence on the role of autophagy in neuronal cell survival and we present evidence that human-immunodeficiency virus may have adapted strategies to alter autophagic pathways in neurons. Moreover, we discuss the usefulness of drugs that facilitate autophagic clearance of proteins that are associated with neurodegeneration.

Keywords

Autophagy; apoptosis; beclin-1; gp120; lysosomes; Parkin

INTRODUCTION

Autophagy (or macroautophagy) is a normal physiological mechanism that plays a crucial role in the degradation of cellular components and damaged organelles. In addition, autophagy has been involved in mammalian cell-autonomous defense mechanisms against pathogens, including bacteria and viruses [1–3]. Autophagy comprises formation of autophagic vacuoles, called autophagosomes, which contain cellular debris and delivery them to lysosomes for proteolytic degradation. Autophagy can also regulate lipid metabolism (lipophagy) including cholesterol, or remove damaged mitochondria

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CONFLICT OF INTEREST

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(mitophagy). Thus, autophagy is implicated in a number of human physiological and pathological conditions.

There are two other types of autophagy, chaperone-mediated autophagy and microautophagy. The former utilizes the chaperone heat-shock cognate protein 70 for specific substrate recognition. Proteins are then delivered to the lysosomal membrane where they are unfolded and translocated into the lysosomal lumen. Microautophagy, on the other hand, promotes direct engulfment of cytoplasmic substrates by the lysosomes through invagination of the lysosomal membrane [4].

Baseline autophagy is a dynamic multi-step process, comprising several compartments, including the pre-autophagosomal sequestering phagophore [5] and the autophagosome [6]. The autophagosome can fuse with endosomes to form amphisomes [7, 8], which may be involved in the degradation of internalized species *via* endocytosis (Fig. 1). Autophagosomes and amphisomes may then fuse with lysosomes to generate autolysosomes or autophagolysosomes, respectively. Therefore, autolysosomes can potentially clear accumulating intracellular debris, like misfolded proteins and defective organelles, while autophagolysosomes may degrade internalized species *via* endocytosis and the endosomal pathways (i.e. extracellular amyloid, pathogens). Although, autophagy can be a cellular survival mechanism to eliminate unwanted materials, it can also be detrimental depending on the cellular environment. For example, cell death may occur when the autophagic flux is blocked, when autophagosome fusion with lysosomes is inhibited, or as a result of loss of the proteolytic lysosomal functions [9, 10].

AUTOPHAGY: A PROCESS INVOLVING MULTIPLE PROTEINS

Autophagosome formation is initiated *via* phosphorylation of several proteins, including autophagy-related (Atg)–Unc51-like kinase complex [11, 12], which is phosphorylated by the serine/threonine protein kinase mammalian target of Rapamycin (mTOR) complex 1 (TORC1) and activated by AMP-activated protein kinase [13]. Unc51-like kinase phosphorylation may trigger molecular cascades to enwrap cytoplasmic materials, resulting in isolation of a sequestering membrane [14], which is enriched with complexes containing ubiquitin-like proteins [15]. Ubiquitin is a small (8.5 KDa) protein that tags and targets other proteins to proteosomes for their disposal [16]. A second process involves the conjugation of microtubule associated protein 1 light chain 3 (LC3) to phosphatidylethanolamine. LC3 undergoes post-translational C-terminal cleavage resulting in the cytosolic LC3-I form, which is then conjugated to phosphatidylethanolamine to generate the autophagosome associated LC3-II form. LC3-II mediates membrane tethering and hemifusion essential for the expansion and closure of phagophores to form autophagosomes. Thus, the levels of LC3-II are often used as a tool to determine the amount of autophagosomes and therefore induction of autophagy [17, 18]. Autophagosome clearance, on the other hand, can be initiated via translocation of a multiprotein complex containing Beclin-1, a Bcl-2-homology domain only protein, and class III phosphoinositide 3-kinase (also known as lipid kinase Vps34) from the cytoskeleton [19, 20] to pre-autophagosomal structures. The activity of hVps34 is enhanced by its interaction with Beclin-1 which, in turn, forms a macromolecule complex consisting of Beclin-1/Atg6, Atg14L and hVps15. Nevertheless, Beclin-1 may bind

to anti-apoptotic proteins such as Bcl-2 and Bcl-XL and negatively regulates autophagy [18, 21].

