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Review

To eat or not to eat: the intricate relationship between autophagy and HIV-1

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ABSTRACT

A consensus is now growing on the fact that autophagy can behave as an antiviral mechanism against incoming pathogens. Therefore, some pathogens have evolved to counteract or benefit from the cellular autophagy machinery. The Human Immunodeficiency Virus (HIV), like many viruses, manipulates autophagy to favor its replication. In particular, HIV-1 envelope (Env) plays an important role in modulating autophagy in $CD4^+$ T cells and phagocytic cells. Env-mediated autophagy is a cell-type dependent mechanism activated in bystander $CD4^+$ T cells but totally inhibited during their productive infection. In both infected myeloid dendritic cells and macrophages, the autophagy flux is progressively shut-down. The modulation of autophagy by HIV in these cell types could be responsible for the onset of HIV-mediated immunopathogenesis and contribute to viral spread. The former effect is responsible for apoptosis of bystander $CD4^+$ T cells and the latter correlates with a viral escape strategy favoring viral replication and transmission by counteracting autophagy-mediated antiviral immunity. Furthermore, HIV-1 can infect different cell types of the central nervous system such as the microglia and, in a restrictive manner, the astrocytes and the neural precursors for which infection has also been correlated with modulation of autophagy. The aim of this review is to provide an overview on the intricate and conflicting relationships that intimately link HIV-1 and autophagy in these different cell types.

KEYWORDS: HIV-1, autophagy, infection

1. INTRODUCTION

Despite more than 30 years of intense basic and clinical studies on HIV infection, the scientific challenge remains, since 35 million people are still living with HIV worldwide (data from UNAIDS, 2013). Although tremendous success has been achieved to date in the treatment (HAART, highly active antiretroviral therapy), no cure has yet been found, mainly due to viral resistance and persistence of viral cell reservoirs. It has progressively become clear that HIV infection is highly complex, explaining the failure to cure this disease. HIV infection induces the early establishment of a chronic immune activation that plays a crucial role in HIV pathogenesis by triggering progressively the exhaustion and collapse of the whole immune system ([1] for review). Moreover, this infection triggers the progressive depletion of $CD4^+$ T cells,

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leading to increased susceptibility to opportunistic infections [2-4]. A residual chronic immune activation persists even in HIV-infected patients in whom viral replication is successfully inhibited by HAART, and the extent of this residual immune activation is associated with $CD4^+$ T cell loss [5]. However, a causal link between chronic immune activation and $CD4^+$ T cell loss has not yet been formally demonstrated.

During sexual transmission, viruses cross epithelia and encounter their target cells, mainly $CD4^+$ T cells, dendritic cell (DC) subsets and macrophages. $CD4^+$ T cells, macrophages and only a small proportion of DC are productively infected. Most of the DC capture the virus via cell surface C-type lectin receptors, such as DC-SIGN, and migrate toward lymph nodes or other secondary lymphoid organs, where they can transmit HIV-1 to $CD4^+$ T cells [6]. HIV also crosses the blood-brain barrier (BBB) and enters the central nervous system (CNS) early after the initial systemic infection, leading to cognitive deficits known as HAND (HIV-associated neurocognitive disorders) that appear in up to 50% of HIV patients in the later stages of infection [7].

In most instances, to enter and productively infect a target cell, HIV must bind to CD4 and specific co-receptors, like CCR5 or CXCR4, belonging to the chemokine receptor family and related to viral tropism (for a recent review see [8]). Co-receptor usage is correlated, at least in part, with the different phases of the disease. R5-tropic viruses, which utilize CCR5, are predominantly represented during the early stages of HIV-1 infection. In infected patients, the emergence of X4-tropic variants, which use CXCR4, is almost invariably associated with faster decline of circulating $CD4^+$ T cells, accelerated disease progression and poor prognosis for survival [9, 10]. However, the presence of X4 viruses is not an obligatory prerequisite for disease progression and a significant proportion of individuals who progress to AIDS exclusively harbors R5 variants. The selective and dominant transmission of R5-tropic viruses is not fully understood, but it may depend on the superimposition of multiple imperfect gatekeepers that restrict HIV-1 X4 transmission at different steps of the infection process [11].

In this review, we detail the past and recent research aimed at investigating the complex interplay between the cellular autophagy pathway and HIV infection.

1.1. $CD4^+$ T cells

$CD4^+$ T lymphocytes represent the main target cell population for HIV-1 infection [12, 13] and their progressive destruction is the hallmark of its associated disease [14]. $CD4^+$ T cell activation is thought to be a major factor in facilitating HIV-1 infection of these cells [15, 16].

$CD4^+$ T lymphocytes constitute a highly heterogeneous population divided by functional and phenotypic differences, and their activation, immune status and localization directly influence their susceptibility to HIV-1 infection and the consequent viral-mediated immunopathogenesis. They can be broadly divided into naive and memory subsets, and both subsets are composed of many subpopulations. Upon microbial infection, naive $CD4^+$ T cells are activated after their interaction with DC carrying and presenting specific pathogen-derived antigens. Depending on the cytokine environment and the source of antigens, the antigen-specific $CD4^+$ T lymphocytes can then rapidly proliferate and differentiate into several cell subsets with highly specialized immunologic functions, such as Th1, Th2, Th17 and regulatory T cells [17]. Quiescent naive and resting $CD4^+$ T cells were reported to be relatively resistant to HIV-1 infection [16, 18] possibly due to the low metabolic rate and antiviral mechanisms present in these cells [19, 20]. On the other hand, non-quiescent memory $CD4^+$ T cells were shown to support active HIV-1 replication with differences in their half-life, mainly depending on their maturation level [21, 22]. Although the majority of these cells die rapidly, a small proportion can undergo a transition toward a resting state [23]. Nevertheless, rapidly after acute infection, viral dissemination to lymphoid tissues and exponential HIV-1 replication throughout the lymphatic system lead to the depletion of most mucosal $CD4^+$ memory T cells [24-28] and to the subsequent establishment of reservoir cells in mucosa-associated lymphoid tissues (MALT) [29-31]. Importantly, this major effect was observed in non-human primate models [32-34] as well as

in humanized mice models of HIV infection [35, 36]. Therefore, resting memory $CD4^+$ T cells could represent a significant viral reservoir seemingly responsible for the failure to eradicate HIV infection upon antiviral therapies [24, 37-40].

Interestingly, an immature memory T cell population with stem cell-like properties called $CD4^+$ T memory stem cells (T_{SCM} cells), the most long-lasting central memory $CD4^+$ T cells, were reported to harbor high levels of HIV-1 DNA, thus potentially providing a viral reservoir over time [41].

HIV-1 entry in $CD4^+$ T cells relies mainly on a receptor-dependent process, leading to the fusion of the target cell membrane with the viral or the HIV-infected cell membrane [42]. More precisely, the HIV-1 envelope (Env), expressed at the surface of free virions or infected cells and composed of the glycoproteins gp120 and gp41, binds to CD4. This interaction triggers structural changes leading to increased exposure of gp120 regions (including the V3 loop) that can bind to the co-receptors, mainly CCR5 or CXCR4 [43]. Finally, interaction of gp120 with the co-receptor induces a structural rearrangement of the trans-membrane Env-subunit gp41 and insertion of the fusion domain at the N-terminus of gp41 into the target cell membrane. At this stage, gp41 adopts a trimeric extended pre-hairpin intermediate conformation before the formation of a stable six-helix bundle structure, thus facilitating virus/cell-to-cell fusion. Although occurring with cell-free viruses, the efficiency of HIV-1 infection is higher when the virus is delivered through cell-to-cell contacts upon formation of the so-called virological synapses (VS) [44-49]. Indeed, DC and macrophages, the first cells encountering HIV-1, efficiently transmit HIV-1 to $CD4^+$ T cells through these synaptic structures in which viral proteins and cellular receptors were shown to be polarized. This way of spread also occurs between HIV-1-infected and uninfected $CD4^+$ T cells [44, 50]. Notably, cell-to-cell fusion can lead to the formation of giant, multinucleated cells [3] called syncytia, which could precede apoptotic events *in vitro* [51]. *In vivo*, syncytia could generate long membrane tethers, thus facilitating viral dissemination [52].

