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Using death to one's advantage: HIV modulation of apoptosis

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Abstract

Infection by human immunodeficiency virus (HIV) is associated with an early immune dysfunction and progressive destruction of CD4⁺ T lymphocytes. This progressive disappearance of T cells leads to a lack of immune control of HIV replication and to the development of immune deficiency resulting in the increased occurrence of opportunistic infections associated with acquired immune deficiency syndrome (AIDS). The HIV-induced, premature destruction of lymphocytes is associated with the continuous production of HIV viral proteins that modulate apoptotic pathways. The viral proteins, such as Tat, Env, and Nef, are associated with chronic immune activation and the continuous induction of apoptotic factors. Viral protein expression predisposes lymphocytes, particularly CD4⁺ T cells, CD8⁺ T cells, and antigen-presenting cells, to evolve into effectors of apoptosis and as a result, to lead to the destruction of healthy, non-infected T cells. Tat and Nef, along with Vpu, can also protect HIV-infected cells from apoptosis by increasing anti-apoptotic proteins and down-regulating cell surface receptors recognized by immune system cells. This review will discuss the validity of the apoptosis hypothesis in HIV disease and the potential mechanism(s) that HIV proteins perform in the progressive T cell depletion observed in AIDS pathogenesis.

Keywords

HIV; AIDS; T cell depletion; apoptosis

Introduction

Human immunodeficiency virus (HIV), a member of the primate lentivirus family of retroviruses, is the etiologic agent of AIDS. AIDS has become the major cause of death in individuals 25 to 44 years of age in the United States.¹ As of December 1999, 16.3 million people have died worldwide since the first cases were identified in 1981 and the total number of people living with HIV/AIDS is currently estimated at 35 million.¹ At the present rate, HIV is spreading faster in the human population than any infectious agent in the last 100 years.

HIV has become the most intensely studied infectious agent in history and the research generated has contributed to our increased understanding to several other fields of biology including virology, immunology, oncology, and cell biology. The primary receptor for these viruses is CD4 which was identified early in the studies of HIV pathogenesis.² CD4 is a necessary, but not a sufficient component for HIV-1 entry and replication.^{3,4} Chemokine receptors, in conjunction with CD4, mediate the efficient entry of HIV into cells⁵⁻⁹ and the use of different chemokine receptors by HIV accounts for the distinct cell tropism associated with various isolates.

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The viral replication cycle of HIV is highly regulated by viral gene products. The HIV-1 genome encodes the Gag, Pol, and Env structural proteins and two regulatory gene products, Tat and Rev. In addition, there are a number of accessory proteins encoded in the HIV-1 genome (Nef, Vif, Vpr, and Vpu) that are dispensable for *in vitro* replication but necessary to varying degrees for pathogenesis. As common for all retro-viruses, attachment of the HIV virion to specific receptors (CD4 and coreceptors) is followed by penetration of the core into the cell.^{3,4} After entry, the single stranded RNA genome is converted into a double stranded DNA molecule by the viral RNA-dependent DNA polymerase, reverse transcriptase (RT). The proviral DNA form is transported to the nucleus and it integrates into host chromosomes, where synthesis of viral RNA is initiated and regulated by both cellular and viral proteins (Tat and Rev). Structural proteins (Gag-Pol and Env) are assembled and the viral genomic RNA is encapsidated into the newly synthesized virion. Post budding and processing, the new virions are released from the infected cell.

AIDS, T cell depletion, and the apoptosis hypothesis

HIV-1 infection results in the progressive destruction of CD4⁺ T lymphocytes. The pathogenic importance of the loss of these T cells correlates with disease progression and increases opportunistic infections, which subsequently leads to AIDS.¹⁰ The mechanisms of CD4⁺ T cell depletion during HIV infection are incompletely understood, however, this depletion can be mediated directly by HIV infection as a consequence of viral gene expression or indirectly through priming for apoptosis of uninfected 'bystander' cells.¹⁰

The mechanism of T cell-induced death involves the complimentary proteins Fas, also known as CD95 or Apo-1 and Fas ligand (Fas-L).¹¹⁻¹⁴ The ligation of Fas/Fas-L is the main mechanism for maintaining the appropriate number of T cells in the body. Therefore, this apoptotic pathway has been hypothesized to enhance HIV-1 expression in infected patients and subsequently to disrupt T cell homeostasis that eventually leads to the depletion of CD4⁺ T lymphocytes. Several lines of evidence support this hypothesis. First, the proportion of Fas expressing T cells in HIV-1-infected individuals increase with disease progression.^{15,16} Second, Fas-L message can be detected in freshly isolated HIV-infected T cells.¹⁷ Third, therapies with anti-Fas antibody and soluble Fas-L induce T cells to undergo apoptosis.¹⁸ In addition, induction of Fas message in peripheral blood lymphocytes (PBL), particularly in T cells and monocytes, from uninfected people can be upregulated when cocultured with HIV infected cells.¹⁹ The significance of this last finding in terms of HIV-1 pathogenesis is unclear and has remained controversial. Other researchers have generated data to indicate that Fas-dependent activation does not induce T cell death in HIV-1-infected patients.^{20,21}

This hypothesis, that apoptosis is important for HIV pathogenesis, has been proposed by several researchers.²²⁻²⁶ A better understanding of HIV-mediated apoptosis is critical for the development of anti-retroviral therapies and vaccines. In this review, we will discuss the importance of apoptosis in HIV-1 disease and the role that HIV viral proteins contribute to the regulation of apoptosis.

