The Role of Mitochondria in HIV Infection and Its Treatment

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Mitochondria play a dual role in the life of the cell, being capable of producing either energy (in the form of ATP) or potentially dangerous reactive oxygen species (ROS), and they also contain molecules that, when released into the cytoplasm, cause apoptosis. There is a growing interest in the importance of these organelles during the infection caused by the human immunodeficiency virus (HIV), as well as during its treatment. Indeed, several drugs that are capable of blocking HIV can also interact with the enzyme responsible for the replication of mitochondrial DNA and inhibit its activity. Cytokines produced by the immune system can alter ROS production. Furthermore, the virus as such can trigger different mechanisms that interfere with mitochondrial functionality and induce alterations, ultimately causing cell death. As a result, mitochondria can be severely altered by HIV infection and by its treatment.

1. Introduction

The crucial role of mitochondria in higher eukaryotes was recognized more than 100 years ago, when these organelles, originally defined "bioplasts", were renamed because of the typical, threadlike shape assumed during spermatogenesis. Starting with the 1940s, the importance of mitochondria in respiration was clarified, and Mitchell proposed the chemiosmotic theory, according to which the energy obtained by respiration is converted into an electrochemical gradient across the inner membrane and used for ATP synthesis.1

Mitochondria are divided into compartments with specialized functions, including the outer mitochondrial membrane, the intermembrane space, the inner mitochondrial membrane, the cristae and the matrix. Human mitochondria contain more than 600 proteins;1 most of which are codified in the nucleus and transported into mitochondria, whereas only 13 are codified by mitochondrial DNA (mtDNA), which is a circular DNA molecule of 16,659 bp present in several copies per mitochondrion.2 mtDNA is replicated autonomously and in a unique fashion, and the sole enzyme responsible for its replication is DNA polymerase-γ.2

Some mitochondrial proteins are encoded by mtDNA while others are encoded by nuclear DNA; thus, mitochondrial dysfunctions can be due to mutations in either nuclear or mitochondrial genes. mtDNA is characterized by a high mutation rate (up to 20 times higher than nuclear DNA) because of the mutagenic action of reactive oxygen species (ROS) that are produced during electron chain transport, and because of the low capability of mitochondrial enzymes to repair DNA mutations.3 The major tissues affected by mitochondrial dysfunction are tissues with a high energy demand such as the brain, muscles, heart and endocrine glands.

Mitochondria are usually seen as the “power plant” of the cell; indeed, more than 90% of cellular ATP is...
produced by mitochondrial respiration. The mitochondrial microenvironment provides the right context not only for the respiration and synthesis of ATP, but also for many other biochemical reactions that are crucial for cell metabolism, including tricarboxylic acid cycle, beta-oxidation, some steps for steroid synthesis, synthesis of heme group, and some steps of the urea cycle. A crucial role for the aerobic respiration in the mitochondria is played by the inner mitochondrial membrane, where the protein complexes involved in electron transfer are embedded. The inner mitochondrial membrane is characterized by a biochemical composition that renders it impermeable to $H^+$, thus providing the barrier for the generation of proton gradient used for ATP synthesis.

Alongside these crucial functions in cell metabolism, mitochondria play other roles in processes that are not directly related to cell metabolism. In particular, during the 1990s, it was shown that mitochondria are crucial for triggering apoptosis, and in particular that loss of membrane potential ($\Delta \psi_{m}$) and changes in mitochondrial membrane permeabilization (MMP) often represent crucial steps for the execution of apoptosis. In fact, MMP causes the release of several molecules that are: (1) direct activators of caspases (such as cytochrome c); (2) indirect activators of caspases (e.g., SMAC/DIABLO; OMI/HTRA2); or (3) activator of apoptosis independently from caspases (e.g., apoptosis inducing factor). It is well known that the human immunodeficiency virus type-1 (HIV) is able to profoundly act on the regulation of apoptotic pathways, either directly (that is, through the action of different viral gene products) or indirectly (through changes in cell metabolism that can alter regulation of extrinsic or intrinsic apoptosis pathways). Until a few years ago, infection with HIV caused an inexorable decline in immune function, leading to fatal consequences. Until 1995, the only drugs that had some (modest) effects on HIV infection were a few compounds in the category of nucleosidic reverse transcriptase inhibitors (NRTIs), molecules that act as competitive inhibitors of the viral reverse transcriptase, the enzyme that copies HIV-RNA to cDNA. In the mid-nineties, other drugs were developed that were able to inhibit another viral target, the viral protease; this class of drugs is known as protease inhibitors (PIs). Nowadays, in most patients the combination of PIs and nucleosidic, non-nucleosidic or nucleotidic reverse transcriptase inhibitors can block viral replication, and is defined as highly active antiretroviral therapy (HAART), which is able to restore the immune system and block the onset of opportunistic infections. Other categories of drugs, such as inhibitors of viral entry into the cell and inhibitors of the chemokine receptor CCR5, are now available and being used in therapy; other drugs, including inhibitors of viral integrase, are under testing in Phase III clinical trials.