During HIV-1 infection, many different mechanisms seem responsible for the death of infected and uninfected $CD4^+$ T cells. However, during the prolonged, asymptomatic phase of the disease, the death of uninfected bystander $CD4^+$ T cells is thought to be the main cause of $CD4^+$ T cell depletion. It is now well admitted that the gp41-mediated fusion process triggers activation of the intrinsic pathway of apoptosis, with activation of the caspases 9 and 3 [53-56]. Recently, abortive HIV-1 infection, occurring in the vast majority of bystander quiescent $CD4^+$ T cells, was shown to be responsible for an inflammatory form of cell death called pyroptosis, with activation of caspase 1 and release of inflammatory cytokines [57, 58] upon viral DNA sensing by the interferon-γ-inducible protein 16 (IFI16) [59, 60].

1.2. Macrophages

Macrophages are terminally differentiated, non-dividing cells, derived from circulating monocytes. They are present in most tissues with different denominations, e.g. microglia in the brain, alveolar macrophages in the lung, or Kupffer cells in the liver. Although circulating monocytes are relatively resistant to HIV-1 infection, macrophages can be productively infected and play important roles in the different phases of HIV-1 infection. In addition, they have been reported to be resistant to HIV-1-induced cytopathic effects, thus representing a significant viral reservoir population which can survive for a long time [61].

First, they act as antigen presenting cells (APC), leading to the presentation of HIV-1-derived peptides via major histocompatibility complex class II (MHC-II) and MHC class I (MHC-I) molecules to $CD4^+$ and $CD8^+$ T cells, respectively (for review see [62]). HIV-1-infected macrophages can also attract $CD4^+$ T cells through secretion of cytokines, thus favoring HIV-1 transfer upon cell-to-cell contacts or directly influencing viral spread by migrating and infiltrating other organs, including the brain.

Like $CD4^+$ T cells, macrophages express the main HIV receptors and co-receptors and can thus be infected by X4-tropic or R5-tropic viruses. However, they are more frequently and efficiently infected by HIV-1 R5 strains, which exploit low levels of

CD4 and/or CCR5 to enter macrophages [63, 64]. While macrophages express HIV-1 receptors, allowing viral entry via gp41-mediated plasma membrane fusion, the low level of CD4 seems to favor virus uptake through endocytosis, presumably due to the barrier formed by the actin cortex and the presence of intrinsic antiviral factors [65]. The favored model for productive HIV-1 entry in these cells would rely on endocytosis via lipid microdomains of the plasma membrane containing CD4 and CCR5, and the efficient gp41-dependent fusion after endocytosis, therefore allowing viruses to escape degradation [66-68]. This complex and yet unclear pathway was called "pathway of HIV endocytic entry in macrophages" (PHEEM) [66, 67]. Alternatively, productive infection of macrophages may occur after endocytosis of HIV-1 through a CD4-independent mechanism, although the inherent mechanisms are not yet fully elucidated and most of the incoming virions are mainly degraded by this way [69].

Additional cellular membrane receptors were described to support HIV-1 entry in macrophages, including syndecan, a heparin sulfate proteoglycan [70, 71]; gp340, a cysteine-rich scavenger receptor [72]; the macrophage mannose receptor [73-75]; elastase [76]; and α-v-integrin [77] supposedly by facilitating viral attachment, binding, entry and/or fusion. Another potential membrane ligand of HIV-1 is annexin II, which is expressed on the membrane of macrophages, but not of T cells. Annexin II, which binds to phosphatidylserine (PS), an anionic phospholipid captured during HIV-1 budding, contributes to the early events of macrophage HIV-1 infection [78]. Other candidate host cell surface proteins that are incorporated in HIV-1 membranes and are potentially needed for HIV-1 entry might include CD28, CD44, and CD62L [79].

In infected macrophages, striking observations were reported on infectious viruses accumulating in cytoplasmic virus-containing compartments (VCC), the nature of which is still debated. Although first described as internal compartments that possess the characteristics of late endosomes/ multivesicular bodies (LE/MVB), these VCC are not acidified with a fraction of them connected to the plasma membrane [80-82]. Are these compartments required to maintain a viral reservoir or do they represent a potential source for a rapid transfer of viral infection? The answer to this will require further investigation but an interesting recent report demonstrated that targeting these VCC with antibodies directed against CD36 could block HIV-1 release and viral transfer toward $CD4^+$ T cells [83].

1.3. Dendritic cells

The two main DC subsets in the blood can be identified based on specific receptor expression. The most represented subset, although constituting only 0.5% of the whole peripheral blood mononuclear cells, belongs to the conventional or myeloid DC (cDC or myDC) and is usually characterized by the specific expression of CD1c (BDCA1) or CD141 (BDCA3) in parallel to myeloid markers like CD11c, CD13 and CD33. These cells migrate rapidly to inflamed tissues and secrete high levels of cytokines (like IL-6, IL-12, IL-15) and chemokines (CCL17, CCL22 for example) in response to microbial stimuli. Plasmacytoid DC (pDC) represent a rarer subset of cells (0.1-0.2% of the whole PBMC) mainly characterized by a plasma cell morphology and the specific expression of CD303 (BDCA2), CD304 (BDCA4/Neuropilin-1) and CD123 (IL-3 Receptor alpha-chain). Although pDC share some common markers, they are thought to be from lymphoid origin and were previously coined as interferon (IFN)-producing cells (IPC) due to their unique ability to rapidly secrete massive amounts of type 1 IFN following viral stimulation (for review see [84]), thus placing these cells at the forefront of innate immune response initiation.

Environmentally exposed surfaces of the body are privileged sites for other unique DC subsets. The human epidermal and mucosa upper layers are homogeneously populated with Langerhans cells (LC) mainly characterized by the specific expression of the C-type lectin receptor (CLR) CD207 (Langerin) whereas the subjacent layers present various dermal DC subsets (dDC), some of them expressing CD209 (DC-SIGN).

Although all these subsets could differ in phenotype and functions, one of their hallmarks is their propensity to regulate innate and adaptive immunity. Indeed, most of these subsets express a

plethora of pathogen recognition receptors (PRR) facilitating their antigen sampling activity and contributing to their quickness in efficiently igniting adapted immune responses.

There is now increased evidence that mucosal DC subtypes, macrophages and $CD4^+$ T cells are involved in the early events of HIV transmission [85, 86]. Indeed, in non-human primate models of simian immunodeficiency virus (SIV) infection, while $CD4^+$ T cells would represent the majority of infected cells, mucosal DC could be carriers of infection and amplify viral transmission rates [87-90]. Some reports also evidenced the involvement of other DC subsets early after acute infection and seemingly contributing to viral spread. In fact, pDC and myDC were shown to be recruited to infected tissues via the secretion of specific chemoattractants like CCL20 or thymic stromal lymphopoietin (TSLP), respectively [91, 92]. Activation of these DC subsets on site would lead to secretion of other chemokines (CCL3, CCL4 etc.), thus fueling the infected tissues with $CD4^+$ viral target cells and also contributing to the onset of immunopathogenesis linked to chronic immune activation. Indeed, recent reports clearly evidenced a systemic redistribution of pDC to mucosal tissues of chronically infected macaques [93, 94] which could account for the chronic immune activation. Accumulation of pDC and myDC was also observed in lymphoid tissues of chronically HIV-infected patients [95].

Although most myeloid DC subsets appeared to be refractory to productive HIV infection, these cells can readily capture and internalize virions via different receptors expressed at the surface. All DC subsets express the main HIV receptor CD4 and the co-receptors CCR5 and CXCR4, although the levels of expression vary depending on the subsets [96-99]. It is now becoming clear that productive HIV-1 infection of most DC subsets is profoundly impeded at a post-entry level due to the expression [100, 101] and phosphorylation status [102, 103] of the deoxy-nucleoside triphosphate triphosphohydrolase SAMHD1, an important regulator of the intracellular dNTP pool [104-106]. DC subsets also possess other important antiviral activities affecting different steps of the viral replication cycle [107-110]. Although not supposed to lead to an efficient productive infection, there are other portals for viral entry via binding to other receptors, some of them being specifically expressed in DC subsets. Indeed, carbohydrate-binding receptors are widely expressed on all DC subsets and were reported to represent the dominant HIV-envelope receptors in myeloid DC [111, 112]. Some of these receptors, like CD209 (DC-SIGN), CD206 (Mannose receptor), CD207 (Langerin) or CLEC4A (DCIR) belong to the C-type lectin receptor (CLR) family and were demonstrated to internalize HIV and regulate DC-mediated viral transmission toward $CD4^+$ T cells [112-115]. Interestingly, most of these CLR were also reported to contribute to immune responses upon pathogen capture and internalization [116-120]. Outstandingly, targeting antigens to DC via these CLR was reproducibly showing enhanced humoral and cellular immune responses thus rendering this approach attractive for innovative prophylactic strategies [118, 121, 122]. However, some of these CLR can be used by HIV to escape immune recognition and to favor viral spread. For instance, upon internalization into DC, some virions can escape lysosomal-mediated degradation and remain over time in a tetraspanin-rich compartment [123], suggested to be a source for viral transfer toward $CD4^+$ target T cells. The complexity of HIV entry into DC and the inherent intracellular viral trafficking require further investigation in order to find potential treatments able to tackle the early events of HIV infection and transmission.

