Universidade de Lisboa

Faculdade de Farmácia

Autophagy in the pathogenesis of HIV infection

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Resumo

O Vírus da Imunodeficiência Humana (HIV) é um dos patogenos humanos mais fatais do mundo, com 940 mil mortes por ano. Desde o início da epidemia, na década de 1980, mais de 70 milhões de pessoas foram infetadas pelo HIV e cerca de 35 milhões de pessoas morreram devido ao HIV.

Ao longo dos anos, a autofagia, um processo lisossomal catabólico essencial para a manutenção da homeostase celular, tem emergido como um importante mecanismo de defesa do hospedeiro contra a infeção pelo HIV, não só através da degradação dos patógenos invasores (xenofagia), mas também pela indução da imunidade inata e adaptativa. No entanto, há evidências de que o vírus possui vários mecanismos contra a autofagia que podem evitar e perturbar o mecanismo de autofagia.

O HIV-1 infecta linfócitos T $CD4^+$, macrófagos e células dendríticas, causando a falha do sistema imunológico. O vírus pode também infetar células do Sistema Nervoso Central, o que provoca neurodegeneração. Vários estudos indicam que a neurodegeneração observada na infeção pelo HIV-1 está associada à desregulação da autofagia neuronal. Por todas estas razões, a modulação da autofagia está a ser alvo de investigação para o tratamento de doenças infeciosas.

Devido à capacidade da autofagia de atuar ao nível da degradação intracelular de patógenos, a modulação da autofagia pode potencialmente melhorar o resultado dos tratamentos atuais antirretrovirais para tratar a infeção pelo HIV.

Esta monografia através de pesquisa bibliográfica aborda os principais aspetos da autofagia na patogénese da infeção pelo HIV, agrupando a informação mais relevante sobre a temática, sendo o principal objetivo reunir evidência de que a autofagia é uma via celular que pode ser modulada para novas terapêuticas e vacinas contra o HIV-1.

Palavras-chave: HIV; autofagia; neurodegeneração; terapêutica HIV-1; Resposta imunológica.

Abstract

Human Immunodeficiency Virus (HIV) is among the most lethal human pathogens worldwide with 940 thousand deaths per year. Since the beginning of the epidemic, in the 80's, more than 70 million people have been infected with HIV and about 35 million people have died of HIV.

Over the years, autophagy, a lysosomal catabolic process essential for maintaining cellular homeostasis, has emerged as a major host defence mechanism against HIV infection not only through lysosomal degradation of invading pathogens (xenophagy) but also in the induction of innate and adaptive immunity pathways. However, there is evidence that the virus proteins deploy various countermeasures against autophagy, which can perturb and avoid autophagy mechanism.

HIV-1 infects $CD4^+$ T cells, macrophages and dendritic cells, causing the failure of the immune system. Furthermore, the virus can also infect nervous system cells, which leads to neurodegeneration. Several studies have also indicated that neurodegeneration seen in HIV-1 infection is associated with dysregulation of neuronal autophagy.

Therefore, the modulation of autophagy is being investigated for the treatment of many infectious diseases. Given the capacity of autophagy to act at the host cellular level to improve intracellular killing pathogens, modulating autophagy may potentially improve the outcome of the current antiretroviral therapies to treat HIV infection.

The purpose of this monography is to develop a bibliographic review focusing on the key points of the autophagy in the pathogenesis of HIV infection, grouping the most relevant information on this matter. The main objective is to gather evidence that autophagy is a cellular pathway that could potentially be modulated for HIV-1 therapy and vaccine.

Keywords: HIV; autophagy; neurodegeneration; HIV-1 therapy; immune response.

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Abbreviations

AIDS	Acquired Immune Deficiency Syndrome
AMPK	5' Adenosine Monophosphate-Activated Protein Kinase
APOBEC3G	Apolipoprotein B mRNA Editing Enzyme Catalytic Polypeptide-like 3G
ART	Antiretroviral Therapy
ATG	Autophagy-related Protein
Bcl-2	B-cell Lymphoma 2
BST2	Bone Marrow Stromal Cell Antigen 2
CA	Capsid Protein
cART	Combined Antiretroviral Therapy
CCR5	C-C Chemokine Receptor Type 5
CD4	Cluster of Differentiation 4
CMA	Chaperone-Mediated Autophagy
CNS	Central Nervous System
CQ	Chloroquine
CXCR4	C-X-C Chemokine Receptor Type 4
DAMP	Danger-Associated Molecular Pattern
DAPK	Death Associated Protein Kinase
dCA	Didehydro-Cortistatin A
DCs	Dendritic Cells
DNA	Deoxyribonucleic Acid
EC	Elite Controllers
EECA	Eastern European and Central Asia
Env	Envelope protein
ER	Endoplasmic Reticulum
FI	Fusion Inhibitor
FIP200	Family-interacting protein of 200 kDa
GAPR-1	Golgi Associated Pathogenesis Related Protein 1
HAART	Highly Active Antiretroviral Therapy
HAND	HIV-Associated Neurocognitive Disorders
HDACs	Histone Deacetylases
HDACi	Histone Deacetylases Inhibitors
HIV	Human Immunodeficiency Virus
HSC70	Heat Shock Cognate 71KDa Protein

HSV-1	Herpes Simplex Virus type I
IAP	Antiapoptotic Inhibitor of Apoptosis
IFN	Interferon
IL	Interleukin
IN	Integrase
ISG	Interferon Stimulated Genes
IRF	Interferon Regulatory Factor
JNK	Jun N-Terminal Kinase
kDa	Kilodalton
LAMP2A	Lysosome-Membrane Protein 2
LAP	LC3-Associated Phagocytosis
LC3	Light Chain 3
LIR	LC3-Interacting Region
LRR	Leucine-Rich Regions
LTR	Long Terminal Repeats
MA	Matrix Protein
MAL	MyD88 Adapter-Like
MAPK	Mitogen-Activated Protein Kinase
MHC	Major Histocompatibility Complex
MyD88	Myeloid Differentiation Primary Gene 88
mTOR	Mammalian Target of Rapamycin
mTORC1	mTOR Complex 1
mTORC2	mTOR Complex 2
NC	Nucleocapsid Protein
NDP52	Nuclear Dot Protein 52Kda
Nef	Negative Regulatory Factor
NF-κB	Nuclear Factor κB
NLR	Nod-like Receptor
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NO	Nitric Oxide
Nod	Nucleotide-binding and Oligomerization Domain
NRTI	Nucleoside/Nucleotide Reverse Transcriptase Inhibitor
OAT	Opioid Agonist Therapies
ORF	Open Reading Frame
PAMP	Pathogen-associated Molecular Pattern

PAS	Phagophore Assembly Site
PBMC	Peripheral Blood Mononuclear Cell
PE	Phosphatidylethanolamine
PI	Protease Inhibitor
PI3K	Phosphatidylinositol 3-Kinase
PI3P	Phosphatidylinositol-3-Phosphate
PR	Protease
PRR	Pattern Recognition Receptor
RIG-I	Retinoic acid-Inducible Gene I
RLR	RIG-I like Receptor
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
RRE	Rev Responsive Element
RT	Reverse Transcriptase
SAHA	Suberoylanilide Hydroxamic Acid
siRNA	small interfering RNA
SIV	Simian Immunodeficiency Viruses
SQSTM1	Sequestosome-1
SSP	Syringe Service Program
ssRNA	Single-stranded RNA
STAT1	Signal and Transducer and Activator of Transcription-1
Tat	Transactivator Protein
TFEB	Transcription Factor EB
TLR	Toll-like Receptor
TOR-KI	ATP-competitive mTOR
TRAF6	Tumor Necrosis Factor Receptor-Associated Factor 6
TRAM	TRIF-related Adapter Molecule
TUFM	Tu Factor of Mitochondria
ULK1	Unc-51 Like Kinase 1
VC	Viremic Controllers
VDR	Vitamin D Receptor
Vif	Viral Infectivity Factor
Vps34	Vacuolar Protein Sorting 34
VSV	Vesicular Stomatitis Virus
WHO	World Health Organization

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1. Materials and methods

In order to write this monography, I consulted search platforms that comprise biomedical literature from various areas, including Pubmed (https://www.ncbi.nlm.nih.gov/pubmed/), Google Scholar (https://scholar.google.com/) and Sciencedirect (www.sciencedirect.com). The search was made using English terms and mainly the following keywords: HIV, autophagy, neurodegeneration, HIV-1 therapy, immune responses, pattern recognition receptors, HIV proteins and viral regulation of autophagy. The research was initiated on March 2019 and lasted until July 2019.

Besides review and research articles, fonts such as the sites of the World Health Organization (https://www.who.int/), Centers for Disease Control and Prevention (https://www.cdc.gov/) and United Nations Programme on HIV/AIDS (https://www.unaids.org/en) were also crucial, proving HIV and AIDS statistics, insights on the disease and its treatment and some of the latest news regarding this subject, keeping this monography as updated as possible.

2. Introduction

2.1. Human Immunodeficiency virus (HIV)

AIDS (Acquired Immune Deficiency Syndrome) is a global epidemic caused by two genetically diverse lentiviruses from the *Retroviridae* family, HIV-1 and HIV-2, that were introduced through cross-species transmissions of simian immunodeficiency viruses (SIV) from African primates to human (1). The most commonly accepted theory for this transmission is that SIV was transferred to humans as a result of primates being killed and eaten or their blood getting into cuts on people in the course of hunting (1). The hunter's body would have fought off SIV, but on a few occasions the virus adapted to the new human host and became HIV (1).

HIV-1 is one of the most genetically diverse pathogens due to its high mutation and recombination rates, large population size and rapid replication rate, making it difficult to development a cure for HIV (2). This evolutionary process has resulted in several HIV subtypes that are worldwide and heterogeneously distributed (2).