Several cellular stress signals may induce autophagy, including alterations of amino acid concentration, growth factors such as insulin, ATP, hypoxia, and accumulation of molecular and cellular debris [22]. However, it appears that different cell types differentially respond to stress signals. For example, inhibition of TORC1, which induces autophagy in non-neuronal cells, mediates translational control necessary for memory reconsolidation, long-term potentiation [23], and dendritic arborization [24] in the central nervous system (CNS). Therefore, TORC1 inhibition may serve different functions in peripheral dividing cells (muscle, blood, liver) versus post-mitotic neurons. This property of TORC1 is important because pharmacological inhibitors of TORC1 kinase activity such as rapamycin and analogs can be used as possible anti-cancer drugs as well as to ameliorate diseases of the CNS.

AUTOPHAGY AND CNS DISEASES

Neurons are unique among other cell types in that they do not re-enter the cell cycle and thus cannot use mitosis as a method for clearing abnormally aggregated proteins. When proteins aggregate and accumulate in the cytoplasm, cytotoxicity emerges. Inefficient recognition of aggregated proteins or defective organelles has been described in models of neurodegeneration [25], whereby molecular steps of autophagy are activated, but autophagosome clearance, due to inefficient fusion with the lysosome, is defective [9]. Thus, accumulation of abnormal protein aggregates has been suggested to be a central common mechanism underlying human neurodegenerative diseases. These include Alzheimer's, Parkinson's, and Huntington's diseases, amyotrophic lateral sclerosis, and others. All these diseases have in common the pathological hallmark of the presence of intraneuronal ubiquitylated protein inclusions or aggregates. Consequently, clearance of protein aggregates could provide a novel therapeutic approach for these diseases. Can up-regulation of autophagy be used as a therapeutic strategy for neurodegenerative diseases? To address these questions, we present a brief summary of autophagy in CNS.

Alzheimer's disease

Alzheimer's disease (AD) is characterized by degeneration of neurons and synapses in the neocortex and some subcortical areas, such as the hippocampus and basal forebrain. Cholinergic neurons are particularly affected. Amyloid β -peptide ($A\beta$) plaques and intraneuronal aggregates of hyper-phosphorylated tau proteins are believed to cause loss of neurons in AD. Neurons in the AD brain exhibit autophagosomes containing hyper-phosphorylated Tau and $A\beta$ [26] which may be due to deficient lysosomal degradation [27]. Thus, it has been suggested that impaired clearance of autophagosomes contributes to AD. Indeed, deletion of Beclin-1 in mice decreases neuronal autophagy and promotes neuronal loss whereas overexpression of Beclin-1 induces autophagy and inhibits amyloid deposition and associated pathology [28].

Several studies reported that inhibition of mTOR leads to induction of autophagy [29–31], and increases $A\beta$ degradation by the lysosomal system [32]. Conversely, accumulation of

intraneuronal A β also increases mTOR activity [33] and accumulation of autophagic vacuoles [34, 35], while inhibition of mTOR attenuates A β levels in a mouse model of AD [33], suggesting mTOR involvement in autophagic induction. In patients with AD, Beclin-1 levels are altered [36, 37], suggesting inefficient execution of Beclin-1-dependent autophagy and increased A β and p-Tau accumulation. Lentiviral Beclin-1 expression activates autophagy and improves neurodegenerative pathology in animal models of AD [36]. Intraneuronal A β accumulates in the brain of AD mice and may lead to lysosomal pathology, while enhancement of lysosomal proteolytic degradation may increase the rate of autophagic protein clearance [38]. Thus, up-regulation of autophagy is considered a therapeutic strategy for AD.

The final step of autophagy following induction and maturation of autophagosomes is lysosomal degradation, which is indispensable for clearance of cellular debris. Autophagosome accumulation can be obtained by elevation of lysosomal pH. With an altered pH, lysosomes cannot recycle/degrade efficiently, resulting in an excess storage of various molecules within the lysosomes [39]. In AD, there is evidence that aberrant lysosomal function leads to abnormal sphingolipid ceramide metabolism. This abnormal accumulation of undigested material within neurons can lead to cell death. Thus, autophagy impairment in AD could lead to protein accumulation as well as impaired lysosomal function.