HIV+ patients on HAART survive by suppression of virus replication, not by total elimination of the virus. As a consequence, the therapy must be taken throughout life with a very strict adherence in order to avoid the appearance of viral resistance to the drugs. However, in a large number of cases, the chronic consumption of antiretroviral drugs causes the onset of several side effects, including metabolic and aesthetic alterations, which can have serious consequences for the health of the patient. As mitochondria are involved in so many different metabolic and regulative pathways, it is not surprising that drugs that affect one or more reactions in mitochondria can deeply affect cellular functionality, leading to important alterations in different tissues and organs.

In this review, we will focus on the role of mitochondria in HIV infection and its treatment, analyzing the effects of drugs used for the treatment of HIV infection on mitochondria metabolism and functionality, the consequences of their consumption in treated patients, and the direct effects of HIV on mitochondria and mtDNA.

2. Antiretroviral Therapy and Mitochondria: The Role of NRTIs

NRTIs are analogs of endogenous 2'-deoxyxynucleosides and 2'-deoxynucleotides, the molecules necessary for the synthesis of DNA. Since the discovery of the antiretroviral capabilities of zidovudine (AZT) in 1985, seven nucleosides and one nucleotide have been approved for the treatment of HIV infection, i.e., AZT in 1987, followed by didanosine (ddl), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), tenofovir disoproxil fumarate (which is converted in vivo to tenofovir), and emtricitabine (Table 1).

NRTIs are inactive in their parent form, and are phosphorylated by host cell kinases and phosphotransferases to form deoxynucleoside triphosphate (dNTP) analogs. In the triphosphate form, NRTIs compete with their corresponding endogenous dNTPs for incorporation by viral reverse transcriptase and, once incorporated, cause the termination of reverse transcription. Since all these drugs act as competitive inhibitors of nucleotides during the synthesis of cDNA from HIV-1 RNA by reverse transcriptase, they can also potentially act as competitive inhibitors of the DNA polymerases responsible for the replication of the host cell DNA. While such an inhibitory effect is negligible for the DNA polymerases that replicate or repair nuclear DNA, this is biologically significant for DNA polymerase-\(\gamma\) (pol-\(\gamma\)), the sole enzyme that replicates mtDNA. As a consequence, NRTIs can inhibit replication of mtDNA.

Within mitochondria, a wide set of enzymes (such as deoxyguanosine kinase and thymidine kinase)
phosphorylate nucleosides to nucleotides, which are necessary for mtDNA replication, in a way similar to that present in the cytoplasm. Differently from their cytoplasmic counterparts, mitochondrial enzyme expression is not linked to the cell cycle, and their levels are not reduced in post mitotic cells.\textsuperscript{43} The traffic of nucleosides and nucleotides from and toward mitochondria is not linked to the cell cycle, and their levels are maintained by the transmembrane transporters "ent" nucleoside transporter" (ENT) and "deoxynucleotide carrier."\textsuperscript{44–46} Such enzymes work in both directions, allowing the direct import of dNTPs when the concentration of cytoplasmic dNTPs exceeds that of mitochondria, and \textit{de novo} synthesis of dNTPs through ENT nucleoside import, followed by phosphorylation inside the mitochondria, when cytoplasmic concentration of dNTPs is low. Thus, the intramitochondrial concentration of nucleotides derived from nucleoside analogs can be greatly affected by the dNTP pool present in the cytoplasm (whose concentration is strictly linked to the cell cycle) and by the activity of ENT and deoxynucleotide carrier, as well as that of mitochondrial kinases.