1.4. Neuronal cells

The BBB isolates the CNS from the blood stream and thus may favor viral escape from immune surveillance and persistence for pathogens that have the ability to cross this cellular structure. HIV can access the CNS soon after the initial infection by using cells of the immune system such as T cells and macrophages, a process coined “trojan horse” [124, 125]. Once in the brain, HIV infects and replicates in macrophages and microglial cells as well as, in a restrictive manner, in astrocytes and neural precursors (NPC). Following this neuronal invasion, and in correlation with the viral load, cognitive deficits known as HIV associated neurocognitive disorder (HAND) will appear in up to 50% of HIV patients [126].

Due to the lack of CD4 expression, neurons are not direct targets for HIV infection. Nonetheless, neurotoxicity is found in HAND patients and is associated with lower quality of life and decreased lifespan. In the most severe cases, patients develop HIV-associated dementia (HAD), which is one of the most important complication associated with AIDS. In this light, HAD closely resembles pathologies such as Alzheimer's and Parkinson's diseases [127-129].

It is commonly accepted that HAND results from two intertwined effects due to viral and host factors. By infecting cells of the circulating and local immune systems, HIV triggers neuro-inflammation associated with selective neuronal apoptosis, mostly through chemokine and cytokine release [130, 131]. Moreover, some viral proteins such as gp120 and Tat can induce specific signals that are associated with neurotoxicity [132-135]. Notably, these proteins can directly interact with neurons, be internalized and activate signaling cascades.

As delivery of HAART in the CNS can be poorly efficient, infected brain cells are often referred to as reservoirs for HIV and thus represent particularly important therapeutic targets.

Microglial cells are considered as the equivalent of resident macrophages in the brain and represent 10-15% of glia cells [136]. They are distributed in a widespread manner in the brain and spinal cord but occupy a defined territory. After activation, they can change their morphology, migrate, proliferate and phagocyte. They are the main brain target for HIV as they represent the main source of active HIV infection in the CNS [130, 137]. Like macrophages, they can be directly activated by HIV infection, or by the interaction of viral proteins or host factors released by surrounding infected cells. In turn, activated microglia cells will secrete host factors such as chemokines, cytokines and excitatory amino acids that will have key roles in HIV-associated neurotoxicity [130, 137, 138].

Astrocytes are the most abundant cell type in the brain and can be infected by HIV but harbor non-productive infection. Only a small population of astrocytes seems to be infected and the exact relevance in HAND mechanisms is still poorly described. Evidence suggests that the BBB integrity will be impaired by infected astrocytes [139].

NPC have the ability to generate neurons and glial cells both during development and adulthood. Indeed, adult neurogenesis allows, in defined regions of the CNS, the generation and integration of new neurons, which can modulate existing neuronal networks and complex cognitive tasks such as learning and memory. Contrary to fully differentiated neurons, HIV has been shown to infect developing and adult NPC. These cells are also believed to act as a major reservoir for HIV [140]. Moreover, infected NPC have been shown to be able to differentiate into astrocytes, which still contain viral genomes and therefore allow (restrictive) viral replication [141, 142]. Here also, viral proteins may have a direct effect on NPC: gp120 is shown to trigger mental retardation in rodent models [143] and induces quiescence in NPC [144] and cell cycle arrest in adult NPC [145], whereas Tat is reported to affect NPC proliferation and differentiation [146, 147].

To fight invading microorganisms, such as HIV-1, the cells have a combination of essential defense mechanisms. One of the most ancient and powerful mechanisms is autophagy, a dynamic and tightly regulated pathway that degrades intracellular components upon fusion with lysosomes.

2. Autophagy

Autophagy is a fundamental and highly regulated lysosomal degradation mechanism which is dependent on specialized autophagy-related proteins (Atgs) [148]. This mechanism plays essential physiological roles in survival, homeostasis and development. It is also involved in the defense against invading intracellular pathogens and acts in both innate and adaptive immunity. Autophagy can be classified into at least three different types: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) [149]. Macroautophagy is the major lysosomal route for the turnover of cytoplasmic constituents, and will be hereafter referred to as autophagy. It is characterized by a highly dynamic flux initiated with the formation of intracellular isolation membranes, called phagophores. The origin of the membranes is still

highly debated and may involve different sources such as endoplasmic reticulum (ER), mitochondria, Golgi, plasma membrane and recycling endosomes [149]. Phagophores engulf cytoplasmic material through membrane elongation and the formation of autophagic vacuoles, called autophagosomes [148, 150, 151]. Then, autophagosomes fuse with lysosomes to form autolysosomes. Before this step, autophagosomes can also fuse with endosomes to form amphisomes, making a direct connection between the endo-lysosomal and autophagic pathways. Upon degradation of the sequestered material by lysosomal hydrolases, constituents are recycled through lysosomal transporters toward the cytosol [152, 153]. It is important to note that several autophagy-related proteins might have autophagy-independent functions, including cell division, cell death, regulation of the inflammatory immune response and resistance to pathogens [154-156].

Several specific protein complexes are successively involved in this process (Figure 1) (for reviews, [157-159]). The first complex involved in the initiation step of autophagy is the ULK1/2 complex, composed of ULK1/2, Atg13, Atg101 and FIP200 ([160, 161] for review). It is under the control of the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) [162], which represses autophagy under nutrient rich conditions whereas, in most instances, AMP-activated protein kinase (AMPK) activation can inversely promote autophagy initiation ([161] for review). Of note, the ULK1/2 complex controls the trafficking of Atg9 from the plasma membrane to phagophores [163].

The second complex is composed of class III phosphatidylinositol 3-kinase (PI3KC3), Beclin 1, p150 and Atg14L, which produces an autophagy-specific pool of phosphatidylinositol 3-phosphate (PI3P) [164, 165]. Several additional proteins interacting with Beclin 1 ensure a timely and spatial regulation of PI3P formation during the autophagy process. These proteins can act as autophagy repressors, such as Bcl-2 present in the ER, or as autophagy stimulators, such as Ambra 1 [166], WIPI2 [167] and DFCP1 (double FYVE domain containing protein 1) [168] and are the major effectors of the PI3P produced.

Two unique ubiquitin (Ub)-like conjugation systems drive the elongation and closure of the phagophore. In the first conjugation system, Atg12 is conjugated to Atg5, in a reaction mediated by the E1-like enzyme Atg7 and the E2-like enzyme Atg10 [169]. However, in contrast to the conventional Ub conjugation system, no specific and exclusive E3-like enzyme has been discovered so far. Another autophagy-related protein, Atg16L1, was demonstrated to interact with the Atg12-Atg5 conjugate in order to regulate the localization of the protein complex to isolation membranes while also specifying the site of LC3 lipidation. The second conjugation system is quite unusual by the fact that proteins of the Atg8 family could be directly conjugated to the lipid phosphatidylethanolamine (PE) through a mechanism controlled by Atg7 and Atg3, another E2-like enzyme [170, 171]. In humans, the Atg8 family comprises three microtubule-associated protein 1 light chain 3 (LC3A, B and C), one gamma-aminobutyrate receptor-associated protein (GABARAP) and three GABARAP-like proteins (GABARAPL1-3) that can be linked to PE [172]. However, only LC3B has been extensively studied, and will be hereafter referred to as LC3. Interestingly, the Atg12–Atg5 conjugate acts as an E3 for the conjugation of LC3 to PE [173]. LC3 is synthesized as pro-LC3 and is very rapidly processed by the protease Atg4 to expose its C-terminal glycine [174, 175]. During autophagosome formation, this cytosolic, soluble form of LC3 called LC3-I, can be conjugated to PE to generate the lipid-conjugated form of LC3, or LC3-II, which becomes tightly associated with the autophagosomal membrane while the Atg5-Atg12 conjugate is removed from the vesicle. Importantly, LC3-II can also return to an unlipidated state via the proteolytic activity of Atg4, suggesting that this process could be reversible. Other autophagy-related proteins, like UV radiation resistance associated (UVRAG) and ATG9 were described to participate in autophagosome formation via their contribution in recruiting the PI3KC3-Beclin 1 complex to the autophagic precursor structures [176]. Of note, Atg14L and UVRAG bind to Beclin 1 in a mutually exclusive manner and ATG14L was shown to be critically required for autophagy induction [177, 178]. The UVRAG-Beclin 1-PI3KC3 complex seems to modulate the