2.1.1. Epidemiology

HIV is among the most lethal human pathogens worldwide with 940 thousand deaths per year. Since the beginning of the epidemic, in the 80's, 77.3 million people have been infected with HIV and 35.4 million people suffer from AIDS-related illnesses since the start of the epidemic (3). The origin of HIV-1 has been documented nearby Kinshasa, nowadays named Democratic Republic of Congo around the 1920s (4). However, it was only in 1959, that the Aaron Diamond AIDS Research Center in New York diagnosed the first case of HIV-1 infection to a man living in the same region (5). From then on, AIDS became an epidemic. In 1981, cases of rare diseases were being reported among homosexual men in New York and California, such as Kaposi's Sarcoma and a lung infection called *Pneumocystis carinii* Pneumonia*,* which currently has a new nomenclature called *Pneumocystis jirovecii* Pneumonia (6–9)*.* In 1982 scientists realized the 'disease' was also spreading among other populations such as haemophiliacs and heroin users and by September of that year, the 'disease' was finally named AIDS (10–13). The global spread of the virus has been associated with the currently human mobility due to exploration, conquest, commerce, international travel and migration (14). Hence, in 2017 there were 258 million migrants and approximately one-quarter of whom were forced migrants (15,16), whose majority come from areas with a high prevalence of infectious diseases and may have associated poor health outcomes (17,18). Therefore, in 2017, 36.9 million people were living with HIV worldwide with most of these infections in sub-Saharan Africa, with 19.6

million people infected with the virus (3). However, the WHO-defined Eastern European and Central Asian (EECA) region is the only region globally where HIV incidence and mortality continue to rise (3). While global HIV incidence and mortality have continued to decline, they have increased by 58% and 25%, respectively, in EECA from 2010 to 2015, due to low HIV treatment and prevention coverage (19,20) and the high number of people who inject drugs in this region (4). Therefore, to control the spread of HIV, primary and secondary prevention is needed, which includes adequate coverage with syringe service programs (SSPs), opioid agonist therapies (OAT) linked to an effective HIV response that increases access to HIV testing and prescription of antiretroviral either as a treatment and prevention strategy (1,3,4).

	2000	2005	2010	2012	2014	2015	2016	2017	2018
People living with HIV	**24.9 million** [21.5 million–28.9 million]	**28.5 million** [24.5 million–33.0 million]	**31.7 million** [27.3 million–36.8 million]	**33.2 million** [28.7 million–38.6 million]	**34.8 million** [30.0 million–40.4 million]	**35.6 million** [30.7 million–41.3 million]	**36.4 million** [31.4 million–42.3 million]	**37.2 million** [32.1 million–43.2 million]	**37.9 million** [32.7 million–44.0 million]
New HIV Infections (total)	**2.8 million** [2.2million–3.6 million]	**2.4 million** [1.9 million–3.2 million]	**2.1 million** [1.6 million–2.7 million]	**2.0 million** [1.5 million–2.6 million]	**1.9 million** [1.5 million–2.5 million]	**1.9 million** [1.5 million–2.4 million]	**1.8 million** [1.4 million–2.4 million]	**1.8 million** [1.4 million–2.3 million]	**1.7 million** [1.4 million–2.3 million]
New HIV infections (aged 15+)	2.3 million [1.8 million–3.1 million]	2.0 million [1.6 million–2.6 million]	1.8 million [1.4 million–2.4 million]	1.8 million [1.4 million–2.3 million]	1.7 million [1.3 million–2.2 million]	1.7 million [1.3 million–2.2 million]	1.7 million [1.3 million–2.2 million]	1.6 million [1.3 million–2.1 million]	1.6 million [1.2 million–2.1 million]
New HIV infections (aged 0–14)	450 000 [300 000–700 000]	410 000 [270 000–640 000]	280 000 [190 000–430 000]	230 000 [150 000–350 000]	200 000 [130 000–310 000]	190 000 [120 000–290 000]	180 000 [120 000–280 000]	170 000 [110 000–270 000]	160 000 [110 000–260 000]
AIDS-related deaths	**1.4 million** [1.0 million–1.9 million]	**1.7 million** [1.3 million–2.3 million]	**1.2 million** [860 000–1.6 million]	**1.0 million** [770 000–1.4 million]	**920 000** [680 000–1.3 million]	**880 000** [650 000–1.2 million]	**840 000** [620 000–1.1 million]	**800 000** [600 000–1.1 million]	**770 000** [570 000–1.1 million]
People accessing antiretroviral therapy	**576 000** [507 000–599 000]	**2.0 million** [1.8 million–2.1 million]	**7.7 million** [6.8 million–8.0 million]	**11.2 million** [9.9 million–11.7 million]	**15.1 million** [13.3 million–15.7 million]	**17.0 million** [15.0 million–17.7 million]	**19.1 million** [16.8 million–19.9 million]	**21.3 million** [18.8 million–22.2 million]	**23.3 million** [20.5 million–24.3 million]
Resources available for HIV (low- and middle-income countries)*	**US$ 4.8 billion****	**US$ 9.4 billion****	**US$ 15.0 billion****	**US$ 17.4 billion****	**US$ 18.1 billion*****	**US$ 18.0 billion*****	**US$ 18.4 billion*****	**US$ 19.9 billion*****	**US$ 19.0 billion*****

Figure 1 - Global HIV data 2000-2018 (Adapted from reference (3))

2.1.2. Infection Pathogenesis

During HIV infection, usually there are three main stages: acute, latent and AIDS stage. Furthermore, HIV infection can be subdivided into three types: rapid progression, where AIDS develops after 3 years of infection, intermediate progression, where AIDS develops slowly between 3 and 10 years and long-term non progression (LTNP) where HIV infected people maintain high $CD4^+$ and $CD8^+$ T-cell counts, remaining clinically asymptomatic without therapy for at least 8 to 10 years (21). The long term non-progressors are less than 5 % of the total HIV population (21). These

patients can be also divided into 2 groups: viremic controllers – VC (50-2000 HIV RNA copies/mL) and the elite controllers – EC (less than 50 HIV RNA copies/mL) (22).

Acute phase of HIV-1 infection (Window period)

After infection with HIV-1 (sexual contact, significant exposure to body fluids or infected tissues, vertical transmission or during breastfeeding) it takes 3 to 6 weeks for anti-HIV antibodies to reach detectable levels in peripheral blood (23). This period is called “window period” because the diagnostic tests that detect anti-HIV antibodies are ineffective during this period (23). However, during the “window period”, a high level of viraemia is observed with plasma viral load reaching a peak in 2-3 weeks (23). Simultaneously, a significant loss of T helper cells is detectable, causing a decrease in circulating $CD4^+$ T lymphocytes (23). The host initiates immune responses during this period in order to control the virus multiplication leading to a decline in plasma viraemia (23). Some infected people may suffer flu-like clinical symptoms during this period such as fever, headaches, arthralgia and rashes (23). About 4-6 months post-infection, steady-state of viraemia (virologic set point) is achieved and this plasma viral load set point is crucial for the future course of the disease since low plasma virus load is usually associated with slower disease progression (23).

Clinically latent phase of HIV infection

The plasma virus load level remains stable for several years after establishment of the plasma virus load set point and the infected person remains asymptomatic during this period (23). Although, a constant multiplication of virus is maintained leading to the destruction of $CD4^+$ T lymphocytes and a gradual depletion of $CD4^+$ T lymphocytes in peripheral blood (23). Plasma viral load and $CD4^+$ T lymphocytes counts are hence two important parameters of HIV disease progression. However, the decline in $CD4^+$ T lymphocytes counts may be influenced by various factors such as plasma viral levels, opportunistic infections, nutritional factors (23). The molecular mechanisms that determine whether a virus produces new viral particles or, instead, performs a latent infection are still unclear (24).

AIDS stage

The gradual decrease in $CD4^+$ T lymphocytes cells (below 200 cells/mm3) ultimately results in loss of control over the immune response and several opportunistic infections emerge (23). This is considered the terminal stage of HIV infection and is

called AIDS. Common opportunistic infections include *Pneumocystis jirovecii* pneumonia, *cryptococcal meningitis*, oral and *esophageal candidiasis* (23). Recurrent activation of Herpes zoster and malignancies such as non-Hodgkin's lymphoma are commonly seen during this stage (23). Symptoms such as night sweats, fever, diarrhea, weight loss and fatigue are also commonly seen during this period (23).

2.1.3. Structure and genome

Virions are spherical with 100-120 nm diameter formed by a lipid bilayer membrane that surrounds the nucleocapsid (core), which contains the genomic RNA molecules, the viral protease (PR), reverse transcriptase (RT), integrase (IN) and other viral proteins (24). The HIV-1 genome consists of two single stranded RNA (ssRNA) molecules within the virion, but the latent form of the HIV-1 genome is the proviral double-stranded DNA within infected cells (24). For the binding of nucleic acids and rearrangement of the most stable conformation of these genomic RNA is required the nucleocapsid protein (NC) or p7 (24–26). p7 is derived from the multidomain 55 kDa Gag precursor protein, which contains matrix (MA), capsid (CA), NC and p6 (26). The p7 bonds to the RNA as well as enzymes necessary to viral replication such as RT, PR, IN and ribonucleases (27). To ensure virion integrity, surrounding the capsid, is localized the MA or p17, forming the matrix. The capsid is one of the proteins forming the core constituted by N-terminal domains of capsid protein (CA) or p24, which assemble in hexameric rings (24,28). During the late stages of the HIV-1 replication cycle, p17 domain has a key role by targets the Gag polyprotein to the host-cell membrane for particle assembly (27,29). Above this matrix, there is a lipid bilayer called viral envelope, where is *env*-encoded proteins composed by two glycoproteins, gp120 and gp41, that allows the fusion with membrane and penetration in cells (27).

The RNA genome consists in nine genes (*gag*, *pol*, *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu* (for HIV-1), or *vpx*, for HIV-2, which encode nineteen different proteins. The genes *gag*, *pol* and *env* coding for structural proteins essential for the emergence of new viral particles (such as integrase, protease and reverse transcriptase enzymes) and the remaining genes (tat, *rev*, *nef*, *vif*, *vp*r and *vpu* / *vpx*) regulate proteins that control the ability of HIV to infect cells or replicate themselves (produce new copies of viruses) (30). The end of each RNA chain contains a RNA sequence called the long terminal repeat (LTR) and regions of this sequence act as "switches" to control the production of new virions being activated by proteins from HIV or from the host cell (31).

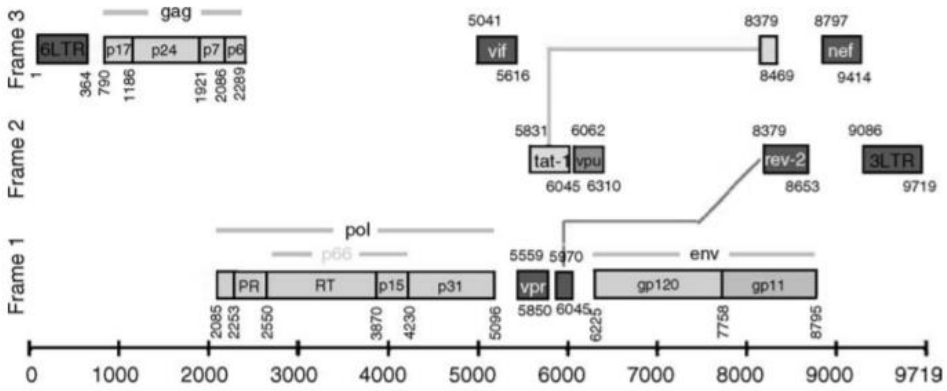

Figure 2 – Organization of the proviral HIV-1 genome (Adapted from reference (24).