The capability of NRTIs to inhibit DNA pol-\textit{γ} is directly correlated with their rate of incorporation into DNA during mtDNA replication. The hierarchy of inhibition is ddC > ddA (that is, the active metabolite of ddl) > d4T > 3TC > AZT > ABC,\textsuperscript{30} thus, drugs of the so-called "d" category, i.e., dideoxy-NRTIs such as ddC, ddI and d4T are the most potent inhibitors of pol-\textit{γ}, as reported by several basic and clinical studies.\textsuperscript{47–55} The order reported above is quite similar to the level of toxicity of the drugs, with the notable exception of AZT, whose toxicity is quite high despite the low rate of incorporation into DNA by DNA pol-\textit{γ}. This discrepancy is likely due to the reduced capability of the enzyme to remove AZT from DNA using its proofreading activity.\textsuperscript{56} Recognition of the role of d4T and AZT in the onset of lipoatrophy has led to a shift in the prescription of NRTIs with a lower impact on mtDNA, such as tenofovir disoproxil fumarate, 3TC or ABC, with consequent benefits for patients who can switch therapy.

\subsection*{2.2. AZT, the first drug used in the treatment of HIV infection, alters mitochondria}

As far as mitochondrial metabolism is concerned, AZT is likely the most studied NRTI. AZT is a derivative of the natural nucleoside thymidine, and was the first drug used for the treatment of HIV infection. Since its introduction, it has been observed that AZT displays several side effects, including general myopathy and cardiomyopathy,\textsuperscript{57,58} which were attributed to a toxic activity on mitochondria. The first description of the toxic effects of AZT was reported in 1999.\textsuperscript{59} A 57-year-old patient who had been on AZT for 3 years showed muscular and hepatic disturbances and lactic acidosis, fatigue and weight loss. He became confused and febrile and died 8 days after detection of high blood lactatemia. Liver biopsy showed diffuse macrovacuolar and microvacuolar steatosis; muscles had mitochondrial abnormalities with ragged-red fibers and lipid droplet accumulation. Southern blot analysis revealed depletion of mtDNA, affecting skeletal muscle and liver tissue, suggesting that AZT can induce mitochondrial multisystem disease.\textsuperscript{59}

In the following years, intensive studies were performed to elucidate the interactions of this drug with DNA pol-\textit{γ}, the enzyme responsible for the replication of mtDNA.\textsuperscript{60} Indeed, AZT is able to inhibit the activity of DNA pol-\textit{γ} in mitochondria in at least three ways: (1) once AZT is incorporated into a growing strand, DNA replication is halted; (2) AZT competes with natural nucleotides to be incorporated into growing DNA chains; and (3) AZT inhibits mtDNA reparatory exonuclease activity as it

\begin{table}[h]
\caption{List of nucleosidic reverse transcription inhibitors (NRTIs) approved for the treatment of HIV infection and their main effects on mitochondria.} \label{tab1}
\begin{tabular}{|l|l|l|l|}
\hline
NRTI & Analog of & Effects on mitochondria & References \\
\hline
AZT (zidovudine) & Thymidine & Inhibits mtDNA pol-\textit{γ}; depletes mtDNA; reduces dTTP pool; increases incorporation error rate in mtDNA. & 23–25 \\
/ddl (didanosine) & Adenosine & Inhibits mtDNA pol-\textit{γ}; depletes mtDNA; impairs complex II and IV activity; increases lactate production. & 26–28 \\
/ddC (zalcitabine) & Cytidine & Inhibits mtDNA pol-\textit{γ}. & 29, 30 \\
/d4T (stavudine) & Thymidine & Inhibits mtDNA pol-\textit{γ}; depletes mtDNA; increases ROS; impairs complex I, II, IV activity. & 31–36 \\
/3TC (lamivudine) & Cytidine & No significant alterations of mitochondria \textit{in vitro}; no morphological changes of mitochondria. & 37–39 \\
/ABC (abacavir) & Guanosine & Increases lactate production \textit{in vitro}. & 40 \\
/TDF (tenofovir) & Adenosine & No significant alterations of mitochondria \textit{in vitro}; increases lactate production. & 39, 41 \\
/FTC (emtricitabine) & Cytidine & No significant alterations in mtDNA content; no morphological changes of mitochondria. & 37 \\
\hline
\end{tabular}
\begin{flushright}
\textit{pol-\textit{γ}} = DNA polymerase-\textit{γ}; dTTP = deoxythymidine triphosphate; ROS = reactive oxygen species.
\end{flushright}
\end{table}
can resist exonucleolytic removal by the exonuclease activity of pol-γ because of the lack of a 3-OH group.61 Once incorporated in the nascent chain of mtDNA, compared to other NRTIs, AZT is removed at a slower rate by the proofreading activity of DNA pol-γ.27