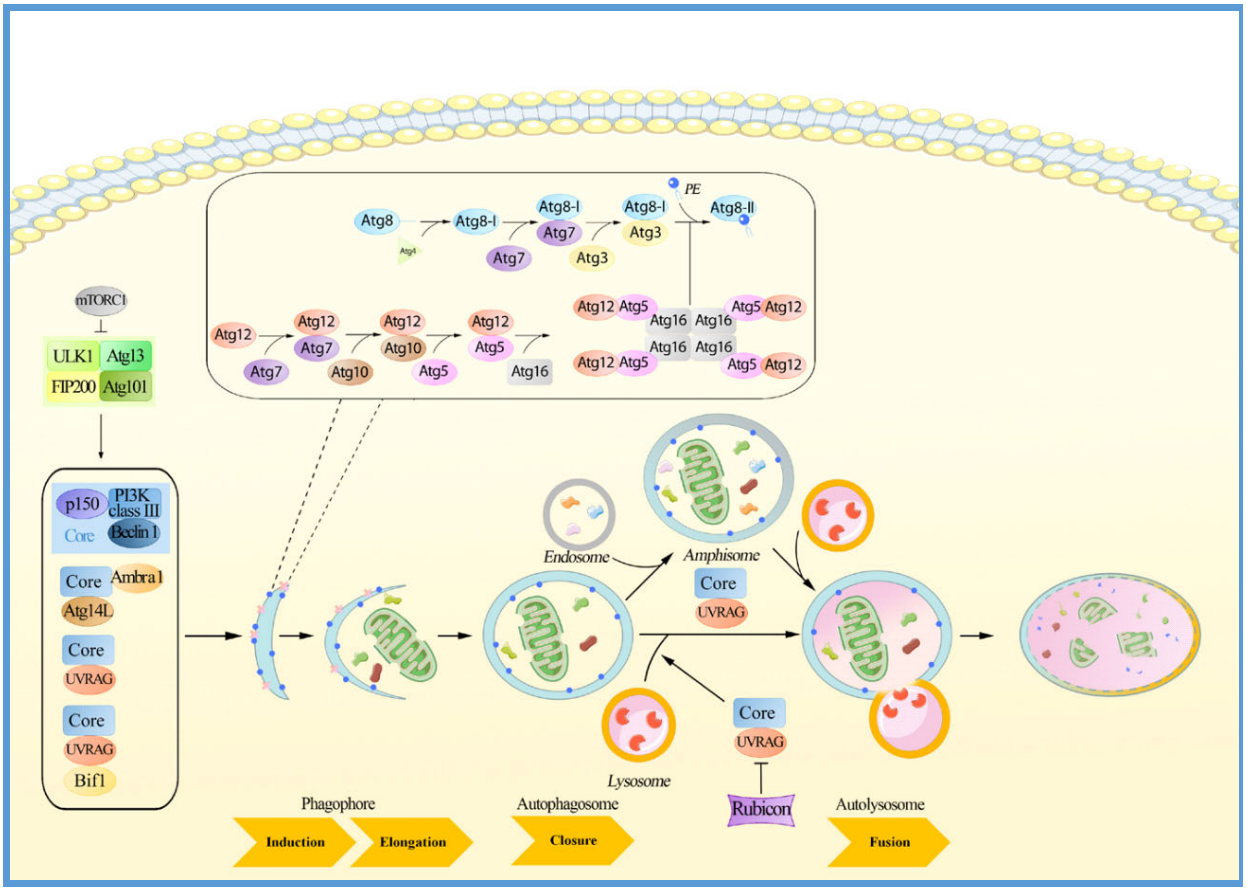

Figure 1. Overview of the autophagic pathway.

The autophagic process begins by the formation, in the cytoplasm, of a double membrane called the phagophore. This structure elongates and sequesters cellular constituents such as organelles, proteins and pathogens. After the phagophore closure, the vacuole called autophagosome can fuse with a lysosome in order to digest and recycle the sequestered material. Autophagy is connected to endocytosis because autophagosomes can fuse with endosomes, to form amphisomes, which then fuse with lysosomes.

Three signaling complexes are involved in the induction, the elongation and the closure steps of autophagy, leading to the formation of autophagosomes:

- The ULK complex, composed of ULK1/2-Atg13-FIP200-Atg101, responsible for autophagy initiation, is regulated by the mTOR kinase.
- The class III PI3Kinase, associated with p150 and Beclin 1 (core complex), is responsible for the formation of the phagophore. Ambra1 and Bif-1 are essential for induction of autophagy, through direct interaction with Beclin 1 and UVRAG, respectively, whereas Bcl-2 binds to Beclin 1 and disrupts the Beclin 1-associated PI3K class III complex, thereby inhibiting autophagy.
- Two ubiquitination-related conjugation systems leading to the formation of the Atg12-Atg5-Atg16L complex and the LC3-II-PE complex, are required for the elongation and closure of the autophagosome. Atg12 is conjugated to Atg5 by the action of the E1 enzyme Atg7 and the E2 enzyme Atg10. The resulting conjugate forms a complex with Atg16L. Likewise, Atg8, which is first processed by Atg4, is conjugated to a lipid, the phosphatidylethanolamine (PE), by the action of Atg7 and the E2 enzyme Atg3. The Atg12-Atg5-Atg16L complex acts as an E3 ligase facilitating the conjugation reaction of Atg8 to PE.

The core complex containing UVRAG is also involved at the level of fusion between autophagosomes and lysosomes. Rubicon inhibits this step by acting on UVRAG.

expansion and curvature of autophagosomal membranes, with the transient association of Bif-1 to UVRAG [179]. Another UVRAG-Beclin 1-PI3KC3 complex plays a major role in the maturation step of autophagy [176, 180, 181] which could be negatively controlled by the RUN domain-containing protein Rubicon [178].

Autophagy has long been considered to be a nonselective bulk degradation pathway. However, there is growing evidence that it can act as a selective pathway leading to the degradation of specific organelles, such as mitochondria and peroxisomes, misfolded proteins and protein aggregates as well as pathogens. By definition, the selectivity should rely on a specific recognition of potential substrates. Indeed, this function is supported by cargo receptors that bind to both "eat-me" signals and Atg8 family members present on the autophagosomal membrane. The "eat-me" signals are generally molecules of ubiquitin, with the exception of galectin-8 present on bacteria [182]. Binding to Atg8 family engages a specific motif called a LC3-interacting region (LIR), with the general sequence W/F/YxxI/L/V preceded by acidic residues [183-188]. The first selective autophagy receptor to be identified was p62 (SQSTM1) [187, 189, 190], and then the related neighbor of BRCA1 gene 1 (NBR1) [191], nuclear dot protein 52 kDa (NDP52) [192] and optineurin [193]. A noncanonical LIR motif termed CLIR, which preferentially binds to LC3C was identified in the NDP52 sequence [194]. Several autophagy receptors specific for the degradation of mitochondria, termed mitophagy, have also been discovered ([195] for review).