Parkinson's disease

In addition to AD, dysfunction in autophagy is common in several other neurodegenerative diseases [40–43], including Parkinson's disease (PD) and Lewy body dementia which are characterized by intracellular accumulation of α -Synuclein (Syn). It has been shown that in degenerating dopamine neurons in PD brains, there is a breakdown of lysosomal membranes [39] and mislocalization of lysosomal receptors and autophagy components. Because Syn is primarily degraded in lysosomes by multiple pathways, including chaperone-mediated autophagy [44–46], it has been proposed that a lack of Syn degradation is a pathogenic event in PD.

PD is also characterized by mitochondrial dysfunction which is believed to be the major pathological feature in this disease [47]. Thus, one would expect to see mitophagy in PD brains. Nevertheless, a functional correlation between recessive early-onset PD caused by mutation in the *Parkin* gene [48, 49] and the *PINK1* gene and a defect in mitophagy has been established. Parkin is an E3 ubiquitin ligase that facilitates proteasomal degradation of misfolded proteins [50] and its expression contributes to autophagic clearance in several animal models [51, 52]. *PINK1* encodes for PTEN-induced putative kinase 1, a mitochondrial serine/threonine-protein kinase. PINK1 mutations in the kinase domain fail to translocate Parkin to mitochondria and to induce mitochondrial aggregation [53–56] and enhance autophagy *in vitro* [57]. Parkin is crucial for neuronal survival because experimental findings have shown that Parkin over-expression attenuates A β ₁₋₄₂ and Syn toxicity [37, 58]. Moreover, activation of autophagy improves dopaminergic cell survival in Parkin deficient and Tau over-expressing mice [59]. Parkin ubiquitinligase activity modulates Beclin-1 LC-3 mediated autophagy [60] and lentiviral delivery of Parkin activates

Beclin-1-mediated autophagy in mice over-expressing β -amyloid precursor protein [34]. More recently it was demonstrated that autophagosome accumulation is cleared *via* treatment with tyrosine kinase inhibitors (TKIs) that increase the Parkin-Beclin-1 interaction, leading to amyloid clearance in neurodegeneration models [37, 61, 62]. Taken together, these findings suggest that autophagy-associated cell death should be carefully interpreted with distinction between induction and/or arrest of autophagy.

AUTOPHAGY AND THE HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

Several publications demonstrated that some intracellular pathogens (i.e., bacteria, viruses, protozoa) are targeted for autophagosomes, which acidify and destroy invading pathogens in peripheral macrophages [63]. However, microbes and other pathogens often have specific adaptations to overcome autophagic clearance. This appears to be the case for the Human Immunodeficiency Virus type-1 (HIV). Reduced HIV levels are associated with a blockade in the processing of a key precursor protein for virus assembly, Gag, which localized to LC3 [64], suggesting that Gag may alter autophagosome maturation in macrophages. A functional genomics screen also linked autophagy proteins with increased HIV replication [65], further suggesting that HIV exploits multiple host proteins during infection. Thus, HIV not only thwarts the host defense mechanisms, but also uses immune defense for its own protection. Specific pathogen effectors targeting autophagy are well described outside the CNS [63], but the interplay between autophagy and HIV in the CNS is not very well understood. For example, many of the viral factors characterized so far, including HIV, mainly target Beclin-1 or other parts of the molecular cascade of autophagy to inhibit autophagosome maturation [66]. Paradoxically, induction of early stages of autophagy in HIV-infected macrophages promotes Gag processing and promotes infectious HIV proliferation [66], suggesting that inhibition of autophagosome maturation is beneficial in early stages but may prevent autophagic degradation of the virus. Furthermore, unlike macrophages that are normally spared, T cells infected with HIV die due to impaired autophagy [67]. Therefore, a better understanding of the role of autophagy in HIV infection in the CNS is imperative to assist in the design of therapies that alleviate HIV-associated neurodegeneration.