Apart from its direct effect on DNA pol-γ, AZT is able to affect mitochondrial metabolism in other ways. First, AZT can compete with thymidine for its phosphorylation, so reducing the capability of thymidine kinase-2 to maintain the deoxythymidine triphosphate pool in the mitochondria.24,25 Second, it can alter the relative ratio in the dNTP pool, so increasing the incorporation error rate during mtDNA replication.62 Third, the increase in the error rate during mtDNA synthesis by DNA pol-γ causes an increased number of mutations in mtDNA that in turn leads to a reduced capability of mitochondria to produce the mtDNA-encoded subunits of respiratory chain complexes. Finally, the observation that polymorphisms in three amino acid residues of pol-γ can lead to a reduction in mtDNA copies in peripheral blood mononuclear cells (PBMCs) and favor the onset of lipodystrophy63 is in agreement with the observation that the capability of NRTIs to interact with DNA pol-γ is crucial for the side effects of antiretroviral therapy mediated by mitochondrial toxicity.

2.3. d4T is extremely potent in altering mitochondrial functionality

d4T (2',3'-dideoxy-3'-deoxycytidine) is a pyrimidine nucleosidic analog, approved for the treatment of HIV-1 infection in 1994. The first studies showed that its principal adverse effect was peripheral neuropathy.64–66 Further reports published after the advent of HAART showed that d4T is one of the most toxic drugs for mitochondria, and clarified its mechanism of action. Studies in animal models have shown that d4T causes impairment of complexes I, II and IV in mitochondria,33 along with mtDNA depletion in liver; in patients, such mitochondrial alterations are often accompanied by hyperlactatemia.33,34 In vitro studies on human cells demonstrated that d4T not only causes marked mtDNA depletion, but also induces an increase in the level of ROS, causing mitochondrial oxidative stress.35 Our group has further confirmed such results in cells of hepatic and adipocytic origin, and showed that d4T-induced oxidative stress activates compensatory mechanisms through the upregulation of Lon protease, a mitochondrial protein that is able to degrade oxidized substrate proteins.36

In the HAART era, several clinical studies have suggested that the mitochondrial toxicity of d4T causes serious side effects, particularly the loss of fat tissue. Indeed, d4T is strongly correlated with lipodystrophy,67–70 and its consumption is associated with a reduction in the mtDNA content of lipodystrophic adipose tissue.71,72 Furthermore, it has been shown that its substitution with other NRTIs that have a lower impact on mitochondria significantly reduces or even reverses this side effect.73,74

2.4. ddI has been studied in vitro and in vivo
dDI (2',3'-dideoxyinosine) was approved for the treatment of HIV infection in 1991, becoming the second drug available for therapy. While first studies on animal models did not report any significant evidence of mitochondrial toxicity,75 further analyses indicated that ddI exerts a cytotoxic effect on human cultured muscular cells, evidenced by increased lactate production; such an effect is accompanied by a decrease in mtDNA content, and a reduction in the activity of complex IV, and part of complex II, of the respiratory chain.27 Interestingly, the same effect was observed in cultured CD4 and CD8 T lymphocytes, in a dose- and time-dependent manner.28

Recently, a single-blind clinical study of 49 HIV+ patients naïve for antiretroviral therapy and treated for a mean of 15 months with ddI-containing regimens showed that those taking such drugs had a lower amount of mtDNA in highly purified peripheral blood CD4+ and CD8+ T lymphocytes.28 In the same cells, the expression of different mitochondrial RNAs showed significant differences that were dependent on the drug used. However, no alterations in mitochondrial membrane potential and mitochondrial mass in peripheral lymphocytes were noted, and the viroimmunological efficacy of ddI-containing HAART was equivalent to that of regimens devoid of this drug.