2.1. Autophagy during viral infections

Autophagy is usually activated rapidly after a viral infection by different mechanisms depending on both viruses and host cells. The interaction between the virus and its receptors can directly trigger autophagy. This is the case for the attenuated strains of the measles viruses for which engagement of its cellular receptor triggers autophagy [196]. Furthermore, viral entry relying on a fusogenic envelope protein can also induce autophagy, as it is the case for HIV-1 and as described in section 3, subsection 3.1.1. Also, many viral infections trigger a cellular stress, such as ER stress, due to accumulation of unfolded proteins, cell damage, production of reactive oxygen species (ROS), and/or mitochondrial depolarization, leading to autophagy induction. It was also recently shown that activation of RNase L during viral infections induces autophagy [197]. Autophagy could as well be induced upon engagement and stimulation of various pattern recognition receptors (PRR), including several Toll-like receptors (TLR) and the double-stranded RNA-dependent protein kinase (PKR) [198, 199]. Viral proteins can also induce autophagy. For example, the non-structural measles virus protein C was shown to participate in autophagy induction upon viral replication [200] and the hepatitis C virus (HCV) nonstructural proteins 4B (NS4B), 5A (NS5A) and 5B (NS5B) were shown to co-localize with autophagosomal membranes or autophagy-related proteins and positively regulate autophagy initiation [201, 202]. Recently, a study from the group of F. Randow demonstrated that the influenza viral protein M2 can redirect LC3 toward the plasma membrane supposedly to facilitate virion budding and stability [203]. Interestingly, viral replication concomitantly with the synthesis of viral proteins can increase energy requirements, thus triggering autophagy [204]. Finally, autophagy is indirectly triggered by IFN-γ which supports its role in innate immunity, hence contributing to inflammation by facilitating an IFN-γ response and signal transduction [205].

Since autophagy is a fundamental and general process involved in fighting viral infections, the viruses have evolved strategies to counteract or to exploit autophagy for their own benefit ([204, 206-209] for reviews). In this review, we intend to describe several mechanisms by which viruses can manipulate autophagy. However, the relationship between a virus and the autophagy response is often more complex. Indeed, autophagy can be differentially subverted by viruses depending on target cells and viral strains. Pathogens can also sequentially block or control autophagy during their life cycle or take advantage of the formation of autophagy membrane while blocking the later step of lysosomal-mediated degradation. Ultimately, viruses can also interfere with several autophagy-related proteins without acting on the canonical, autophagy process. Because autophagy is involved in a complex cross-talk between cellular homeostasis

and apoptosis, usurping or modulating autophagy could therefore benefit viruses in controlling the survival of infected cells.

2.2. Autophagy as an antiviral mechanism

Autophagy is an essential pathway of host defense against viral infection that can degrade entire viruses or specific viral proteins by a process termed xenophagy [210].

There are growing reports on autophagy-dependent lysosomal-mediated degradation of viruses or viral components. Indeed, the autophagy pathway was reported to restrict infection *in vivo* by herpes simplex virus 1 (HSV-1) [211], sindbis virus (SIN) [212] and rift valley fever virus (RVFV) [213], although these observations were not fully recapitulated when investigating viral replication *in vitro*. This apparent discrepancy could be explained by the cellular-dependent context as well as by potential antiviral countermeasures developed by those viruses. Indeed, the importance of autophagy in antiviral immunity is strongly supported by the countermeasures and adaptations that viruses have evolved to dysregulate or limit specific steps of autophagy. For example, the viral protein ICP34.5 from HSV-1 and some viral proteins encoded by the γ-herpesviruses (HV68 M11, KSHV vBcl-2) were reported to inhibit autophagy through a physical interaction with Beclin 1, thereby impeding autophagy initiation (reviewed in [214]). The conjugation of LC3 to PE was also shown to be impaired upon binding of the FLICE-like inhibitory protein (FLIP), encoded by the kaposi sarcoma-associated virus (KSHV), to Atg3 [215]. Later stages of autophagy, and particularly fusion with lysosomal compartments, could also be targeted by viruses as evidenced by the action of the HIV viral protein Nef [216], the influenza virus matrix protein 2 (MP2) [217] or the hepatitis B virus X protein (HBX) [218]. Additionally, human cytomegalovirus (HCMV) infection of human fibroblasts was reported to inhibit autophagosome formation [219, 220] and vaccinia virus (VV)-mediated aberrant conjugation system and LC3 lipidation were suggested to be the cause of autophagy inhibition [221]. Also, recent reports on human papillomavirus-16 (HPV-16) infection revealed that viral envelope-mediated signaling events leading to activation of the mTOR pathway correlated with inhibition of autophagy [222]. This would, however, need confirmation because in the same time another study reported a HPV-16-mediated induction of antiviral autophagy upon infection of primary human keratinocytes [223].

Furthermore, specific degradation of viral components by autophagy has been described for only three viruses, the sindbis virus (SIN), the chikungunya virus (CHIKV) and the herpes simplex virus I (HSV-I). The sindbis capsid protein was shown to be targeted for autophagy degradation via the interaction with p62 and with the participation of the HECT domain-containing E3 ligase, Smad-ubiquitin regulatory factor 1 (Smurf1), also important for the mitophagy process [224]. Smurf1 also appeared to be required for the degradation of HSV-I, but seemingly dispensable for general autophagy. Interestingly, the Ub E3 ligase domain of Smurf1 is dispensable for its function in selective autophagy. In the same way, it has been shown that p62 binds to ubiquitinated capsid of CHIKV and targets it to autophagic degradation [225].

Besides the direct role of autophagy in degrading viruses or viral components, autophagy can also stimulate the production of antiviral cytokines through production of type-I IFN. Indeed, in a cell-dependent context, virus-induced autophagy was shown to be important for the delivery of viral nucleic acids to endosomal and cytosolic PRR, thus contributing to the global innate immune response against invading pathogens. Interestingly, while autophagy was shown to be required for type I IFN secretion in response to DNA-immune complexes [226], a recent study reported that autophagy-related proteins could also be involved in the negative feedback regulation of innate immune responses due to the direct interaction between the cytosolic DNA sensor cyclic GMP-AMP (cGAMP) synthetase (cGAS) and Beclin 1 [227]. At a glance, the underlined mechanisms reveal a high level of complexity but the coincidental relationship between autophagy, inflammation and antiviral innate immunity warrants, undoubtedly, further investigation to appreciate all the parameters and cellular factors linked to autophagy involved in such processes.

While autophagy can be an important component of innate antimicrobial immunity, it is becoming clear that this pathway and associated autophagy-related proteins are readily involved in the regulation of antigen processing and adaptive immune responses. It is already known that MHC-II-mediated presentation of endogenous antigens was dependent on cytoplasmic proteases, including the proteasome and calpain as well as non-proteasomal proteases [228, 229]. Interestingly, previous studies reported that autophagy was regulating the trafficking of the invariant chain li, a specialized MHC-II chaperone, towards endo-lysosomal compartments [230] and an inhibitor of autophagy could prevent MHC-II-mediated presentation of the biosynthesized endogenous fifth component of mouse complement C5 [231]. This suggests that autophagy flux modulation was regulating the intracellular antigen flux toward lysosomal compartments before reaching MHC-II molecules. Thereafter, major publications reported that autophagy flux modulation was indeed influencing MHC-II cross-presentation of endogenous antigens [232-235], reinforcing the hypothesis of a non-proteasomal proteolytic machinery involved in cytosolic or nuclear antigens presentation by MHC-II molecules. Further consolidating evidence came from data showing that the viral protein MP1 from influenza fused to LC3 led to an increased MHC-II-mediated antigen presentation to influenza-specific $CD4^+$ T cell clones [236]. These observations were supported by other models of viral infection [235, 237-239], bacterial infection [234, 240, 241] and even in the context of thymocytes selection driven by intracellular self-antigens loaded on thymic epithelial cell MHC-II molecules ([242-244] and see Figure 2). Of note, another autophagy-related process, CMA, was also reported to facilitate MHC-II-mediated presentation of cytoplasmic antigens in B cells via the lysosomal Lamp2A/ Hsc70 complex [245].

Autophagy-related organelles and proteins were also known to intersect with internalized pathogens and endocytic trafficking [246-248], possibly also involving this pathway in extracellular antigen presentation. Indeed, it seems clear now that autophagy can also regulate conventional MHC-II-mediated antigen presentation [249-254], MHC-I-mediated presentation of endogenous antigens [238, 255, 256] and also MHC-I cross-presentation of antigens derived from dying or tumor cells [257, 258]. All these data therefore support a more selective process regulating autophagy-mediated antigen presentation, although the mechanisms brought into play in such a process require further investigation.