2.1.4. Replicative cycle

The infection process begins with the attachment of virions to cells by an interaction between HIV-1 gp120 and cellular receptor CD4 and a coreceptor, usually the chemokine receptor CCR5 (C-C receptor type 5) or CXCR4 (C-X-C receptor type 4) (24,32–34). After the anchoring of gp120 and binding with the coreceptor CCR5/CXCR4, the subunit gp41 forms pores to allow the viral and cellular fusion (24,35–37). Afterwards, the viral core is released into the cell and the viral RNA genome is retrotranscribed into a double-stranded DNA by the viral RT, which can be integrated into the host cell genome (24,38,39). The action of the reverse transcription can be blocked by the presence of a cellular protein named APOBEC3G but the viral protein, Vif, counteracts the antiretroviral effect of APOBEC3G by inhibiting its translation and by accelerating the posttranslational degradation (24,40–44).

Then, the preintegration complex composed by the integrase (45) docks to the nuclear membrane by HIV-1 Vpr (46) and enters the nucleus through a nuclear pore (24,47,48). Before the integration, the viral DNA can be found in three forms: linear, 1-LTR (Long Terminal Repeats) or 2-LTR circles, which produce Nef, Tat and Rev through activation of the LTR promoter by cellular factors such as NF-κB (24,49,50). Linear double stranded-DNA in the preintegration complex is inserted into the host chromosome by the viral IN (24,51–53). Once proviral DNA is integrated, the first rounds of proviral transcription by cellular RNA polymerase II occur producing again Nef, Tat and Rev (24,54). When a sufficient amount of Tat has been produced, Tat controls further transcription of HIV-1 genes by binding to the TAR element of the LTR and to other transcriptional activators of cellular origin (39,55). Finally, when enough Rev is produced, it binds to a structure designated RRE (Rev Responsive Element) present in the singly-spliced and unsliced viral RNA molecules and facilitates their

transport through the nucleus membrane, saving them from splicing and allowing them to be translated in the cytoplasm, leading to the production of other viral proteins and genomic RNA (24,28,56,57).

The *env* gene is translated into the precursor protein gp160, which is glycosylated within the endoplasmic reticulum. Also, the *gag-pol* gene is primarily translated to produce the Gag and Gag-Pol polyproteins. Gag polyprotein (p55) is proteolytically processed during maturation of the virus into six structural proteins which rearrange and produce the mature virion. On the other hand, Gag-Pol protein when cleaved leads to the production of protease (PR), reverse transcriptase (RT) and integrase (IN). After translation, the Env proteins migrate and insert into the plasma membrane, as well as, Gag and Gag-Pol polyproteins which assemble directed by the Gag polyprotein (24,28,56–58). Also, viral enzymes, full-size genomic RNA, the cellular tRNAlys3 primer for initiation of reverse transcription and cellular compounds associate to the immature core (24,59–62). Later on, this complex buds through the plasma membrane producing an immature virion (24). For viral assembly and budding, there must be a decrease in the number of the CD4 molecules present in the plasma membrane to avoid interactions with the newly synthesized gp120 (24). Indeed, in the early stages of infection, Nef accelerates endocytosis and, therefore, degradation of CD4 molecules as well as MHC class I and II molecules on the surface (24,63). In the later stages, the envelope precursor gp160 traps the newly synthesized CD4 molecules within the endoplasmatic reticulum (24). Moreover, Vpu induces the degradation of these CD4 molecules and releases the gp160 molecules allowing their maturation and trafficking. (24,64,65) Also, Vpu forms ion conductive pores leading to enhanced virus release (66). Budding triggers the activation of the PR that autocatalytically cleaves the Gag and Gag-Pol polyprotein releasing the structural proteins and enzymes (24,28,67). The individual proteins undergo further interactions, with CA and NC forming the conic nucleocapsid, and MA remaining associated with the viral envelope. (24,28,67). After all this process, it can occur “cell-to-cell” infection, in which viruses spread through contact of infected cell with surrounding uninfected cells (68).

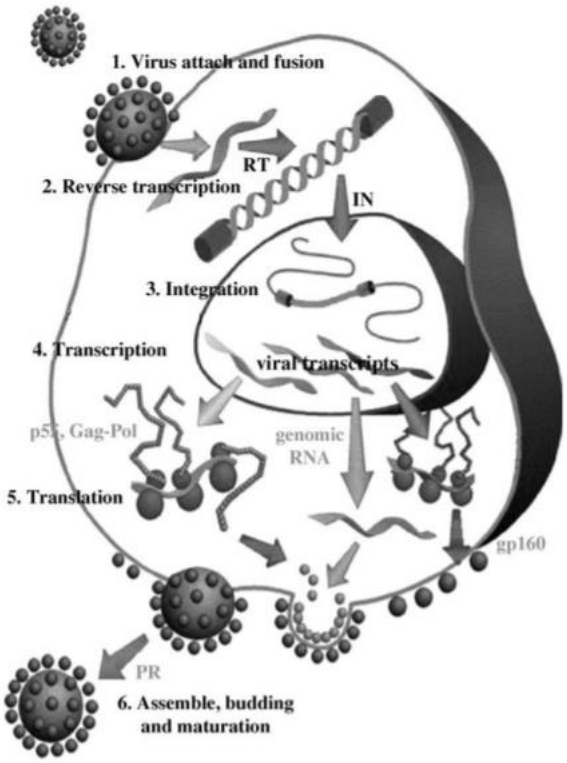

Figure 3 – Replicative Cycle of HIV (Adapted from reference (24)).

2.1.5. HIV-1 Tropism

HIV-1 infects mainly $CD4^+$ T lymphocytes, macrophages and Dendritic Cells (DCs) leading to the failure of the immune system (69–71). The virus can also access the brain, where it infects and replicates in macrophages, microglial cells, astrocytes leading to neurodegeneration known as HIV-Associated Neurocognitive Disorders (HAND) (71,72).

2.1.5.1. $CD4^+$ T cells

$CD4^+$ T cells are a lymphocyte subpopulation also known as helper T cells. They are involved in the immune response by helping B cells in the production of antibodies, as well as in enhancing the cellular immune response to antigens (73). Concerning the interaction between these cells and HIV, after a few hours of exposure to the virus, the $CD4^+$ T lymphocytes are infected and viral replication initiates (74). The infected $CD4^+$ T cells release virions, which subsequently infect uninfected $CD4^+$ T cells (74). These cells are also important viral reservoirs since a part of these cells carries HIV proviruses integrated into the host DNA without active viral replication (74). The circulating $CD4^+$ T cell count is used not only as a tool to

monitor disease progression but also to evaluate the efficacy of antiretroviral therapy (ART) (74).

2.1.5.2. Macrophages

A macrophage is a type of phagocyte, which is responsible for detecting and destroying pathogens and apoptotic cells (removing dead or necrotic cells) (75). Macrophages are produced through the differentiation of monocytes, which turn into macrophages when they leave the blood (75). Macrophages also play a role in alerting the immune system to the presence of pathogens (75).

The HIV infection in macrophages induces the secretion of cytokines, which recruits T lymphocytes, therefore infected macrophages increase the number of surrounding target cells and transmit the infection to various tissues and organs, thanks to its ability to cross the blood-tissue barrier(76). Although the replication cycle is the same in macrophages and T lymphocyte, macrophages are much more resistant to the cytopathic effects of the virus, which means that, in the absence of cell death, the infected macrophages are preserved, producing and accumulating virions for long periods of time, becoming viral reservoirs (76).

2.1.5.3. Dendritic Cells

Dendritic cells are antigen-presenting cells that are present in tissues such as the skin, where a specific dendritic cell called the Langerhans cell is found, inside the nose, lungs, stomach, intestines and are also found in the blood in its "immature" form (77). When activated, DCs migrate to the lymph nodes, where they interact with T cells and B cells to initiate an immune response (77). At a certain point, branched projections grow, the dendrites, and that´s why they called Dendritic cells (77). During sexual transmission of HIV, it is thought that dendritic cells are among the first to find the virus and these include non-migrating Langerhans cells from the epithelial and mucosal tissue, as well as immature myeloid dendritic cells from the submucosa (78,79). Therefore, immature dendritic cells capture HIV and migrate to lymphoid tissues full of CD4 + T cells, where HIV trans-infection of activated CD4 + T cells occurs, facilitating viral spread (78,79).

2.1.5.4. Central Nervous System Cells

The migration of infected cells across the blood-brain barrier ("Trojan horse" hypothesis) is considered the main cause of Central Nervous System (CNS) infection and affects half of all adults with AIDS (80–82). In the brain, macrophages, microglia

and astrocytes are the major types of cells infected with HIV-1 and potentially serve as viral reservoirs (82,83). Microglia, immune cells of CNS, are the main players in the development of neurological disorders related to HIV-1 (82,84,85). Besides phagocytosis, microglia are involved in surveillance of the microenvironment, communicating with neurons and with other glia, which in turn initiate immune responses (82,85). Infected microglia not only produces viral particles but also secretes HIV Tat protein, which affects bystander neurons causing an accumulation of autophagosomes and leading to neurodegeneration (82,86,87). Neurons are highly specialized for intercellular communication. However, neurons are post-mitotic, therefore it's important to avoid the accumulation of toxic proteins and organelles. As such, autophagic control of proteins and organelles is crucial for neuronal function (82,88). HIV-1 can also infect astrocytes which serve as viral reservoirs with low levels of viral replication by suppressing autophagy (82,89). Astrocytes are the most abundant cell type and are responsible for maintaining brain homeostasis, by function as neurotransmitter trafficking and recycling, nutrient and ion metabolism and defence against oxidative stress (80,82).

2.1.6. Current therapy

The current therapeutical strategy, called highly active antiretroviral therapy (HAART) or combined antiretroviral therapy (cART), involves the use of agents from at least two distinct classes of antiretrovirals (24,90). The classes being used in the clinical practice are inhibitors of the reverse transcriptase (nucleoside/nucleotide, NRTI, and non-nucleoside, NNRTI), protease inhibitors (PI), fusion inhibitors (FI), antagonists of the CCR5 coreceptor and integrase inhibitors (24,90,91).

In the majority of HIV-1 infected patients, HAART is capable of increasing $CD4^+$ T cell counts and reducing plasma viraemia to undetectable values (92,93). At present, the international guidelines recommend treatment of all HIV-positive patients regardless of their $CD4^+$T-cell count and early initiation of ART (91,94–96). Initiating ART as early as the day of HIV diagnosis is a strategy to control HIV epidemic and optimize the health of people living with HIV due to the reduce morbidity and mortality from HIV infection and reduce HIV transmission (97). However, the long-termed virological success of HAART is limited by resistance development, side effects which also affect treatment adherence and patients' personal choice (24,93,97). Therefore, new treatments acting on alternative targets, to avoid resistances, and with better systemic tolerability profiles are required (24).