2.5. Other NRTIs have a low impact on mitochondria

NRTIs other than d-drugs have a lower, if at all, toxic effect on mitochondria and mtDNA.38,76 In vitro studies on hepatoma HepG2 cells did not show any significant effect of tenofovir or 3TC on cell growth, lactate production, mtDNA levels and expression of proteins encoded by mtDNA, such as COXII. On the contrary, ABC impaired proliferation and increased lactate, but did not induce mtDNA depletion.39

These results are in agreement with several clinical observations reporting that tenofovir and ABC have a lower impact on adipose tissue, and do not significantly affect lactatemia. Moreover, the switch from d-drugs to non d-drugs typically leads to a general amelioration of a number of symptoms present in patients with lipodystrophy.55,73,77–79

3. Antiretroviral Therapy and Mitochondria: Low Mitochondrial Toxicity of PIs

While a large number of studies exist on the effects of NRTIs on mitochondria, less attention has been devoted to the effects of PIs on the organelle. At present,
nine PIs are approved for clinical use (saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, tipranavir, darunavir) and others are in development.\textsuperscript{90} This class of antiretroviral agent targets the aspartyl protease of HIV, inhibiting the cleavage of viral polyproteins and the subsequent generation of individual viral proteins, which are required for virion assembly.

As reported above, CD4\textsuperscript{+} T lymphocytes from HIV + patients are characterized by an enhanced tendency to undergo apoptosis. Since PI-based HAART significantly increased CD4 T cell count in treated patients, researchers asked if PIs were, in a direct or indirect way, able to inhibit apoptosis, so contributing to counteract CD4 T cell loss, and found a positive answer.\textsuperscript{81} Several different mechanisms have been proposed to explain the effect, including the direct inhibition of caspases by PIs,\textsuperscript{83}–\textsuperscript{86} the capability of PIs to reduce expression of proapoptotic molecules or increase that of proliferative ones,\textsuperscript{83}–\textsuperscript{86} the capability to inhibit calpain,\textsuperscript{87}–\textsuperscript{89} and finally, the capability to counteract the loss of mitochondrial membrane potential.\textsuperscript{84}

It is of note that contrasting results have been obtained for all but the latest mechanism, and different \textit{in vitro} studies have demonstrated that a possible major role in such action is played by mitochondria. Indeed, a study on Jurkat T cell lines have shown that nelfinavir inhibits apoptotic signaling by preventing loss of $\Delta \psi_m$ and the consequent release of apoptotic mediators such as cytochrome c.\textsuperscript{84} These data were confirmed by observations on resting lymphocytes treated with different PIs (lopinavir, indinavir, saquinavir), whose mitochondria were protected from $\Delta \psi_m$ loss compared to control lymphocytes after challenge with different apoptotic stimuli (Fas, tumor necrosis factor-$\alpha$, TRAIL or UV irradiation). Of note, treatment with PIs reduced the toxic effect of AZT on mitochondria.\textsuperscript{90,91} Data from patients on HAART regimens that include nelfinavir corroborated these observations,\textsuperscript{92} and studies in different mouse models further confirmed these results, showing that nelfinavir and ritonavir are able to reduce cytochrome c and apoptosis-inducing factor release.\textsuperscript{93}

4. HAART-related Lipodystrophy is a Main Clinical Manifestation of Mitochondrial Toxicity

In 1998, 2 years after the introduction of HAART, it was shown that such treatment can have a significant side effect, i.e., lipodystrophy,\textsuperscript{94} which rapidly became an important problem in the treatment of HIV infection. Depending on the criteria used for defining the syndrome, its overall prevalence was estimated to be from 18\% to 83\%.\textsuperscript{95} As in lipodystrophy syndromes of genetic origin, in HAART-related lipodystrophy fat redistribution may precede the development of metabolic alterations\textsuperscript{96} that include (but are not limited to) increased serum total and low-density lipoprotein, cholesterol and triglyceride levels (observed in about 70\% of patients), insulin resistance and type 2 diabetes mellitus (observed in 8\%–10\% of patients),\textsuperscript{97–99} and lactic acidemia.\textsuperscript{100} HAART-related lipodystrophy is characterized by an increase in visceral adipose tissue and a reduction in subcutaneous adipose tissue; as a consequence, fat is completely lost in the periphery (cheeks, arms, legs) and accumulates in the abdomen (including vessel walls) and behind the neck, in the so-called buffalo hump.\textsuperscript{101}