Interestingly, some recent reports shine some light on the possible involvement of cell surface PRR in driving specificity toward autophagy-mediated lysosomal degradation which could thus regulate antimicrobial immunity [249, 259].

2.3. Autophagy as a pro-viral mechanism

Several data converge toward the fact that viruses, mainly RNA ones, can use autophagy-related organelles as replication platforms, thereby promoting viral replication. Interestingly, some of them, including coxsackievirus B3 [260, 261] and rotavirus [262] are able to induce autophagy while blocking the fusion between autophagosomes and lysosomes to avoid their degradation. Some viruses, including dengue virus, poliovirus and CHIKV seem to require the whole autophagy process in defined cell types, *i.e.*, the acidic maturation step (for review [263]), while others might require only components of cellular autophagy, like for the murine hepatitis virus (MHV) [264] although this was not confirmed by others [265].

Induction of programmed cell death is also an important host defense mechanism against intracellular pathogens. Indeed, for a multicellular organism, it is advantageous to let infected cells die to thwart viral replication and dissemination. Considering the numerous interconnections between autophagy and apoptosis [266], and the fact that autophagy was already shown to contribute to cell survival after different stresses, including infections [209], virus-induced autophagy may thus counteract the antiviral effect of apoptosis by keeping infected cells alive until the viral replication cycle has been completed. This phenomenon was shown for several viruses, such as the human parvovirus B9, the influenza A virus, CHIKV, and SIN, as they can downregulate apoptosis through activation of autophagy [212, 267-269].

Autophagy is also a recycling pathway that provides free amino acids or fatty acids from

degradation of proteins or lipids, respectively. These new metabolites are then reused either as sources of energy or building blocks for the synthesis of new macromolecules. The dengue virus is the only one described until now that exploits the recycling function of autophagy. Its infection induces lipophagy (selective degradation of lipid droplets by autophagy) and thus release of free fatty acids, which undergo β-oxidation in mitochondria to generate adenosine triphosphate (ATP) [270].

Even if many viruses may utilize autophagy to degrade host restriction factors or to maturate proteins involved in the viral life cycle, the data available are very few. One example is the cleavage of the capsid protein VP0 of the poliovirus in autolysosomes, leading to the maturation of a noninfectious virus to an infectious one [271].

3. Autophagy during HIV-1 infection

Data on the relationships between HIV-1 and autophagy are still fragmentary and further investigation is required. However, from the current literature, autophagy is recognized as an important anti-HIV-1 process manipulated by the virus for its own replication. This process is at the center of the innate and adaptive immune responses against HIV-1. Its regulation depends on the cell type ($CD4^+$ T cells, macrophages or DC) and the status of the cells (*i.e.*, infected or non-infected cells). However, several Atgs including Atg7, Atg12, Atg16L, and GABARAPL2 appear to be required for HIV-1 infection [272] and strikingly, autophagy is also responsible for Env-mediated apoptosis of bystander $CD4^+$ T cells.

3.1. Regulation of autophagy in $CD4^+$ T cells

3.1.1. Autophagy in uninfected $CD4^+$ T lymphocytes

HIV-1 infection is characterized by a progressive decline in the number of $CD4^+$ T lymphocytes in untreated infected patients, ultimately leading to AIDS. Several mechanisms induced by HIV-1 infection are involved in the death of these cells by apoptosis, and Env exerts a major role in this process. HIV-Env can be considered as a pathological ligand as its interaction with cellular receptors (CD4, CCR5 or CXCR4) constitutes the primary interface between viruses and $CD4^+$ T cells. Interestingly, the majority of cells dying during HIV-associated disease progression are not infected. In accordance, several studies have demonstrated that infected cells, expressing Env at the cell surface, are able to induce apoptosis of bystander uninfected $CD4^+$ T lymphocytes upon binding to either CXCR4 or CCR5 [56, 273-275].

There are multiple cross-talks between autophagy and apoptosis. We have demonstrated that Env expressed on HIV-infected cells induces autophagy, subsequently required to trigger $CD4^+$ T cell apoptosis [276]. Indeed, the blockade of autophagy at different steps, by either drugs (3-methyladenine or Bafilomycin A1) or by short interfering RNAs specific for *beclin 1/atg6* and *atg7* genes, inhibited the Env-induced apoptotic process. Even if the presence of CD4 and the co-receptor on uninfected target cells was required for Env-mediated autophagy, this process was independent of these receptors signalling pathways but rather depended on the gp41 fusion activity [277]. Both hemifusion and complete fusion, which leads to the formation of syncytia triggered autophagy in bystander $CD4^+$ T cells. Notably, the deletion of the C-terminal part of gp41 enhanced Env-induced autophagy and apoptosis [277].

Recently, Doitsh *et al.* have identified a new mechanism of uninfected $CD4^+$ T cell death during HIV-1 infection called pyroptosis, defined as a caspase-1-mediated cell death involving the release of pro-inflammatory cytokines. Pyroptosis could be triggered by abortive viral infection in quiescent lymphoid $CD4^+$ T cells [58]. It would be interesting to analyze the possible involvement of autophagy in pyroptosis-associated uninfected $CD4^+$ T cell death.

3.1.2. Autophagy in productively infected $CD4^+$ T lymphocytes

After its entry in $CD4^+$ T lymphocytes, the RNA viral genome of HIV-1 is retro-transcribed into DNA before integration into the host cell genome. In quiescent naive $CD4^+$ T cells, this last step does not occur and in this case, autophagy-dependent cell death would occur. In contrast, activated $CD4^+$ T cells are permissive to HIV replication with *de novo* production of viral particles. Interestingly, in these productively infected $CD4^+$

T cells, autophagy appeared to be completely blocked [278-280]. Indeed, after a co-culture between target $CD4^+$ T cells and HIV-1-infected cells, two target cells populations were observed: a population of highly autophagic uninfected cells and a population of productively infected cells in which autophagy was impaired. Moreover, the level of LC3-II and Beclin 1 were dramatically decreased in the population of productively infected cells [279, 280]. These results suggested that a newly synthesized viral determinant, produced after the provirus integration step, was able to block autophagy [279]. Interestingly, induction of autophagy in productively infected $CD4^+$ T cells with drugs acting on the mTORC1 complex, decreased the production of new viral particles (our unpublished data), indicating that autophagy could behave as an anti-HIV process, however counteracted by the virus in order to replicate efficiently. To date, the mechanism by which HIV-1 could interfere with this essential cellular pathway is still under investigation. As seen before for other viruses, the way to understand the relationship between HIV-1 and autophagy is seemingly paved with complexity. Indeed, data from a genome-wide RNAi screen [272] and silencing of 30 candidate cofactors [281] indicated that HIV-1 replication in cells might require the presence of several Atgs (Atg7, GABARAPL2, Atg12, and Atg16L). A very recent study underlined the role of several Atgs, in particular Atg5 and Atg16, in HIV-1 replication in $CD4^+$ T cells [282].

It is worth noting that autophagy-related proteins can also function independently of the autophagic process, and this aspect has to be taken into consideration when trying to decipher the link between autophagy, autophagic proteins and HIV-1 infection.

3.2. Regulation of autophagy in macrophages

3.2.1. Autophagy in uninfected macrophages

In contrast to bystander $CD4^+$ T cells, autophagy induction was not observed in uninfected cells from the monocyte/macrophage lineage after contact with cells expressing HIV-1 R5 or X4 Env, although these cells were definitely susceptible to autophagy induced by pharmacological means [279]. The state of differentiation was seemingly not responsible for their intrinsic resistance to Env-mediated autophagy. Furthermore, Van Grol *et al*. demonstrated that the absence of autophagy observed in these target cells could be due to the simultaneous activation of Src-Akt and STAT3 by HIV-1 Tat and IL-10, pathways previously known to inhibit autophagy [283]. As Tat upregulates IL-10, the release of Tat from HIV-1- infected cells in the presence of increased concentrations of IL10 can lead to a blockade of autophagy in neighboring, uninfected macrophages, independently of the presence of Env and Nef [283]. HIV-1 Tat was also shown to perturb IFN-γ signaling in monocytes through the suppression of STAT1 phosphorylation [284] and consequently inhibiting MHC-II antigen expression. Recently, Li and collaborators demonstrated that Tat could suppress IFN-γ-induced autophagy in primary macrophages [285], suggesting that inhibition of autophagy could impair the immune defenses by blocking the antigen processing for the recognition and killing of intracellular pathogens. This result correlated well with the physiopathology of HIV-1 infection. Indeed, macrophages do not undergo Env-mediated apoptosis and are not subjected to depletion during HIV-1 infection. However, it raises many unsolved questions, including why the fusogenic function of gp41 induces autophagy only in $CD4^+$ T cells and why Tat and IL-10 do not inhibit autophagy in the bystander $CD4^+$ T cells. While further investigation is needed to elucidate these major differences, one hypothesis would rely on the differential gp41-induced perturbations triggered at the membrane of macrophages or $CD4^+$ T cells. Indeed, HIV-1 can enter macrophages by endocytosis, and additional specific interactions with host membrane molecules following Env binding to the receptor/co-receptor could be involved (described in the paragraph 1.2.). The functions of these two types of cells are also highly different, and macrophages are more prone to autophagy triggering than $CD4^+$ T cells, which may also explain the observed differences.