HIV associated dementia complex

Acquired Immunodeficiency Syndrome is common in HIV positive subjects. Less common but equally devastating is the development of HIV associated dementia complex (HAD), an array of neurological alterations characterized by motor and cognitive abnormalities [68, 69]. Neuroimaging studies of HAD brains show atrophy of the cortex and subcortical regions such as the caudate nucleus and hippocampus [70]. It is now apparent that these patients may be suffering from protracted forms of HIV encephalitis (HIVE), a neuroinflammatory condition characterized by the presence of HIV infected microglial cells, formation of microglial nodules, multinucleated giant cells, astrogliosis and myelin loss [71]. In addition, these subjects exhibit profound neuronal degeneration and loss of synaptodendritic connections, which persist despite the highly active combination antiretroviral therapy (HAART) [72]. Paradoxically, as HIV-infected individuals live longer because of HAART, the prevalence of HAD is increasing. In addition, viral persistence in

these subjects has been demonstrated in cerebrospinal fluid even when treated with HAART that lowers HIV RNA to undetectable levels in plasma [72]. Nevertheless, HAD, like other neurodegenerative diseases, develops over time. Therefore, one therapeutic strategy to prevent HAD is to modify biological events downstream of the etiopathological processes at a point when neurons are already infected but before they are committed to die. Key to develop such therapeutic compounds is the understanding of the molecular and cellular mechanisms of how HIV leads to HAD.

HIV is a retrovirus that, after initial infection, produces profound T cell depletion [73]. The end result is severe cellular immunodeficiency, with the development of opportunistic infections and cancers. Macrophages may also serve as an important reservoir for HIV, especially because HIV infection is latent and not cytopathic in these cells, in contrast to T lymphocytes. HIV utilizes CD4 as its primary receptor followed by engagement with chemokine receptors CXCR4 and CCR5 [74–76]. CCR5 is found in macrophages and mediates macrophage (M-tropic) HIV infection, whereas T-tropic HIV binds to CXCR4, which is abundant in T cells [77]. There are also HIV isolates that use both CCR5 and CXCR4 [78]. The importance of chemokine receptors in HIV pathogenesis is underscored by the observation that individuals deficient in CCR5 are resistant to infection by HIV [79]. HIV penetrates the CNS a few weeks after infection. Microglia, unlike neurons, express CD4, CXCR4, and CCR5 [80] and thus, are the primary target cells for HIV in the brain [81]. However, astrocytes can also be infected [82]. Yet, numerous *in vitro* and *in vivo* studies have revealed that HIV can cause neuronal cell death [83–86]. Thus, it has been suggested that infected microglia and infiltrated macrophages could produce several factors that are directly or indirectly toxic to neurons [84]. Moreover, astrocyte function could become disturbed in HIV positive subjects and influence neuronal cell viability. For instance, the uptake of neurotoxic glutamate could be impaired [87]. Furthermore, several HIV proteins have been shown to be released from HIV-infected microglia and/or present extracellularly in the HIV-infected brain. These proteins include at least i) the envelope glycoprotein gp120, which is crucial for the initial binding of the virions to CD4 and chemokine receptors, ii) the activator of HIV transcription Tat, and iii) Nef, the regulatory protein that promotes viral replication [88, 89]. Among these proteins, gp120 appears to be the most potent neurotoxin with lethal activities in the picomolar range [90, 91]. Most importantly, either transgenic mice expressing gp120 [92] or neurons exposed to recombinant gp120 [93] exhibit neuronal cell loss and dendritic simplification, which are pathological features of HAD [94].

Gp120 neurotoxicity

The ability of gp120 to promote pruning of the synaptic network has been widely accepted [92]. However, debate exists on whether gp120 induces neuronal cell damage directly or indirectly. The characterization of the neurotoxic mechanisms of gp120 is paramount for a drug discovery program against HAD. Gp120 has been shown to interact with neuronal chemokine receptors CXCR4 and CCR5 to activate neurotoxic signals/pathways or inflammatory responses [91, 95, 96]. These pathways converge on caspase-3, a member of the cysteine-aspartic acid protease family that plays a crucial role in the execution of neuronal cell apoptosis, which is well recognized in HIV [94]. Apoptosis has numerous