2.2. Autophagy

The evolution of the endomembrane system was critical for unicellular eukaryotes that needed to be in continuous contact with their food sources by allowing the storage of nutrient that could be used during periods of starvation (98,99). The term autophagy originates from the Greek expressions αὐτός (autos = self) and φαγεῖν (phagein = to eat), literally meaning the self-eating of a cell (100). Macroautophagy (hereafter named as autophagy) is a mechanism that mediates membrane rearrangements to permit cellular catabolism (99,101). However, this pathway has evolved to respond to many other stressors besides starvation, including hypoxia, high temperature, overcrowding reactive oxygen species (ROS) and endoplasmic reticulum (ER) stress (99,102). It is also a mechanism for lysosomal degradation and recycling of intracellular portions of eukaryotic cells (103,104). Autophagy has been shown to play an important role in the pathogenesis of viral infections and is suggested to act as inducer and effector of innate and adaptive immune responses against intracellular pathogens, including viruses. Evidence suggests that viruses have countermeasures to content with this pathway: some inhibit autophagy and are negatively affected when this interference is eliminated, while others respond positively when it is induced (104). However, some viruses seem unaffected by autophagy and do not appear to regulate the pathway through any apparent mechanism (104).

2.2.1. Types of autophagy

In mammals, there are four distinct autophagic pathways: macroautophagy, microautophagy, chaperone-mediated autophagy (CMA) and selective pathway, each to degrade specific cellular components by distinctive mechanisms (105,106). The macroautophagy degrades substrates inside a double membrane structure termed the autophagosome, which fuses with lysosomes (106,107). The second pathway, microautophagy, directly captures target cytosolic substrates through the invagination of membranes into the lysosomes (106,107). The one referred as chaperone-mediated autophagy, leads to the degradation of proteins harboring the KFERQ domain, which allows them to interact with the lysosomal membrane protein LAMP2A (Lysosome-membrane protein 2) and the HSC70 (Heat shock cognate 71KDa protein) chaperone (106,107).

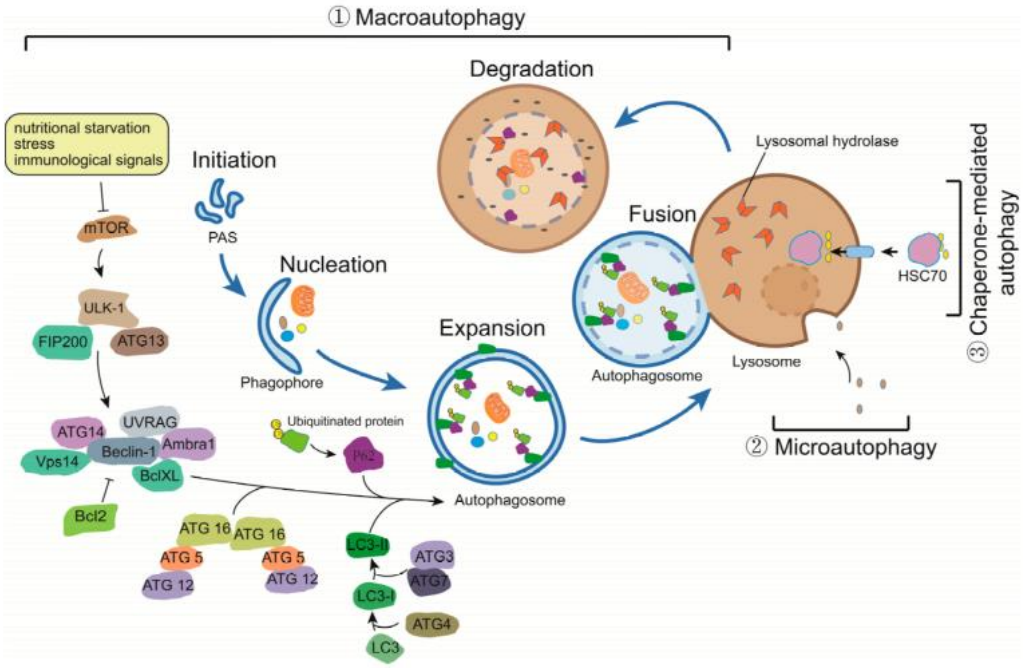

Figure 4 - Three types of autophagy (Macroautophagy, Microautophagy and Chaperone-mediated autophagy) and regulatory mechanisms (Adapted from reference (106)).

Also, a fourth pathway with selective forms of autophagy has been identified leading to the specific degradation of organelles or pathogen (106,107). These selective pathway includes the autophagic degradation of mitochondria (mitophagy), peroxisomes (pexophagy), endoplasmic reticulum (reticulophagy or ER-phagy), ribosomes (ribophagy), protein aggregates (aggrephagy), lipid droplets (lipophagy), spermatozoon-inherited organelles following fertilization (allophagy), secretory granules within pancreatic cells (zymophagy), or intracellular pathogens (xenophagy) (100).

The major form of autophagy is macroautophagy hereafter referred to as “autophagy” and has been first reported in 1962 (108). Furthermore, studies have proved that HIV-1 infection can trigger and restrict this type of autophagy depending on the cell type (106,109).

3. Overview of the mechanisms and regulation of autophagy

There is evidence that autophagy represents a conserved host defence response against diverse intracellular pathogens (99). In mammalian cells, autophagy is regulated by more than thirty genes named autophagy-related proteins (*ATG*) (22,82). The core *ATG* genes can be divided into four groups: *ATG*1/unc-51-like kinase (ULK) and their regulators, Vacuolar Protein Sorting 34 (Vps34) complex I, the *ATG*9 cycling complex and conjugation pathways involving the ubiquitin-like proteins Atg12 and Atg8/LC3 (microtubule-associated protein light chain 3) (110). These genes coordinate autophagosome formation, encapsulation of target cargos and subsequent fusion with the lysosome for degradation (22,82). The process involves three steps: initiation, elongation and maturation (22).

The initiation step starts with the isolation of a cellular membrane (called phagophore) at the ER surface by the formation of an omega-like shape protrusion (22,111,112) and with the formation of the phagophore assembly site (PAS) (82,113). This step is induced by a preinitiation complex composed by Atg101 and Atg13 proteins, FIP200 (a focal adhesion kinase family-interacting protein of 200 kDa) and UNC-51 like kinase (ULK) 1 and ULK2 proteins, which will assemble to the PAS (22,114–117). In the absence of stimuli, the preinitiation complex is inhibited by the serine/threonine kinase mammalian Target of Rapamycin (mTOR) protein, which is a negative regulator of autophagy (22,118,119). mTOR forms two distinct complexes, mTORC1 and mTORC2. In mammalian cells, mTORC1 consists of mTOR, mLST8 (GβL), and raptor, and mTORC2 consists of mTOR, mLST8, mSin1, and rictor (22,119,120). mTORC1, not mTORC2, is a nutrient-sensitive complex that can be inhibited by rapamycin mTOR (120). mTORC1 maintains the preinitiation complex in an inactivated state by phosphorylating ULK1 and Atg13 (22,119,120). Under metabolic stress (e.g. nutrient starvation or amino acid deprived conditions) and immunological signals, mTORC1 is inhibited, allowing the release and the translocation of the preinitiation complex to the site of phagophore formation (22,121). Subsequently, ULK1 phosphorylates Beclin-1 (an essential autophagic protein), which leads to the dissociation of Beclin-1 from B-cell lymphoma 2 (Bcl-2), another inhibitor of autophagy (106,122,123). Beclin-1 under normal conditions remains associated with other proteins, particularly to the antiapoptotic protein, Bcl-2, and sometimes to the 14-3-3 or vimentin 1 (82,124). Dissociation of Beclin-1 from Bcl-2 is mediated either by phosphorylation of the BH3 domain of Beclin-1 by Death Associated Protein Kinase (DAPK) or through the phosphorylation of Bcl-2 by c-Jun N terminal kinase (JNK)

which is essential for Beclin-1 to be incorporated in the class III phosphatidylinositol 3-kinase (PI3K) complex (82,124,125). Afterwards, Beclin-1 interacts with several cofactors (Atg14L, UVRAG, Bif-1, Rubicon, Ambra1, HMGB1, nPIST, VMP1, SLAM, IP(3)R, PINK and survivin) to activate the lipid kinase Vps34 and forming the Beclin-1-Atg14L-Vps34-Vps15 complex, the PI3K complex (106,110,123). This complex is targeted to the ER-mitochondria contact sites, where it produces phosphatidylinositol-3-phosphate (PI3P) triggering phagophore formation and providing a membrane platform for accumulation of autophagosomal proteins (22,126,127).

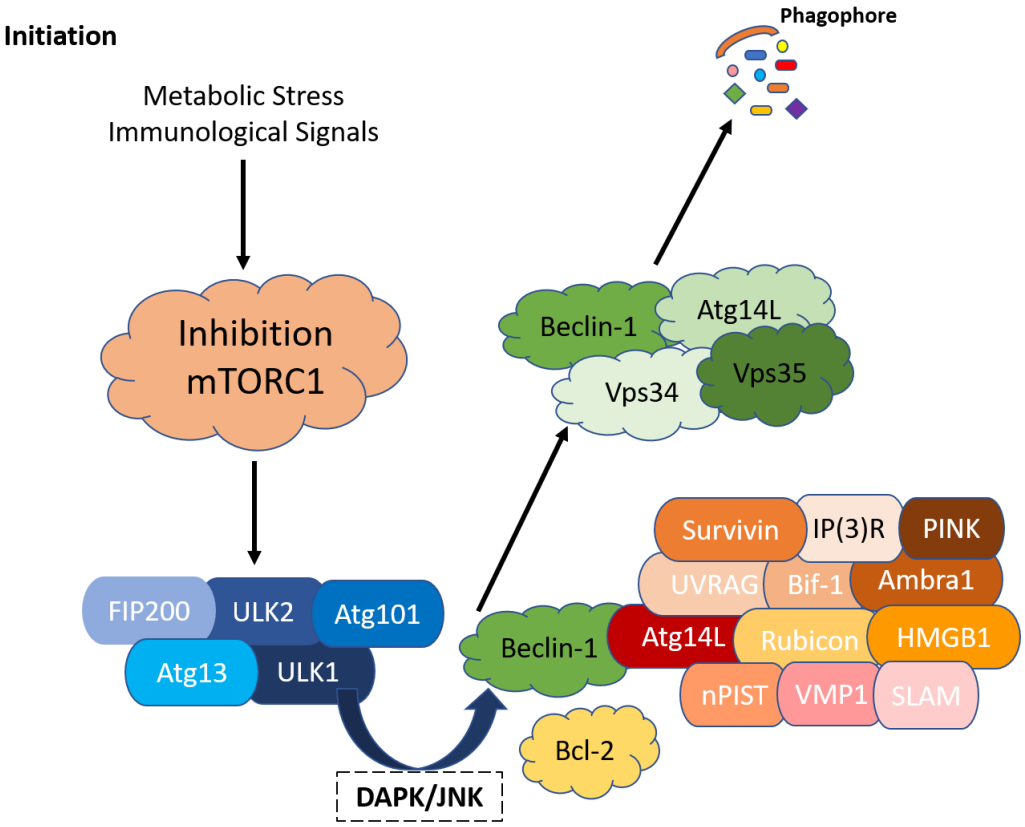

Figure 5 - Schematic model of the initiation step of autophagy.