3.2.2. Autophagy in productively infected macrophages

Conversely, in cells from the monocyte/macrophage lineage, autophagy is induced following productive infection through contact with HIV-1

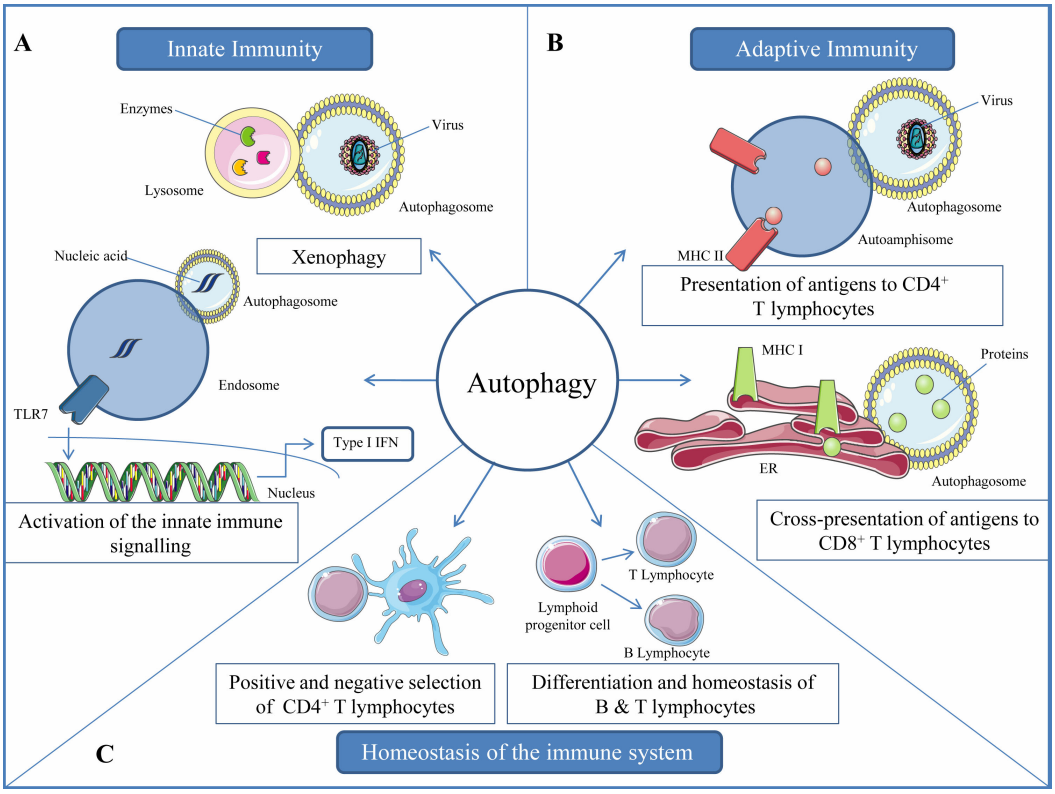

Figure 2

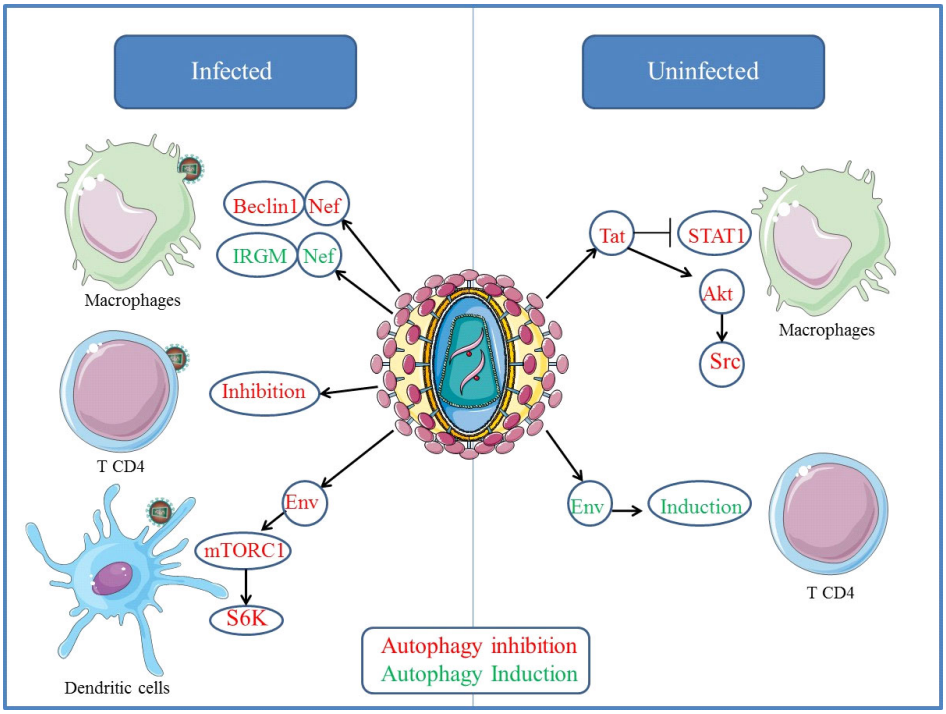

Figure 3

X4 or R5-infected effector cells [279]. Surprisingly, two populations of autophagic cells are present: one highly autophagic and the other weakly autophagic, and viruses could be detected in the weakly autophagic cells but not in the highly autophagic cells, suggesting that autophagy might still be controlled by HIV-1 in these cells to avoid degradation. Interestingly, the early steps of autophagy promoted HIV-1 production since blockade of this process dramatically decreased the quantity of HIV-Gag p24 [216, 279]. In addition, the HIV-1 precursor Gag was found in complexes with LC3 and was also present in LC3-II-enriched membranes, suggesting that autophagy could favor Gag processing and thus production of viral particles [216]. In contrast, the degradative step of autophagy behaves with anti-HIV-1 activity and therefore, needs to be controlled by the virus to prevent its degradation. Indeed, blockade of the degradative step of autophagy increased HIV-1 production [216, 279]. Interestingly, the auxiliary HIV-1 protein Nef was shown to play a major role in the inhibition of the degradative stage of autophagy by binding to Beclin 1 [216]. Nef also interacts with immunity-associated GTPase family M (IRGM), a protein known to play an autophagy-dependent anti-bacterial function [286-288] and to bind to several key proteins of the autophagy process such as Atg5 and Atg10 [289]. Nef/IRGM interaction promotes autophagosome accumulation and improves HIV-1 replication [289]. In contrast, its absence is detrimental for viral production. IRGM also triggers autophagy in cells infected by other RNA viruses, such as Hepatitis C virus (HCV) and Measles virus (MeV), suggesting that different RNA virus families use similar strategies, involving IRGM, to fine-tune autophagy to their own benefit.

In addition to HIV-1 proteins expressed through a transcript initiating from the promoter-harboring 5' long terminal repeat (LTR) region, antisense transcription leads to expression of the antisense protein ASP [290-293]. This protein is unstable in mammalian cells and seems to multimerize *in vitro*. ASP partially colocalizes with LC3 and preliminary data suggest that expression of ASP induces autophagy in the promonocytic U937 cell line and increases viral replication [294]. One hypothesis is that ASP might induce autophagy but then get degraded by autophagy as already shown for the human T-lymphotropic virus (HTLV) protein Tax, thus imposing a positive feedback on its own stability [295].