The pathogenesis of lipodystrophy is extremely complex, as pharmacological, immunological, metabolic, genetic and environmental factors can contribute to its onset. The data regarding mtDNA depletion during lipodystrophy are quite controversial and sometimes misleading, because of the different origins of the cells where mtDNA content was analyzed, and because of the different techniques used for the quantification of mtDNA. In the first study, the mtDNA content of subcutaneous fat tissue from the neck, abdomen and thigh was measured, and a decrease in mtDNA content was found in HAART-treated HIV + patients with peripheral fat wasting compared to HIV + patients without lipodystrophy but with a similar treatment history.\textsuperscript{71} Furthermore, HIV + patients naïve to antiretroviral therapy and healthy controls had similar amounts of mtDNA, suggesting that lipodystrophy with peripheral fat wasting is associated with a decrease in subcutaneous adipose tissue mtDNA content.\textsuperscript{71} In the following years, several studies have indicated that depletion of mtDNA and consequent mitochondrial impairment is the primary cause of lipodystrophy.\textsuperscript{53,71,102}

However, data from several groups suggest that the pathogenesis of lipodystrophy is not based merely on NRTI-induced depletion of mtDNA. The first study on mtDNA content in peripheral blood lymphocytes analyzed mitochondria function and apoptosis in the cells of HIV-infected children, with or without lipodystrophy, who were receiving HAART.\textsuperscript{48} No significant changes were detected in the lymphocytes from children with lipodystrophy, suggesting that normal mitochondrial function and tendency to undergo apoptosis were present in these cells.

Then, analysis of mtDNA content in isolated T cells from adult lipodystrophic patients showed a paradoxical increase in the mtDNA content of CD4\textsuperscript{+} T cells, while no change was observed in CD8\textsuperscript{+} T cells.\textsuperscript{103} It is of note, however, that in the aforementioned cases, the quantification of mtDNA had been performed in circulating CD4\textsuperscript{+} T lymphocytes that were likely to have been newly formed cells, and thus were exposed to damaging drugs for only a relatively short period of time. Studies on fat cells are always needed to better clarify mitochondrial damage. In any case, it is crucial to avoid the use of the most toxic antiretrovirals, particularly d4T.\textsuperscript{104}
5. HIV Exerts a Direct Effect on Mitochondria and mtDNA

In past years, the majority of this type of study has been devoted to the analysis of the effects of antiretroviral drugs on mitochondria. However, it must be noted that HIV per se can directly affect mitochondrial functionality. While the capability of HIV to alter mitochondrial functionality by impairing the functionality of complex I of the electron transport chain has been shown, the capability of HIV to alter mtDNA content remains a matter of debate. Some authors reported a depletion of mtDNA in HIV+ patients naïve for therapy compared to normal controls, at least in PBMCs, suggesting that the reduction in mtDNA can occur with HIV infection alone and precedes the use of NRTIs, and that HIV products or cytokines released in response to HIV infection may make mitochondria more vulnerable to NRTIs. However, other authors did not observe any alteration in mtDNA that could be attributed to HIV in adipose tissue from lipoatrophic patients. The discrepancy in such observations can probably be attributed to the different tissues used for this kind of analysis, as HIV-1 could exert a greater impact on blood cells than on adipocytes.

6. HIV Exerts Direct Effects on Mitochondria-mediated Apoptosis

A large number of studies over the last two decades have demonstrated that during HIV infection, apoptosis is one of the major mechanisms of CD4 T cell depletion. Indeed, in response to a variety of different stimuli, both CD4+ and CD8+ T cells from HIV patients display a high susceptibility to ΔΨm loss and subsequent apoptosis. Such a tendency can also be observed in the earliest phases of the infection, i.e., in patients with acute infection, and the capability of HIV to alter ΔΨm and induce apoptosis in peripheral blood cells plays an important role in this phenomenon. Intensive analyses of the proapoptotic activities of HIV products have shown an important role for viral proteins that are the products of the env, nef, tat and vpr genes.