The elongation step, where occurs phagophore expansion, is regulated by the Atg5-Atg12 conjugate associated with Atg16L and the Atg8/MAP1LC3 (microtubule-associated protein 1 light chain 3, hereafter referred to as LC3) conjugation system (22,128,129). Atg8/LC3 is cleaved by Atg4 protease exposing a C-terminal glycine residue. Cleaved LC3 is then conjugated with phosphatidylethanolamine (PE) by activation of Atg7 (E1-like enzyme), Atg3 (E2-like enzyme), and the Atg5-Atg12 complex, to generate LC3-PE (a membrane-bound form of LC3 also referred to as LC3-II), the level of which is correlated with the number of autophagosomes (128,130,131). Atg8 proteins exist as cytosolic forms, being designated LC3-I.

However, once autophagy is induced, LC3-I is translocated to the phagophore membrane, where the lipidation reaction occurs and binds LC3-I to the membrane (LC3-II) (22,128). The Atg5-Atg12 conjugate, in interaction with Atg16L (E3-like enzyme), controls lipidation of LC3-I by specifying the membrane localization where the reaction takes place (22,132). Therefore, Atg8 proteins induce membrane expansion and phagophore closure, resulting in a double-layered membrane vesicle called autophagosome (22,82,131).

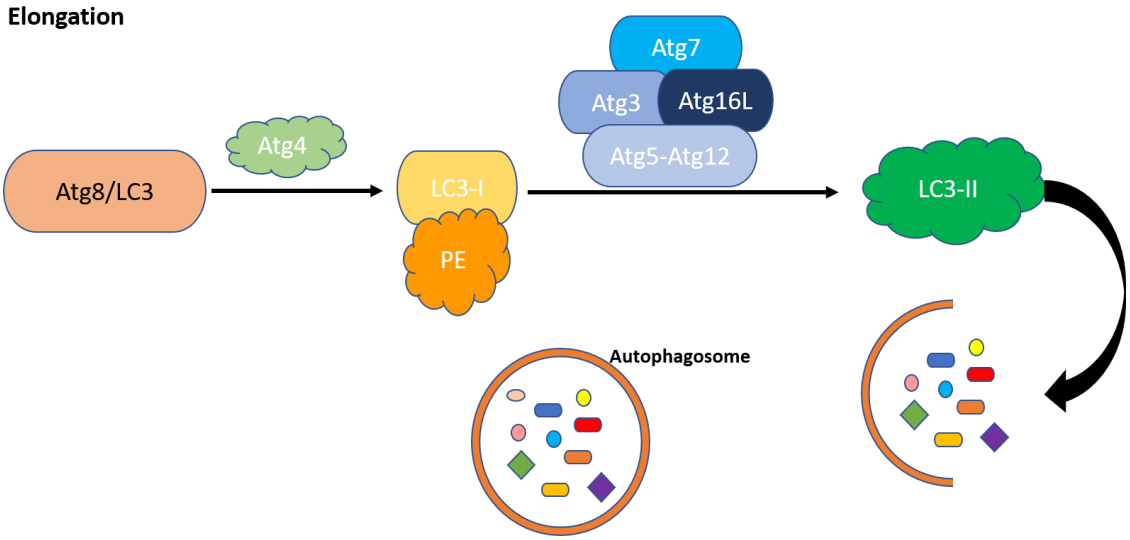

Figure 6 - Schematic model of the elongation step of autophagy.

The maturation step corresponds to the fusion of autophagosomes with lysosomes to form autolysosomes, where the content of the autophagosomes is degraded. This step is regulated by the members of the Rab GTPase family (RAB7, RAB8B, RAB9, RAB11, RAB23 or RAB24) and the soluble N-ethylmaleimide attachment protein receptor (SNAREs) superfamily (VAMP3, VAMP7, VAMP8, VTI1B or STX17), which increases the permeability of membranes and create the actual membrane opening leading to fusion of the contents of two adjacent organelles (22,133–135). In the docking and fusion stages, SNAREs, assemble themselves into complexes between opposite membranes nearby, being this process coordinated by Rab proteins. Consequently, the proteins form a “zipper”-complex which directly triggers the membrane fusion, the “opening” of the bilayers and fusion of vacuoles occurs, either by the so-called “kiss and run” or direct fusions (133).

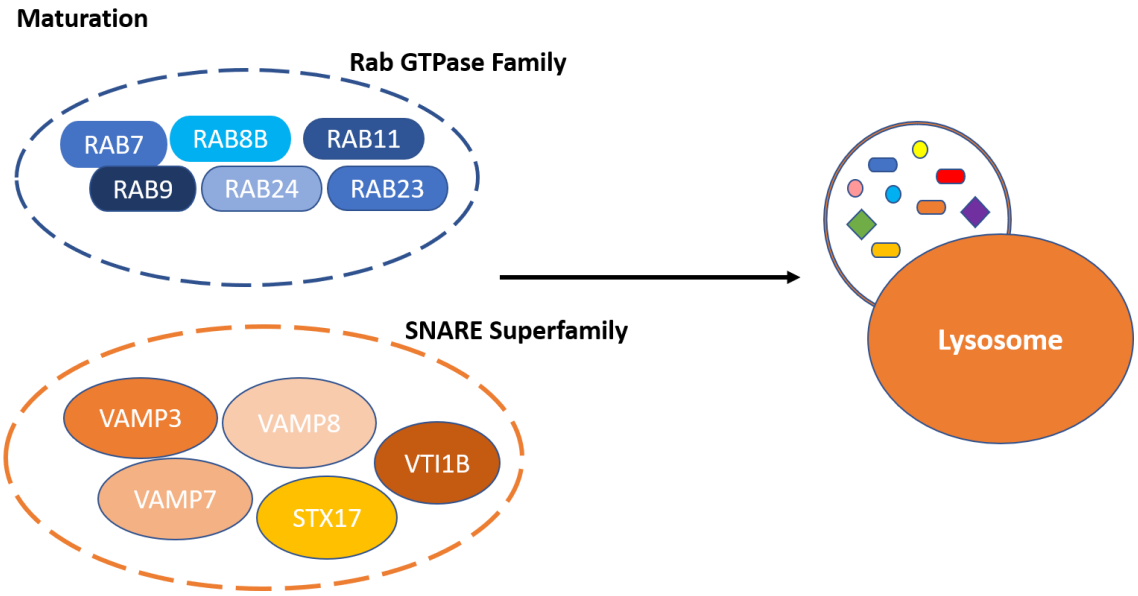

Figure 7 - Schematic model of the maturation step of autophagy.

Selective autophagy needs to have efficient recognition and sequestration of the cargo within autophagosomes. The cargo specificity is regulated by autophagic cargo receptors that specifically bind the cargo material and the autophagosomal membrane (136). Therefore, selective autophagy assembles and degrades substrates specifically recognized by autophagy receptors (sequestosome-1-like receptors), such as p62/SQSTM1 (Sequestosome-1), Optineurin and NDP52 (Nuclear dot protein 52Kda) (106,136). The p62/SQSTM1 has several functional domains, including Phox1 and Bem1p (PB1) domain, an LC3-interacting region (LIR), (recognizing and interacting with LC3-II allowing the packaging of p62/SQSTM1 cargos) and a Ubiquitin-associated (UBA) domain (recognizing and interacting with ubiquitinated substrates) (106,137). p62/SQSTM1 also interacts with Tumor necrosis factor receptor-associated factor 6 (TRAF6) binding domain which regulates mTORC1 translocation and activation to lysosome its subsequent activation (138). Furthermore, autophagosome-packaged p62/SQSTM1 and its cargo are subsequently degraded, making p62/SQSTM1 another marker to monitor autophagy (106). Small molecules resulting from the degradation, particularly amino acids, are then transported to the cytosol for protein synthesis and maintenance of cellular functions under starvation conditions (102).

Numerous signaling pathways have been shown to regulate autophagy, such as nutrient signaling, insulin/growth factor pathway, energy sensing, stress response and pathogen infection (102). However, in this monography, we will only focus on how pathogen infection regulates autophagy by induction of innate and adaptative immunity.

4. Autophagy in induction of innate and adaptive immunity to HIV-1 infection

In the pathogenesis of bacterial and viral infections like HIV-1 infection, evidence suggests that autophagy acts as both inducer and effector of innate and adaptive immunity (104). To face an infection there are two main mechanisms: the intrinsic immunity and the innate immunity (139). The intrinsic immunity is mediated by cellular pathways such as autophagy, RNA interference and restriction factors, which provide an immediate antiviral activity (22). On the other hand, the innate immunity, which is less rapidly, is an antiviral response mediated by pattern recognition receptors (PRRs) (22). These PRRs detect components of pathogens or products of their replication termed pathogen-associated molecular patterns (PAMPs) (99,102). The existence of these receptors was first suggested by Janeway in 1989 (140), but it was only confirmed by the later discovery of several classes of PRRs, such as Toll-like receptors (TLRs), Retinoic acid-inducible gene I (RIG-I) like receptors (RLRs), Nucleotide-binding and oligomerization domain (Nod)-like receptor (NLR) family and the double-stranded RNA binding protein kinase receptor (PKR) (99). Besides PAMPs, there is also another pathway described by Matzing in 1994 to immune activation, the "Danger Model", in which cellular damage activates immune responses (141). These danger-associated molecular patterns (DAMPs) include products of apoptotic cells (extra-cellular ATP and DNA, monosodium urate crystals), hypoxia, production of reactive oxygen species (ROS) and misfolded protein (ER stress) (99,142).

Toll-like receptors (TLRs)

In mammalian innate and adaptative immunity signaling, Toll-like receptors (TLRs) located on the cell surface and endosomes are type I integral membrane glycoproteins containing an extracellular domain with leucine-rich regions (LRR), which recognize PAMPs to activate pro-inflammatory cytokine and type I interferon (IFN) production via NF-κB (Nuclear Factor κB)- and MAPK (mitogen-activated protein kinase)- pathway common to all TLRs (99,143). However, TLR 3, 7, 8 and 9 also activate the interferon-regulatory factor (IRF)-dependent pathway leading to high levels of IFNs (99,143). Production of type I IFN results in the transcriptional regulations of several interferon-stimulated genes (ISGs), which leads to an "antiviral state" by reduction of virus replication (99,144).