physiological functions. In mature organisms, these functions include, among others, negative selection in the immune system, removal of damaged or compromised cells during tissue turnover, protection from viral infection, and protection from cancer. Apoptosis serves its most important physiological role in the nervous system during neurogenesis, when apoptosis reduces by ~50% the number of excess neurons that do not innervate a given target and thus sculpts the developing nervous system [97]. However, when apoptosis occurs inappropriately in the adult CNS where neurons cannot proliferate or regenerate it creates loss of neurons and other neurodegenerative pathologies, including AD, PD, and HAD. Pro-apoptotic molecules or signaling pathways induced by gp120 through activation of chemokine receptors include ceramide [98], p38/mitogen-activated protein kinase, c-Jun terminal kinase and extracellular signal-regulated kinase [95]. Conversely, pharmacological inhibition of the c-Jun terminal kinase pathway prevents gp120-mediated apoptosis [99]. These findings are important because they point to specific signal transduction pathways as potential therapeutic targets. However, drugs that block signaling in all cells may be detrimental as these pathways, especially extracellular signal-regulated kinase, are necessary also for neuronal cell differentiation and survival [100, 101]. In addition, CXCR4 or CCR5 receptor inhibitors, either synthetic or naturally occurring, do not cross the blood-brain barrier. Therefore, additional studies must be carried out to reveal the numerous biological and molecular events that occur after HIV/gp120 binds to chemokine receptors.

Gp120 endocytosis and autophagy

Endocytosis is a fundamental cellular function involved in nutrient uptake, pathogen removal, and transporting signaling molecules from extracellular environment to intracellular cytoplasm. Endocytosis includes phagocytosis, which internalizes large particles, and pinocytosis, which internalizes membrane proteins, lipids, and fluid with soluble molecules [102]. Neurons, through the endocytosis process control intracellular trafficking for internalizing neurotrophic factors, for recycling or down-regulating receptors, for neurotransmitter uptake or for directing information to intracellular biosynthetic pathways. Recent data show a novel mechanism of T-tropic gp120 toxicity that followed gp120 endocytosis by CNS [103] and peripheral [104] neurons. The endocytotic process is not unique for gp120, as other neurotoxic proteins promote neuronal cell degeneration, including prions, A β , and Syn. What is unique for T-tropic gp120 is the fact that this protein relies on the chemokine receptor CXCR4 to be internalized. Gp120 is then axonally transported both anterogradely or retrogradely to accumulate inside neurons, either around microtubules or perinuclearly inside lysosomes [103]. Neurons internalizing gp120 undergo axonal retraction before showing signs of apoptosis. Indeed, quantitative image analysis revealed that neurons internalizing gp120 exhibit shorter processes compared to controls within 6 hours [93]. Thus, gp120 appears to initiate intracellular processes that are crucial for neuronal cell survival before affecting the apoptotic pathway. For instance, accumulation of gp120 may impair neuronal cell homeostasis which involves anterograde axonal transport of proteins. Moreover, anterograde axonal transport must be balanced by a similar rate of retrograde transport and autophagic clearance. Because a connection exists between pathways that regulate autophagy and apoptosis, a simple question is whether gp120 is neurotoxic because it impairs autophagy in neurons.

Autophagy plays a crucial role in gp120-induced T cell death, using CXCR4 [105]. Interestingly, autophagy in T cells does not require CD4. In addition, a CXCR4 antagonist inhibits the formation of autophagic vacuoles supporting the concept that autophagy in these cells requires the presence of CXCR4. Beclin-1 and LC3 are higher in the cortex of young HIVE subjects when compared to aged HIVE [106]. Thus, progressive neurodegeneration, and loss of synaptic processes seen in these subjects may be associated with decreased autophagic clearance. This notion is supported by data showing that Beclin-1 induction by gene transfer in gp120 transgenic mice restores autophagy and reduces neuroinflammation and neurodegeneration. In addition, p53, which is also activated by gp120, is another activator of autophagy [107]. Finally, the ubiquitin proteasome system, which is altered in HAD subjects [108] and is crucial for protein degradation is also implicated in autophagy [109]. Thus, gp120 may play a role in altering autophagy pathways causing neuronal cell death. This would be in line with the notion that impaired autophagy leads to neurodegenerative diseases.