3.3. Regulation of autophagy in DC

As mentioned above, the autophagy pathway can behave as an important effector of DC-mediated antiviral immunity. In the context of HIV-1 infection of DC, an exhaustion of autophagy-related organelles was rapidly observed upon viral challenge [249]. Interestingly, this was paralleled with the appearance of the compartmentalized virus evidenced hours after viral challenge [249, 250].

Legend to Figure 2. Crosstalks between autophagy and the immune systems during viral infection.

A. Autophagy and innate immunity: Autophagy can directly degrade intracellular pathogens after their sequestration in autophagosomes (xenophagy). It can also stimulate the production of type 1 IFN by allowing the delivery of viral nucleic acids to TLR7-containing endosomes.

B. Autophagy and adaptive immunity: Pathogenic antigens can be presented to $CD4^+$ T lymphocytes by MHC II molecules after fusion of autophagosomes with MHC II-containing endosomes. Autophagosomes can allow the delivery of pathogenic antigens to MHC I molecules for a cross-presentation to $CD8^+$ T lymphocytes.

C. Autophagy and homeostasis of the immune system: Autophagy is involved in both the positive and negative selection of $CD4^+$ T lymphocytes, and in the differentiation and maintenance of B and T lymphocytes.

Legend to Figure 3. Interactions between HIV-1 and autophagic proteins.

The figure shows the complex relationship between the different HIV-1 and autophagic proteins in the different cellular types that constitute the HIV-1 target cells. The figure also illustrates the different autophagy modulation behavior of HIV-1 on its target cells according to their infection status (i.e., whether they were infected or not). Pathways leading to inhibition of autophagy are indicated in red, and those triggering autophagy are indicated in green.

Of note, there is now a growing line of evidence that autophagy-related organelles can intersect with internalized virions and behave as an early antiviral mechanism in myeloid cells [249, 296, 297]. Even more interesting is the observation that DC-SIGN level of expression was linked to autophagy flux induction [298] and the receptor could traffic through autophagy-related organelles early upon engagement [249, 259]. The link between CLR and autophagy therefore warrants further investigation and might be of interest for future therapeutic strategies aiming at enhancing DC antiviral immune responses.

Autophagy exhaustion correlates with a signaling cascade induced early upon HIV-Env binding, leading to the activation of mTOR, a well-known inhibitor of autophagy flux initiation. However, the exact mechanism and upstream signaling effectors require further investigation. mTOR kinase is a target of numerous converging signaling pathways with implications on cellular growth, transcription and translation synthesis, autophagy flux regulation and cell survival (for review see [299]). Interestingly, there are now growing evidences that mTOR function and signaling are intriguingly connected to another amino acid sensor pathway converging toward the general control nonderepressible-2 (GCN2) kinase. Of note, a link between inhibition of TOR signaling and activation of GCN2 to phosphorylate eIF2α has been demonstrated in yeast [300]. In mammalian cells, some studies also reported some potential crosstalk between both pathways, although never related to DC-mediated antiviral immunity [301, 302] but still with implications in protective innate immune and stress responses upon bacterial infection [303, 304]. Outstandingly, GCN2 was recently reported to be a target of HIV-1 infection [305, 306] and was shown to exert antiviral effects against RNA viruses [307]. It would thus be of interest to decipher the potential crosstalk of the mTOR and GCN2 pathways in the context of HIV-1 infection of DC and subsequent immune responses.

In DC, reduction of autophagy was shown to impair TLR-mediated innate immune response [249] while also strongly affecting antigen processing and MHC-II-mediated antigen presentation to $CD4^+$ T cells *in vitro* and *in vivo* [249, 252]. Autophagy involvement in innate immunity was previously reported in pDC with the evidence that cytosolic viral replication intermediates could be transported into the lysosome by the process of autophagy and also regulate IFN-γ secretion upon TLR7-mediated ssRNA recognition [308, 309]. Future studies on upstream cellular factors influencing viral trafficking through endocytic compartments linked to immune responses and intersecting with autophagy-related organelles are therefore required to better understand viral recognition mechanisms in DC, which could help in the design of vaccines.

3.4. Regulation of autophagy in neuronal cells

There is now compelling evidence that defects in neuronal intracellular trafficking is linked to neuropathologies [310]. In this light, the autophagy pathway is playing a key role in neuronal homeostasis as it is involved in clearance of protein aggregates that accumulate during aging and damaged organelles. Defects in this process are commonly found in neurodegenerative disorders [311]. Consistent with this, the autophagy machinery has been proposed to participate in HAND, even though conflicting data have been reported. Studies in patients and in transgenic mice expressing gp120 under an astrocyte-specific promoter have shown alteration in the autophagic pathway [312]. Young (under 50 years of age) patients had an increase in the level of key autophagy markers such as LC3 and Beclin 1, whereas these markers were decreased in older patients, suggesting a modification of the autophagy process during disease progression [312]. This report showed that aged gp120-expressing mice had reduced expression of autophagy markers and increased neurodegeneration compared to aged control animals [312]. Similarly, decreased autophagy has been reported in HIV-infected patients and SIV-infected monkeys [313]. Supernatant from SIV-infected microglia also led to decreased autophagy and alteration of autophagosome formation [314]. However, another study showed increased autophagic markers in HIV patient post-mortem brain samples, as well as in a neuronal cell lines exposed to gp120 [315]. Notably, increased expression/detection of markers such as LC3 can also be explained with a perturbed autophagic flux and not only with a global increase of autophagy.

Moreover, as reported recently, disease progression may interfere differently with the autophagic pathway [312], highlighting the balance between the role of autophagy in neuronal homeostasis and in pathogen defense. In this light, the viral load in the blood stream and therefore the severity of neurocognitive defects (i.e., the neurotoxicity) could switch this balance.

The exact mechanisms of how HIV brain infection does alter the autophagic pathway are still under characterization. More mechanistic studies are therefore needed to determine the exact role of autophagy in HAND and HAD, and in particular in which cell types this process is impaired. As HIV can infect various brain cells but not neurons, other neurotoxic factors, viral proteins included, may also interfere with neuronal autophagy. Interestingly, Tat was reported to modulate the endolysosomal and autophagy system in primary neurons [316], whereas recombinant gp120 was suggested to increase autophagosome formation in neural cells [315].

Finally, autophagy is emerging as a key pathway regulating aging. As stated earlier, defects in autophagy have been reported in pathologies such as Alzheimer and Parkinson's diseases. Notably, there is now compelling evidences that HIV patients developing HAND have symptoms usually associated with an aging brain and increased susceptibility to neuropathologies such as Alzheimer disease. Whether the dysregulation of autophagy observed in HAND and HAD patients has a direct effect on this process is therefore a pertinent question.

4. CONCLUSION AND PERSPECTIVES

Taken together, these results, presented in the Figure 3, suggest a complex, cell-type specific relationship between HIV-1 and the autophagic response, and highlight the complexity of HIV-1 pathogenesis.

Development of drugs acting on autophagy to treat HIV-1 infection is not yet achieved but several *in vivo* and *in vitro* data suggest that drugs that activate autophagy and/or inhibit the degradation step of autophagy may improve future prophylactic and therapeutic strategies by enhancing the immune responses [297, 317-320] and may be used as antiviral compounds in association with HAART.

First, sirolimus (rapamycin, Rapamune), a compound that strongly activates autophagy through inhibition of mTORC1 pathway, enhances the efficacy of HIV treatment. Sirolimus represses HIV-1 replication *in vitro* through different mechanisms including, but not limited to, the downregulation of CCR5 [321, 322]. Although this was very surprising due to the known immunosuppressive action of sirolimus, the rapamycin effects might differ depending on the cell type targeted.

Moreover, the group of B. Levine demonstrated that an autophagy activator peptide, composed of the Tat transduction domain attached to an 18 amino acids sequence derived from the protein Beclin 1 that binds to Nef, decreases HIV-1 replication *in vitro* [297]. Finally, chloroquine, a compound that blocks the fusion between autophagosomes and lysosomes, reduced HIV-1 replication *in vitro* and the DC-DIGN-mediated HIV-1 transfer to $CD4^+$ T cells [323].

Modulation of the autophagy flux might thus be part of the currently developed therapeutic strategies against HIV infection as well as a promising approach to improve antiviral prophylactic regimen.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest in this review.

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