To date have been identified 12 members of TLRs (TLR1-TLR12) (99). While most TLRs are located on the surface of the cell, TLRs 3, 7, 8, and 9 are mainly on the endocytic compartments and are involved in the detection of endocytosed viral material

like autophagosomes (99,104,143). In fact, It has already been proved that the delivery of viral nucleic acids to these endosomal TLRs in DCs can occur through autophagosomes (104,145). Therefore, it is expectable that if autophagosomes can facilitate the sequestration, delivery and detection of cytoplasmic viral RNA, thereby they are helping to produce a classical IFN response (104,146).

TLRs signaling depends on TLR adaptors, such as in a Myeloid Differentiation primary gene (88) (MyD88)-dependent manner (TLR1, 2, 5, 6, 7, 8, and 9), TLR3 signals in a MyD88-independent manner by using TRIF (TIR domain-containing adapter-inducing interferon-β) only and TLR4 signals by both MyD88 (assisted by MyD88 adapter-like (MAL)) and TRIF (assisted by TRIF-related adapter molecule (TRAM)) adaptors (143). Another involvement of autophagy in the detection of PAMPS by TLRs is by a mechanism where TLRs themselves can induce autophagy (104). One potential mechanism proposed by which TLR signaling might activate autophagy is upon TLR stimulation, the TLRs adaptors MyD88 interact with Beclin-1, resulting in the interruption of interaction with Bcl-2, which usually suppresses autophagy (99).

HIV-1 presents natural ligands (guanosine and uridine-rich ssRNA) to TLR7 and TLR8, which probably induce autophagy (147). A study demonstrated that when dendritic cells (DC) and macrophages were infected with HIV-1, an increase in the levels of LC3-II was detected. This increase in LC3-II reveals that autophagy induction was TLR8-dependent, as TLR8 knockdown with small interfering RNA (siRNA) decreases LC3-II. Thus, autophagy induction in response to natural TLR7 or TLR8 ligands can be detected during infection with HIV-1 (143,148).

Nod-like receptor (NLR) family

NLRs are cytoplasmic receptors, which also recognize PAMPs and DAMPs (149). The mammalian NLR family has more than 20 members (99) and have a common domain organization with a central NACHT domain (NAIP, CIITA, HET-E, and TP-2), N-terminal effector domain, and C-terminal leucine-rich repeats (LRRs) (99). The NACHT is involved in dNTPase activity and oligomerization, the C-terminal LRR domain is involved in ligand binding or activator sensing and the N-terminal domain performs effector functions by interacting with other proteins (149).

NLRs can be subdivided based on their domain structure into: Nods (activates signaling via NF-κB and MAPK) and NALPs and IPAF/NAIP (components of the “inflammasome” formation) (99).

Two studies have proven that NOD1 and NOD2 can induce autophagy to remove pathogens by recruiting Atg16L1 to the plasma membrane at the site of

bacterial entry (150,151). However, the interaction between NLRs and virus-induced autophagy is not clear yet and there are only two NLRs with documented autophagy induced by viruses. A recent study reported that NOD2 interacts with viral single stranded RNA (ssRNA) inducing IFN-β production utilizing the adaptor protein MAVS (152). It was also discovered that NLRX1 (NLR with N-terminal caspase activation and recruitment domain), located in mitochondria by interacting with the Tu translation elongation factor of mitochondria (TUFM) interacts with Atg5-Atg12 and Atg16L1, inducing autophagy in virus-infected cells (153).

dsRNA-dependent protein kinase (PKR)

PKR is a cytosolic serine/threonine kinase that is one of the mammalians eIF2α kinases, which regulate protein translation in response to cellular stress (hypoxia, ER stress and formation of ROS) (99). In response to viral infections, the antiviral eIF2α kinase signaling pathway is activated and upregulates autophagy (154). Although the mechanisms related to HIV-1 infection are still unclear, in HSV-1 (herpes simplex virus type 1) there is evidence that viral infection triggers autophagy through PKR activation. In HSV-1 the protein ICP34.5, which recruits protein phosphatase 1α to dephosphorylate eIF2α, has been shown to inhibited autophagy by interaction and sequestration of Beclin-1(99,155).

RIG-I-like receptor (RLR)

RLRs are cytosolic receptors which include the RNA helicases RIG-I and MDA-5, inducing activation IRF3, NF-κB and MAPK signaling in response to virus RNA (99). Although it hasn't been proved that RLR directly activates autophagy, there some studies demonstrating that autophagy downregulates RLR signaling, suppressing innate immune responses (104). For example, in VSV (Vesicular Stomatitis Virus) infection the CARD domains of RIG-I, MDA-5 and their downstream adaptor MAVS are conjugated to Atg12-Atg5, inducing downregulation of type I IFN and NF-κB pathway (99,156). Also, a deficiency in the proteins Atg5 and Atg7 make cells more resistant to the infection and increases type I IFN response (104,156).

Autophagy is also involved in adaptive immunity by delivering viral antigens to late endosomes, where they are loaded into MHC class II molecules for presentation to CD4+ T cells (101). This pathway seems crucial during influenza virus infection, due to the targeting of influenza matrix protein 1 to autophagosomes via fusion to the autophagosome-associated protein Atg8/LC3 led to enhanced major histocompatibility complex (MHC) class II presentation to $CD4^+$ T cell clones (157). The clinical relevance of this mechanism is related to the targeting of proteins for autophagic delivery to MHC class II loading compartments that might be effective in improving T helper cell responses and thereby useful for the development of novel vaccines and adjuvant therapies (101,104).

There are some other theories about the mechanisms by which autophagy induces immunity against HIV-1 infection. One potential mechanism was demonstrated in a study with CD4+ T cells, where occurred interaction between p62/SQSTM1 and the viral protein, HIV-1 Tat, triggering the selective pathway of autophagy in an ubiquitin-independent manner, which resulted in the targeting to lysosomal degradation (82,158). Moreover, Tat released from infected cells that enters into uninfected neighbouring cells can also be degraded by autophagy through the same way (82,158). The p62 protein also participates in the anti-retroviral activity of TRIM5α restriction factor, which controls cellular processes like apoptosis, autophagy, innate immunity and intracellular signaling (22). TRIM5α is known to induce pro-inflammatory innate response and accelerates the uncoating of the viral core through binding to retroviral capsid (159) and can also act as an autophagic receptor for HIV-1 p24 (HIV-1 Gag polyprotein) (22). This mechanism was proved by a study, which revealed that autophagy activation in HIV-1 infected CD4+ T cells induced HIV-1 p24 degradation depending on TRIM5α, p62, beclin-1 and Atg7 (22). Furthermore in another study, was found that peripheral blood mononuclear cells (PBMC) of the HIV-1 controllers present a higher amount of autophagic vesicles associated with an increased expression of autophagic markers compared to normal progressors (160). Besides, the treatment of PBMC from the HIV-1 controllers with the mTOR inhibitor rapamycin results in a more efficient autophagic response, leading to a decreased viral production (160). Interestingly, HIV-1 virions were detected in weakly autophagic macrophages, but not in the macrophages with higher numbers of autophagosomes (82,161). These data support the hypothesis that autophagy plays a key role in limiting viral pathogenesis in HIV-1 controllers by targeting viral components for degradation (82,160).

Furthermore, the restriction factor apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (APOBEC3G) produces G to A hypermutations in the HIV-1 genome and inhibits reverse transcription and proviral DNA integration (22,162). This factor is degraded by the viral protein, Vif, but it can be protected by histone deacetylase 6 (HDAC6), which induces degradation of Vif through autophagy proved in a study performed with HEK 293T cells (22,163). In other words, HDAC6 promotes Vif degradation by favouring association of Vif with autophagosomes (22,164).

These are the mechanisms by which cells induce immune responses to HIV-1 infection and in the next chapter, we will see how the virus developed countermeasures.

5. HIV-1 regulation of autophagy

Viral regulation of autophagy can be pro-viral or anti-viral depending on cell type, cellular environment and type of virus (82). However, some viruses evolved in order to circumvent the autophagy pathway for their benefit to replicate and disseminate (165). When autophagy has an anti-viral action, viruses have developed mechanisms to inhibit stages of autophagosome maturation or autolysosome formation (82). Though, sometimes viruses need to induce autophagy pathway to complete their replication cycle inside the cells. Specifically, HIV-1 has a dual role inducing the early stages of autophagy while inhibiting the late stages of autophagy (166). However, this may not be so clear. For example, a recent study has reported that the autophagy protein Atg9A is required for optimal HIV-1 infectivity by removing a factor that inhibits infectivity or by incorporation of this factor into virions, which increases its infectivity (165). Therefore, although the process involves an autophagy protein it doesn´t induce autophagy itself.

During HIV-1 infection the regulation of autophagy depends on the target cell and those responsible for the viral regulation of autophagy are several HIV-1 proteins (22).

5.1. Envelope proteins (Env)

HIV-1 Env (gp120 and gp41) has been shown to induce autophagy in uninfected CD4+ T cells through a fusogenic activity of gp41, leading to apoptosis (167). This phenomenon was proved by a parallel accumulation of Beclin-1 in these same cells (168). Although, as mentioned before, the viral regulation of autophagy can differ with target cells. Therefore, in DCs, gp120 inhibits autophagy to promote viral spread (22,169), through the binding to CD4 receptors activating the Erk-mTOR signaling and inhibiting autophagy (169). Therefore, although a large fraction of HIV-1 is degraded by DCs, a considerable amount of virus escape this degradation allowing the transfer of HIV-1 to CD4(+) T cells, which occurs through DC presentation (22,169).

5.2. Transactivator protein (Tat)

In infected macrophages, Tat impairs IFNγ-dependent autophagy by inhibiting STAT1 (signal and transducer and activator of transcription-1), which is essential for degradation of pathogens by autophagy and consequently inhibits major histocompatibility complex class-II antigen expression (22,170). On the other hand, in uninfected macrophages, Tat is shown to inhibit autophagy by inducing the Scr-Akt signaling pathway through interacting with the surface receptors CXCR4, VEGFR

(vascular endothelial growth factor receptor) and β-integrins (22,171). This blockade of autophagy is also dependent on the activation of STAT3 (171).

Tat was also demonstrated to bind LAMP2A, leading to upregulation of fusion autophagosomes with lysosomes and accumulation of autophagosomes in CNS cells (87).