6.1. Env-induced apoptosis

Env proteins (such as gp120, a glycoprotein exposed on the surface of the HIV envelope, and its precursor gp160) induce apoptosis in a Fas-independent manner, through the so-called intrinsic pathway of apoptosis; in different cellular models, this activity of Env proteins has been correlated with caspase 3 activation, increased expression of Bax, and downregulation of Bcl-2. Indeed, the interaction of Env proteins on the surface of HIV-infected cells with the CD4/CCR5 complex of uninfected cells could mediate a rapid ΔΨm loss that in turn initiates apoptosis in lymphocytes, probably by altering the ion fluxes through the plasma membrane, and the global ion equilibrium of the cell.

Furthermore, Env proteins can induce apoptosis not only in infected cells, but also in bystander T lymphocytes through the formation of syncytia, when the gp120/gp41 complex on infected cells interacts with the CD4/CCR5 complex on bystander cells. Syncytia then undergo apoptosis through a caspase-3 dependent mechanism, mediated by mitochondrial depolarization.

6.2. Nef-induced apoptosis

Nef (negative regulatory factor) is a protein required for the efficient replication of virus. It has been shown that Nef can be both proapoptotic and antiapoptotic, depending on the situation, since it can promote the killing of bystander (non-infected) cells through the activation of the Fas/FasL (CD95/CD178) pathway, while simultaneously preserving viral replication in the HIV-infected host cell by blocking apoptosis in infected T cells. When exerting its proapoptotic activity, Nef acts mainly by triggering apoptosis via Fas/FasL interactions; however, Nef can also trigger apoptosis through the intrinsic pathway, being able to decrease the expression of Bcl-2 and Bcl-XL in infected cells.

6.3. Tat-induced apoptosis

The protein Tat (transactivator of transcription) is necessary for many functions of the virus, including gene transcription and virus replication. As far as apoptosis is concerned, Tat triggers Fas-independent pathways by reducing Bcl-2 expression, and increasing both Bax expression and the levels of caspase 8 and 10. Tat can translocate into mitochondria where it promotes MMP and downregulates mitochondrial superoxide dismutase (SOD2), either at the transcriptional or post-transcriptional level. In turn, SOD2 downregulation reduces the capability of the cell to cope with ROS produced within mitochondria, sensitizing the cell to ROS-induced apoptosis.

6.4. Vpr-induced apoptosis

Viral protein R (Vpr) is a 14-kilodalton molecule that plays an important role in regulating nuclear import of the HIV-1 pre-integration complex. Vpr is essential for productive infection of macrophages and resting PBMCs. Like other viral proteins, Vpr can induce apoptosis by downregulating Bcl-2 and Bcl-XL. However, it is also able to directly interact with the mitochondria: Vpr binds adenine nucleotide translocator, which in turn causes the permeabilization of the inner mitochondrial membrane.

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membrane, swelling of mitochondria and breaking of the outer mitochondrial membrane.\textsuperscript{137,138}

7. Conclusions

During HIV infection and its treatment, a variety of molecular and cellular mechanisms are triggered to cope with the virus and to eliminate dangerous virus-producing cells. The immune system actively fights the infection, and, in the case of viral infections, has to remove infected cells. A large number of immune mechanisms are triggered, including the production of proinflammatory cytokines, among which is tumor necrosis factor-$\alpha$ (TNF-$\alpha$), which increases the production of intramitochondrial reactive oxygen species (ROS). Even proteins derived from the virus can directly affect mitochondria. The result of all the aforementioned mechanisms can be an irreversible alteration of the organelle, with a decrease in mitochondrial membrane potential ($\Delta \psi_m$) and the release into the cytoplasm of molecules such as cytochrome c (cyt-c) and apoptosis inducing factor (AIF), which activate caspases and lead to cell death.

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References


Lynch MD, McKeever EE. 3'-Azido-3'-deoxythymidine (AZT) is a competitive inhibitor of thymidine phosphorylation in isolated rat heart and liver mitochondria. Biochem Pharmacol 2006;72:239–43.


Hawkins T, Veilley W, St Claire RL, 3rd, Guyer B, Clark N, Kearney BP. Intracellular pharmacokinetics of tenofovir diphosphate, carbovir
Mitochondria in HIV infection and its treatment


Mitochondria in HIV infection and its treatment


115. Ohnimus H, Heinkelein M, Jassoy C. Apoptotic cell death upon contact of CD4+ lymphocytes with HIV glycoprotein-expressing cells is mediated by caspases but bypasses CD95 (Fas/Apo-1) and TNF receptor 1. *J Immunol* 1997;159:5246–52.