Gp120 may impair neuronal survival *per se* by inducing proteinopathies, as we have observed accumulation of gp120 inside neurons [103]. To answer this question we exposed primary rat cortical neurons to gp120 and determined Beclin-1 levels in a time-dependent manner. Neurons treated with gp120 (5nM) exhibited a time-dependent increase in Beclin-1, which was induced by 3 hours relative to actin or GAPDH (Fig. 2). This effect was similar to that observed with Rapamycin. However, by 6 hours, Beclin-1 levels returned to control levels, suggesting that gp120 may alter autophagy only for a limited period of time. We also measured the level of LC3 in primary neurons treated with gp120. LC3 is initially synthesized in an unprocessed form, proLC3, but sequential conjugation of Atgs leads to its modification into LC3-II, which is a marker of autophagosomes [110, 111]. Western blot analysis was used to determine the conversion of LC3-I to LC3-II (Fig. 3). Gp120 induced a time-dependent increase in LC3-I conversion to LC3-II but the effect lasted only for 6 hours, suggesting that gp120 induces autophagosome formation early after infection, which may then be fused into the lysosomes, where gp120 was previously detected [103]. The molecular steps of autophagy can be activated, but autophagosome clearance remains insufficient, due to inefficient fusion with the lysosome [9]. In fact, while gp41, a subunit of the envelop protein has been shown to undergo ubiquitination in non-neuronal cells [112] ubiquitin-dependent gp120 degradation has not been yet established in neurons. Lack of gp120 degradation in the CNS, may reflect absence of autophagic degradation in glial and neuronal cells or defects in lysosomal proteolysis, leading to intracellular accumulation of gp120. Such gain of function of gp120 could be deleterious for neurons because it can lead to alterations of many intracellular processes including endoplasmic reticulum stress, mitochondria failure, and axonal retraction. Therefore, it is imperative to better understand the effects of HIV infection and/or gp120 on neurons, which die after exposure to gp120, compared to astrocytes that accumulate gp120 and survive. Taken together this leads to speculation whether gp120 may be able to alter autophagy in brain astrocytes as it does in macrophages; and whether neurons fail to sequester gp120 in a similar fashion to T cells. These speculations lead us to think that neuronal autophagy may be different than the poorly understood astrocytic autophagy. Furthermore, the observed accumulation of gp120 in lysosomes may reflect inefficiency in proteolytic enzymes or alteration of lysosomal paucity,

leading to lysosomes to be overwhelmed by gp120 proteins (Fig. 4). However, other HIV proteins, i.e. Tat, Gag, Nef may affect the autophagic clearance machinery in a different way compared to gp120. In addition, LC3-II and Beclin-1 alone may not be sufficient to estimate autophagy. Thus, more markers (e.g. ATG) and experimental approach (e.g. subcellular fractionation, mitophagy) are needed to characterize a working “mechanism” for gp120-mediated neuronal cell death.

POTENTIAL INTERVENTION

The possibility of autophagic clearance of molecular and cellular debris from neurons was demonstrated in models of neurodegenerative diseases, including PD and AD Parkin-Beclin-1 interaction, leading to increased proteasome function and autophagosomal maturation, which is impaired in HIV. Therefore, Nilotinib and Bosutinib might be used as brain-penetrant drugs to ameliorate autophagic gp120, and perhaps as HIV proteins, clearance.

CONCLUSION

HIV proteins form proteinacious species and impair autophagic clearance in a similar fashion to accumulating or misfolded proteins in neurodegenerative diseases. Although the effects of HIV on autophagy are very well understood in the peripheral organs, there is very little information how viral proteins may behave in the CNS, leading to neuroinflammation and degenerative death. Most importantly, it is necessary to understand how gp120 and other HIV proteins may differentially affect autophagy in neurons versus astrocytes. The short term increase in autophagic markers, including Beclin-1 and LC3-II, in neurons may suggest a [34, 35, 51, 113, 114]. More recently we demonstrated that autophagosome accumulation can also be cleared *via* treatment with TKIs, which penetrate the brain and facilitate autophagic clearance in neurodegeneration models [37, 61, 62]. TKIs such as Imatinib are effective and well-tolerated treatments in patients with Philadelphia chromosome-positive chronic myeloid leukemia [115, 116]. Nilotinib is a second generation Abelson TKI inhibitor, which was approved by the US Food and Drug Administration in 2007 [117–119] following Imatinib resistance and intolerance [115]. Another dual Src/Abelson TKI inhibitor TKI, Bosutinib, (SKI-606) was also approved by the US Food and Drug Administration in 2012 [120–122]. A much lower dose of these drugs can promote autophagic clearance of accumulating proteins, leading to translocation of amyloid from autophagosomes and clearance within lysosomes [37, 61, 62]. Short term administration of these drugs improved amyloid pathology, protected against cell death and enhanced cognitive and motor performance in AD and PD models, respectively. TKIs increased functional parallel process with HIV-infected macrophages (Fig. 4), which are unable to clear the virus due to its highly adaptive nature to overcome lysosomal degradation. It is possible that HIV proteins either impair autophagosome maturation or alter lysosomal degradation in the CNS, ultimately leading to gp120 accumulation and neuronal cell death. Finally, autophagy can be exploited therapeutically *via* treatment of brain-penetrant and clinically tolerated TKIs that promote autophagic clearance of molecular and cellular debris.