5.3. Negative regulatory factor (Nef)

In 2009, It was first reported the autophagy inhibitory activity in the later stages of autophagy (maturation) of Nef in macrophages (22,166). This inhibition is achieved by an interaction with Beclin-1 and the Nef diacidic motif $_{174}DD_{175}$ forming the complex Nef-Beclin-1. (166). Another report has demonstrated that through TLR8, which depends on beclin-1 dephosphorylation and nuclear translocation of TFEB (transcription factor EB), this interaction of Beclin-1 with Nef induced mTOR activation and cytoplasmic sequestration of TFEB (106,172).

Besides inhibition of maturation, a study has indicated that Nef could interfere in the initiation stage too. In one study was found that the Beclin-1–hVPS34 complexes in mammalian cells include potential equivalents of yeast Atg14 (173), which appears to be autophagy initiation specific and VPS38 (UVRAG), acts at maturation by stimulating Rab7 GTPase activity and autophagosome fusion with late lysosomes (166,174).

5.4. Gag protein

The Gag polyprotein regulates autophagy by interaction with LC3-II with the gag polypeptides p24 and p17 in macrophages during the early steps of autophagy (initiation and elongation), which causes inhibition of autophagy (166). Besides, another interesting data in this study was that when autophagy was induced, Gag production was simultaneously increased, which leads us to think that Gag is needed for an optimal viral replication in macrophages (106,166).

5.5. Viral protein R (Vpr)

Vpr has been shown to induce apoptosis, by the activation of the caspase 9 pathway and by fas-induced apoptosis, in target cells of HIV-1 such as lymphocytes, monocytes, DCs (22,175,176) but not in macrophages (177). The mechanism of this macrophages resistance is not very clear yet but one study proved that Vpr did not downregulate the expression of antiapoptotic inhibitors of apoptosis (IAPs) and Bcl2 family members in macrophages, making these cells HIV-1 reservoirs (22,178). However, recently it was found that autophagy was co-related to this resistance in macrophages to Vpr-induced apoptosis (177). This report showed that macrophages

expressing Vpr revealed augmented levels of Beclin-1 and LC3B (22,177). Despite what was expected, a higher number of autophagosomes was not detected, suggesting that only the early stages of autophagy are induced by Vpr (22,177).

5.6. Viral infectivity factor (Vif)

As mentioned before, the main function of Vif is the degradation of APOBEC3G, inhibiting its antiviral activity (162). However, it was reported that Vif could interact with the autophagic marker, LC3B, autonomously of APOBEC3G, blocking autophagy in CD4+ T cells (106,179). Further studies are needed to find out the modulation of autophagy by Vif in other target cells of HIV-1 (106).

5.7. Antisense protein (ASP)

ASP is a relative new protein of HIV-1 replication cycle, although already proposed in 1988 as an open reading frame (ORF) in a region complementary to the envelope gene sequence (180,181). Therefore, it is considered as a recent protein because its detection was only possible through electron microscopy and even more recently by *in silico* analyses (182,183).

However, the ASP biological role remains little known, there is some hypothesis (181). One study has reported the involvement of ASP in the CCR5 (R5) and/or CXCR4 (X4) co-receptor interaction (181) and another one the correlation between the presence of asp and the prevalence of HIV-1 groups M, which is responsible for the human pandemic and spread of the infection (184). Recently, it was reported another biological function in monocytes. ASP through the formation of multimers could induce autophagy assessed by the formation of autophagosomes and higher LC3-II and p62 (SQSTM1) levels (183,185). Furthermore, it was reported that this ASP-induced autophagy increased the HIV-1 replication (106,185).

5.8. Vpu protein

The main function of Vpu is to increase viral release from infected cells by proteasomal degradation of CD4 molecules and BST2 (bone marrow stromal cell antigen 2) (106,186). BST2 is a restriction factor of enveloped viruses which inhibits the release of new virions and is colocalize with the HIV-1 Gag in endosomes and plasma membrane (186). It is thought that BST-2 traps virions and retain nascent enveloped virions on cellular membranes (186). Different studies have indicated a role of autophagy in how Vpu counteracts BST2. For example, Madjo *et al* have found that in CD34+ stem cells (hu-mice) Vpu binds to LC3C antagonizing BST2 restriction by removal of BST2 from the HIV-1 budding site and downregulating it on the cell surface (106,187). This targeting of BST2 is reaching by the ubiquitination of

new molecules and recycling molecules through endosomes (22,188). Besides, it was recent discovered that Vpu can also counteract BST2 through a non-canonical autophagy process, which requires the LC3-Associated Phagocytosis (LAP) (22). Although, through the canonical autophagy pathway, this mechanism doesn't require all autophagy machinery because it is independent of the preinitiation complex (22,189). In this study, they found a direct interaction between Vpu and LC3C and that a decreasing in LC3C levels induced virus sequestration in intracellular single membrane-bound very similar to the LC3-associated phagosomes (22). Interestingly, this mechanism does not lead to a reduction of BST2 at the cell surface, which made us suppose that LAP-dependent Vpu-mediated removing of BST2 is independent of Vpu-mediated BST2 degradation (22).

6. Autophagy modulation by HIV-1 in Central Nervous System cells (CNS)

HIV-1 can infect CNS cells due to the migration of infected monocytes through the blood-brain barrier (BBB) causing neurodegeneration (82). HIV-1 activates stress responses in the brain, what causes induction of autophagy and thereby abnormal accumulation of autophagosomes increasing spread and secretion of virus material (82,190). This neurodegeneration is also caused by chronic inflammation induced by HIV-1, which induces increased glial proliferation (191). The CNS cells infected through this mechanism are mainly microglia and macrophages and to a less extent, astrocytes (82,83). Since about half of all adults with AIDS suffer from HAND and cART is ineffective in avoid neurodegeneration induced by HIV-1, attention should be given in the development of new therapies that can penetrate the BBB (80–82,190). One of these new approaches is the modulation of physiological processes, like the regulation of autophagy.

Neurons are post-mitotic cells and therefore autophagy regulation plays a key role in the neuronal function by avoiding the release of toxic proteins and organelles (82). It is known that bystander neurons are affected by HIV-1 Tat protein, produced by microglia, causing an accumulation of autophagosomes and a decrease in p62/SQSTM1 and LC3II associated with neuron membranes (82,86,87). This accumulation of autophagosomes induces neurodegeneration but it was recently discovered that rapamycin can inhibit this effect (87). Beyond HIV-1 Tat, gp120 also induces autophagy in neurons cells, since the autophagic markers, Beclin-1, Atg5, Atg7 and LC3II were increased in postmortem brains infected with HIV-1 (192). Another type of cell infected is microglia, which are the main responsible for HAND by two reasons. On one hand, HIV-1 directly induces autophagy activation proved by the increasing conversion of LC3I to LC3II and expression of Beclin-1 and Atg5 proteins (82,84) and on the other hand is the production of both HIV-1 Tat and viral particles, which will infect other cells (86). However, through the small interfering gene *beclin-1* was possible to inhibit autophagy in these cells and thereby HIV-1 replication (82,84). Relatively to astrocytes, Nef is known to disturb these cells increasing p62/SQSTM1 and LC3II levels, thereby blocking fusion of autophagosomes with lysosomes allowing viral escape from autophagic degradation (193). For this reason, astrocytes are HIV-1 reservoirs and maintain low levels of HIV-1 replication (82). Autophagy is modulated through different pathways in CNS cells infected by HIV-1, therefore by targeting specifically these mechanisms, autophagic modulation could be a new potential approach in preventing neurodegeneration associated with HIV-1 (82).

7. Therapeutic approaches for HIV-1 based on autophagy

Due to the role of autophagy in the pathogenesis of HIV-1 infection, modulating this process might be used as a new therapy approach and to improve the outcome of antiretroviral therapy (82). Besides the added value to the existing treatments, viral resistance is less likely because it is a cellular process (82). This therapy based on autophagy can be subdivided into two categories: induction and inhibition of autophagy. Furthermore, the effectiveness of this new therapy can be increased by nano-formulation although this is largely unexplored (82).

7.1. Induction of autophagy

There are at least four mechanisms by which induction of autophagy for HIV-1 suppression have been investigated, which include: mTOR inhibitors, Tat-Beclin-1 fusion peptide, vitamin D and histone deacetylase inhibitors (82).

7.1.1. mTOR inhibitors

Rapamycin, a mTOR inhibitor of mTORC1, prevents mTOR of inactivating the preinitiation complex in the initiation step of autophagy being, therefore, an autophagy inducer. One study demonstrated that rapamycin reduces CCR5 membrane expression and interrupts IL-2 receptor signaling in T cells and monocytes, therefore, inhibiting HIV-1 entry (194). Also interferes in basal transcription of the HIV-1 LTR, inhibiting replication of R5 HIV-1 (194,195). Low doses were proved to reduce CCR5 density and enhanced vicriviroc, aplaviroc and enfuvirtide (CCR5 antagonists) antiviral activity (196,197). However, rapamycin is cytotoxic at concentrations above 2 μM and induces apoptosis, leading to secretion of HIV-1 p24 antigen (198). These results suggest that low doses of rapamycin may increase the durability of regimens containing CCR5 antagonists in patients by enhancing viral suppression, thus allowing the use of lower doses of CCR5 antagonists with reduced potential for toxicity, and by controlling emerging resistant variants (194).

Another mTOR inhibitor is an ATP-competitive mTOR (TOR-KI), which inhibits both mTORC1 and mTORC2 (82). INK128, a prototype TOR-KI, was demonstrated as an inhibitor of R5 and X4 HIV-1 in lymphocytes without toxicity levels showed in rapamycin due to its immunosuppressive nature (82,195). By downregulation of CCR5 levels inhibit R5 HIV-1 entry and due to inhibition of mTORC2 blocks transcription of HIV-1 genes through induction of NF-κB (195). As well as rapamycin, INK128 is also used to avoid the emergence of drug-resistant HIV-1 strains in

combination with maraviroc, a CCR5 antagonist, and had favourable antiviral activity with other HIV-1 inhibitors of reverse transcriptase, integrase and protease (195).

The effect of the antidiabetic drug, metformin, has been studied in HIV-1 reservoirs in non-diabetic ART-treated patients (199). Metformin inhibits mTOR signaling independently of the insulin-signaling pathway by activating AMPK, which enhances autophagy by activating ULK1, and acts as an inhibitor of mTOR (199,200). Therefore, metformin modulates T-cell glycolysis by inhibiting mTOR and was shown to improve CD4+ T cell counts (199). Although needing further confirmation, the results of this recent study suggest that metformin can reduce immune activation and/or the size of the viral reservoir in HIV-infected participants doing ART (199).