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LIST OF ABBREVIATIONS

mTOR	Mammalian target of Rapamycin
CNS	Central nervous system
AD	Alzheimer's disease
PD	Parkinson's disease
Syn	α synuclein
HIV	Human immunodeficiency virus type-1
HAD	HIV associated dementia
LC3	Light chain protein-3
HIVE	HIV encephalitis
HAART	Highly active combination antiretroviral therapy
Aβ	Beta-amyloid
TKIs	Tyrosine kinase inhibitors

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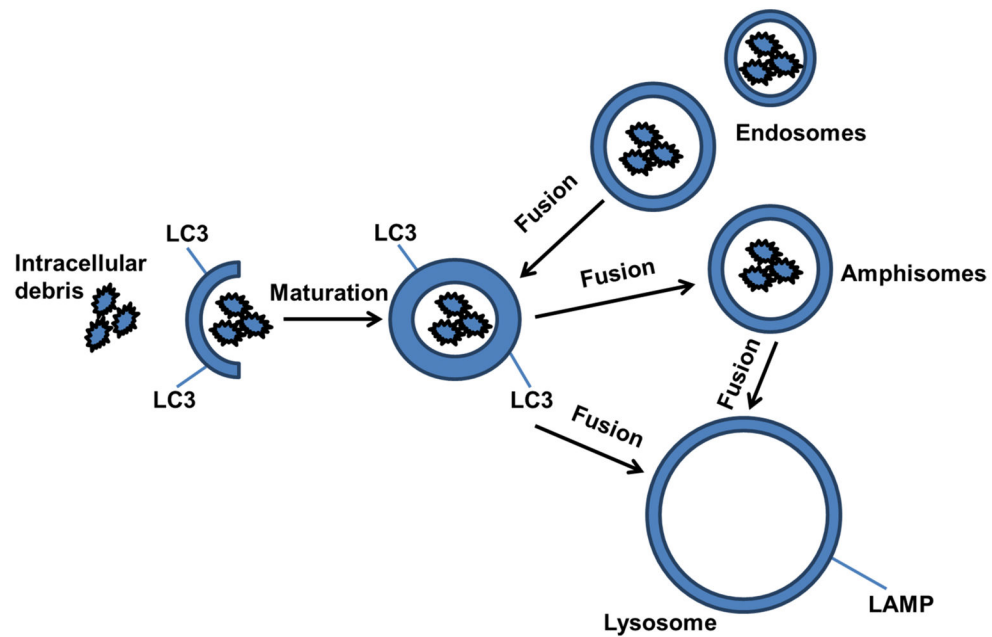


Fig. 1. Schematic pathways of autophagy

Baseline autophagy is a dynamic multi-step process, comprising several compartments, including the pre-autophagosomal sequestering phagophore and the autophagosome, which is enriched in LC3. The autophagosome can fuse with endosomes to form the amphisome, which may be involved in the degradation of internalized species *via* endocytosis. Either autophagosomes or amphisomes may then fuse with the lysosome to generate autolysosomes or autophagolysosomes, respectively. Autolysosomes clear accumulating intracellular debris, like misfolded proteins and defective organelles, while autophagolysosomes may degrade internalized species *via* endocytosis and the endosomal pathways (i.e. extracellular amyloid, pathogens).