7.1.2. Tat-Beclin-1 Fusion Peptide

This fusion peptide is formed by the combination of the transduction domain of the viral protein, Tat and the amino acids 267-284 of Beclin-1 (82,201). The peptide will bind to Nef protein and through interaction with Golgi-associated pathogenesis-related protein 1 (GAPR-1), a negative regulator of autophagy, induces autophagy through the canonical pathway in human macrophages (201). By this study, it was proved that Tat-beclin 1 decreases the replication of HIV-1 *in vitro* by the inhibition of HIV-1 p24 antigen release in the presence of lower doses the fusion peptide (201).

7.1.3. Vitamin D

In HIV-1 infected patients, the prevalence of vitamin D deficiency is estimated to be 70% to 85% (202) due to chronic inflammation and immune activation (203). Also contributes to this deficiency several comorbidities, infectious complications, and hospitalizations of these patients that lead to reduced sun exposure, malnutrition, and less oral intake of vitamin D-rich foods (203). Furthermore, antiretroviral therapy with protease inhibitors and non-nucleoside reverse transcriptase interfere with vitamin D metabolic pathways (202).

The active form of vitamin D, 1α,25-dihydroxycholecalciferol, has been demonstrated to induce autophagy in macrophages through two pathways, which in turn inhibits the replication of HIV-1 (198). After 1α,25-dihydroxycholecalciferol binds to the vitamin D receptor (VDR) is promoted through a PI3KC3-, ATG5-, and Beclin-1-dependent mechanism autophagosome elongation and fusion with lysosomes (198). The other pathway involves cathelicidin (microbial peptide) that after vitamin D binding to VDR activates transcription of the autophagy-related genes beclin-1 and atg5 (204). However, this study was only performed using *Mycobacterium tuberculosis* in human macrophages, though more studies are needed in cells

infected by HIV-1. Thus, supplementation and reestablishment of normal values of vitamin D in HIV-infected patients may improve immunologic recovery during combined antiretroviral therapy, reducing HIV-1 replication, increasing CD4+ T cell counts, controlling opportunistic infections and decreasing the risk of HAND (82,205).

7.1.4. Histone Deacetylase Inhibitors (HDACi)

Histone deacetylases (HDACs) regulate histone acetylation and accessibility of DNA to transcription factors, having a key role in HIV-1 latency (206). The mechanism is processed either by inducing deacetylation at HIV-1 integration sites or through modification of non-histone proteins like NF-κB (82,206). As latently infected cells are undetectable by the immune system and unresponsive to cART, HDACi are being evaluated in a "shock-and-kill" therapeutic approach (172). Through this strategy, HDACi first reactivate latent HIV-1 in CD4+ T cells and macrophages, then the virus is killed by cART and at last HDACi induce autophagy by inhibition of mTORC1 and activation of ULK1 (82,172). Besides valproic acid, a HDACi that depletes resting CD4+ T cells but does not induce autophagy (207), there are two HDACis, Vorinostat and suberoylanilide hydroxamic acid (SAHA), that can induce autophagy and expression of latent HIV-1 from the resting CD4+ T cells in HIV-infected, ART-treated and aviremic patients (208). The main concern related to this therapeutic approach is the off-target effects such as spreading of virus emerging from resting cells to other susceptible cells (82,208).

7.2. Inhibition of autophagy

Since autophagy is crucial for some steps of the HIV-1 replication cycle, its inhibition, especially during the active phase of HIV-1 infection, might be a potential therapeutic approach (82). Though, Nitric oxide (NO) and Chloroquine (CQ) have been reported as autophagy inhibitors in HIV-1 infection.

Nitric oxide (NO) is a cellular messenger of the host defense that has been reported to be augmented in HAND (81,202). NO inhibits autophagy by blocking the activity of the JNK1, which reduces Bcl-2 phosphorylation and increases the Bcl-2-Beclin 1 interaction, thereby impairing hVps34/Beclin-1 complex formation (209). Furthermore, NO inhibits IKKβ and reduces AMPK phosphorylation, leading to mTORC1 activation (82,209). In contrast, NO has also been shown to block HIV-1 replication by inhibiting reserve transcriptase and activation of NF-κB by the viral protein, Tat (210). Thus, more studies are needed to confirm the therapeutical potential of NO and the consequences of its inhibition.

Chloroquine (CQ), known for its antimalarial effects, can also show anti-viral effects in viruses requiring a pH-dependent step for entry in cells, by inhibition of

autophagy at the later stages by lysosomal dysregulation (211). Furthermore, CQ also inhibits HIV-1 replication by preventing the maturation of gp120 due to pH increase (211). One study has demonstrated that CQ can reduce HIV-1 vertical transmission (212). Beyond NO and CQ, several approaches trying to inhibit autophagy in HIV-1 infection have been studied like silencing Beclin-1, downregulation of ATG genes, upregulation of SQSTM1/p62 and targeting viral proteins.

7.2.1. Silencing *beclin-1*

Since HIV-1 leads to accumulation of autophagosomes by targeting *beclin-1* and interfering with Bcl-2 leads to inhibition of maturation step (213). Silencing *beclin-1* can be a new therapeutical approach especially in HAND, since the accumulation of autophagosomes is the main cause for neurodegeneration associated with HIV-1 (82).

7.2.2. Downregulate ATG genes

The genes *atg7*, *atg12*, *atg16l2* and *map1lc3b* are crucial in elongation step of autophagy and genes *cln3* and *laptm*5 in lysosomal function, they were classified as HIV- 1 dependency factors (214). Therefore, these genes are essential for HIV-1 replication and its inhibition or silencing might restrict HIV-1 infection (82,214).

7.2.3. Upregulation of SQSTM1/p62

This approach can be applied to vaccines promoting T-cell-mediated immune responses (215). Due to the binding of p62 and Gag p24, p24 is delivered into the autophagy pathway (82,215). A study *in vitro* demonstrated that immunization of mice with the presentation of p24 to CD4+ T cells leads to virus-specific interferon γ producing T cells (215). This model might be transposed to human to test efficacy and safety.

7.2.4. Target Viral Protein

As shown before, viral proteins regulate autophagy either by induction or inhibition of autophagy. Particularly fusaric acid and picolinic acid, due to ion chelating proprieties, target HIV-1 Tat conserved zinc finger (216). Hence, lower levels of Bcl-2 are detected in infected cells (216). There is also another Tat inhibitor, didehydro-cortistatin A (dCA) that reduces residual levels of viral transcription in HIV-1 latency, breaks the Tat-mediated transcriptional feedback loop, and establishes a nearly permanent state of latency (217). Besides, reducing viral transcription is maintained even after removing the exposure to dCA, indicating that the HIV-1 promoter is epigenetically repressed (217).

8. Conclusion

The autophagy in the pathogenesis of HIV-1 plays a crucial role either in the induction of both innate and specific immune responses to fight HIV infection, as in potentiating viral replication.

During viral infection, innate immunity is activated with the detection of PAMPs and DAMPs by PRRs, which will induce autophagy, promoting the degradation of the virus (22,99,102,141). Particularly in HIV-1, the most relevant PRRs are TLRs 7 and 8, which delivers viral RNA to autophagosomes to later degradation (143,147). Besides, TLRs can induce autophagy themselves by an interaction between MyD88 and Bcl2 (99,104). Furthermore, a Nod-like receptor, NLRX1 has shown to induce autophagy in virus-infected cells, through an interaction with Atg5-Atg12 and Atg16L1, although this study was only performed in cells infected by the vesicular stomatitis virus (153). There is also another PRR, PKR, which directly inhibits autophagy by sequestration of Beclin-1 (99,104,155).

In adaptive immunity, autophagy is essential to deliver antigens to late autophagosomes, where they are loaded into MHC II molecules for presentation to $CD4^+$ T cells (101).

There are also other hypothesis about the mechanisms by which autophagy induces immune responses against HIV-1 infection. For example, in CD4+ T cells viral proteins Tat and p24 are degraded through the selective pathway of autophagy (22,82,158) and in HEK 293T cells was shown that HDAC6 can degrade the viral protein, Vif, by inducing autophagy (163). Finally, one interesting mechanism about the role of the autophagy in the induction of immune responses is the fact that HIV-1 controllers (defined as those that maintain viral load between 50-2000 HIV RNA copies/mL without therapy) have an accumulation of autophagosomes that blocks the viral replication, allowing them to remaining clinically asymptomatic without therapy for at least 8 to 10 years. (160).

To escape these antiviral proprieties of autophagy, HIV-1 had to develop some strategies (106). Therefore, HIV-1 uses autophagy for the early replication steps and then inhibits fusion of lysosomes with the autophagosomes to prevent its degradation. (82,106). On one hand, the viral proteins, Vpr, Asp and Vpu induce autophagy in monocytes (Asp) and macrophages (Vpr) (177,183,185,187) and, on the other hand, Nef, Vif and Gag inhibit autophagy in CD4+ T cells (Vif) and macrophages (Nef and Gag) (106,166,173,179). Besides, HIV-1 Env and Tat, can modulate both depending on the cell type. Env induces autophagy in uninfected CD4+ T cells leading to apoptosis and inhibits autophagy in DCs to promote viral replication (167,169).

Moreover, Tat inhibits autophagy in infected and uninfected macrophages but in CNS cells it induces the accumulation of autophagosomes (87,171).

Neurodegeneration in patients infected by HIV-1 is known as one of the serious consequences of HIV-1 infection and was found to be related with dysregulated autophagy (82,190). This dysregulated autophagy leads to an abnormal accumulation of autophagosomes in CNS cells especially in microglia, macrophages and astrocytes. (83,86,192,193).

Due to all the roles that autophagy has in the pathogenesis of HIV-1, new therapeutic approaches have been developed as potential therapies either by induction or inhibition of autophagy (82). These approaches include inhibition of autophagy through four mechanisms: silencing Beclin-1, downregulating ATG genes, upregulating the selective pathway and targeting viral proteins, specifically, the Tat protein (82). In addition, there are two molecules that were demonstrated to inhibit autophagy: nitric oxide and chloroquine (209,211). Alternatively, a variety of approaches have been investigated such as histone deacetylase Inhibitors, Tat-Beclin-1 Fusion Peptide, mTOR inhibition and vitamin D supplementation in order to induce autophagy (82).

Currently, some of the major barriers to eradication of HIV-1 are the resistance developed by HIV-1 to the antiretrovirals, the establishment of virus latency and the low bioavailability of cART in CNS compartment (82). Therefore, these approaches based on autophagy would add an extreme value to the existing treatments. However, the complex interaction of HIV-1 and autophagy is a challenging task (82,84). For example, autophagy has an antiviral activity but it is also needed to HIV-1 initiate its replication cycle. Therefore, the use of autophagy as new therapeutic alternative to control HIV-1 infection needs to be fine tuned and further studies are needed (82).

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