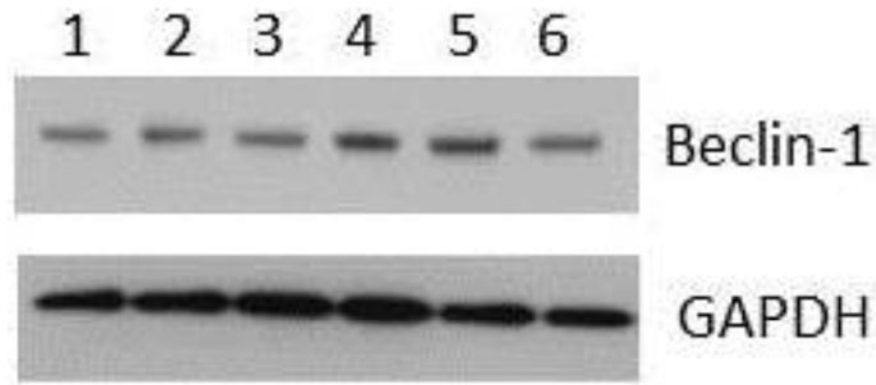


Fig. 2. HIV/gp120 evokes a short-time activation of Beclin-1

Cortical neurons were exposed to gp120 (5 nM) for various times. Lysates were prepared in RIPA buffer and Beclin-1 was analyzed by Western blot on 4–12% NuPAGE SDS gel. 1=DMSO; 2=boiled gp120; 3=gp120 1 hour; 4=gp120 3 hours; 5=rapamycin 3 hours; 6=gp120 6 hours. Results were replicated in three independent experiments.

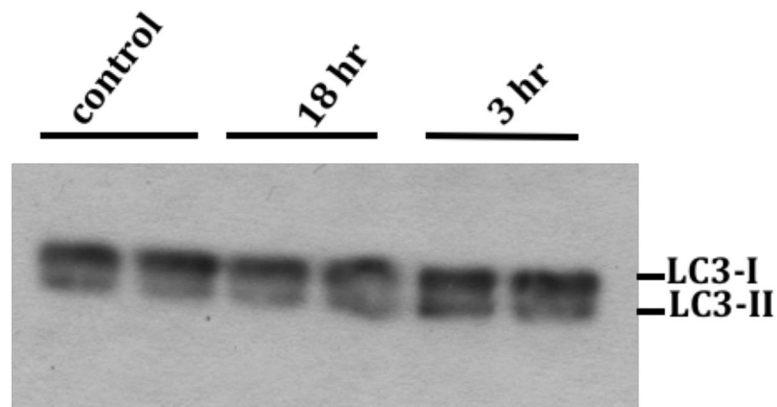


Fig. 3. HIV/gp120 promotes LC3-I conversion to LC3-II

Neurons were treated with 5nM gp120 for the indicated times. Total cell lysates in RIPA buffer were analyzed by Western blot on 4–12% NuPAGE SDS gel using monoclonal anti-LC3 antibody. The level of LC3-II was increased relative to LC3-I after 3 hours of treatment (n=3) but LC3-II levels returned to control at 18 hours, suggesting autophagic induction up to 3 hours and an autophagosome clearance at 18 hours.

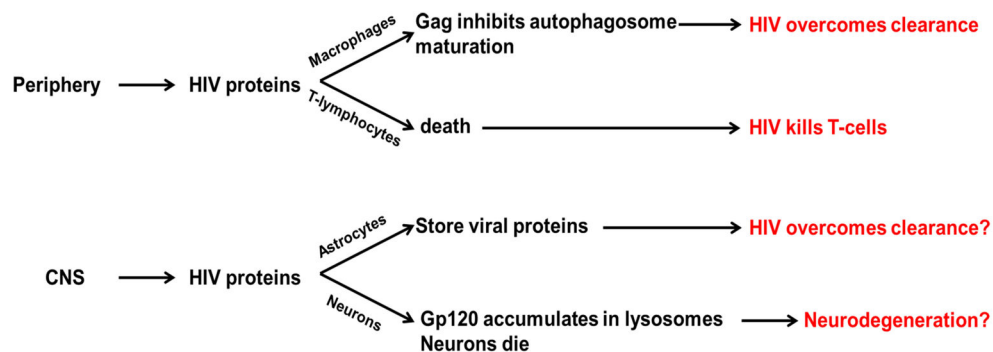


Fig. 4. Possible homology of HIV manipulation of autophagic mechanisms between the immune system and CNS

HIV proteins, including Gag, inhibit autophagosome maturation, turning the cell defense system to their advantage in macrophages. However, T cells die leading to AIDS. HIV proteins may imitate this process in astrocytes, leading to storage of the virus in astrocytes, thus providing a reservoir for future supply. However, neurons cannot clear viral proteins *via* autophagy. This mechanism may lead to neurodegeneration and HAD.