Endogenous and exogenous regulators of apoptosis

Apoptosis is an ordered, suicide mechanism that is characterized by cell shrinkage, loss of membrane integrity, chromosomal condensation, and internucleosomal cleavage of DNA. These changes are associated with the activation of cellular nucleases resulting in the fragmentation of DNA into a ladder of regular nucleosomal subunits.²⁷ It is now widely appreciated that apoptosis contributes to most types of physiological cell death. However, its participation in the pathological forms of cell death by HIV and other viruses is less fully understood and might vary depending on the particular pathological agent.

Studies analyzing the mechanisms responsible for apoptosis in human cells have revealed that the cysteinyl aspartate-specific protease (caspase) family constitutes the effector arm of apoptosis.²⁸⁻³¹ Caspases are synthesized as inactive proenzymes that are activated by upstream proteins in response to an apoptotic stimulus and are characterized by a cysteine located within their active site.³² Following activation, caspases recognize specific sequences within their various target proteins and characteristically cleave these proteins 3' of an aspartic acid residue within the recognition sequence.³³ Thus far, the 13 caspases identified differ with regard to the activation stimuli and to their substrate specificity and sensitivity to various inhibitors.^{34,35} In addition to caspases, other apoptosis-inducing proteases can be activated in response to certain stimuli. One of these proteases, calpain, is a member of the calcium-dependent cysteine proteases and is involved in some forms of apoptosis.³⁶ Calpain substrates are primarily calmodulin-binding proteins.³⁶

The idea that apoptosis could be used by the host immune system as a defense against viruses has been recognized for many years. A major line of defense against viral infections are cytotoxic T cells (CTL), which can recognize and induce apoptosis in virally infected cells.³⁷ CTL use more than one killing pathway: (1) CTL express the ligand for Fas and thus can kill Fas-bearing target cells; and (2) CTL bear granules that they can deliver into their target cells. One of the proteases in these CTL granules, granzyme B, cleaves after aspartate residues and activates many members of the caspase family.³⁷⁻⁴⁰

There are several proteins encoded by human cells and viruses that share structural similarities and can both promote (Bax, Bak, Bok) and inhibit (Bcl-2, Bcl-x) apoptosis.⁴⁰⁻⁴⁵ These proteins are members of the *bcl-2* gene family and share sequence and functional homology to *Ced-9* of *Caenorhabditis elegans*.⁴⁶ The extensively studied Bcl-2 gene product is a major anti-apoptotic regulator and therefore, Bcl-2 itself is highly regulated. Cell-type specific expression of Bcl-2 and its interactions with the apoptotic protagonists, Bax and Bak are two major regulatory mechanisms of this protein.⁴⁷ Several members of this family dimerize via the Bcl-2 homology (BH) domain. Different sets of dimers either inhibit apoptosis (such as Bcl-2/Bax heterodimers) or induce apoptosis (such as Bax homodimers). The Bcl-2 member proteins that promote cell survival inhibit apoptosis upstream of caspase activation.^{47,48}

A large number of viruses have evolved to encode specific inhibitors of caspase activation and apoptosis. Cytokine response modifier A (CrmA) from cowpox virus is similar to the serpin family of proteins and is able to inhibit caspase activation. CrmA acts as a substrate for caspase-1 and interferes with the host immune response to the virus.⁴⁹ The viral protein p35 (encoded by baculovirus) is a broad-spectrum inhibitor of apoptosis by acting as a substrate for several caspases.⁵⁰⁻⁵² To date, p35 blocks the induction of apoptosis by all stimuli tested.^{51,52} Mutation of these genes allows for host cells to respond to infection by undergoing apoptosis that in turn drastically reduces viral spread. A number of adenoviruses encode the protein, E1B, which has similar properties to Bcl-2 by blocking apoptosis and interacting with Bak and Bax.

However, not all data support the important role of Bcl-2 or Fas/Fas-L interaction in HIV-1-induced disease. A recent study showed that during HIV-1 infection of T cells, E1B of adenovirus and Bcl-2 only marginally decreased overall cell death.⁵³ In addition, inhibition of Fas/Fas-L interaction did not prevent HIV-induced cell death.⁵³ Therefore, a non-apoptotic pathway may serve as a major pathway of HIV-induced cell death.

Modulation of apoptosis by HIV infection

Primate retroviruses such as HIV-1, HIV-2, simian immunodeficiency virus (SIV), and human T cell leukemia virus (HTLV), have considerably smaller genomes compared to other viruses (herpesviruses, poxviruses, and baculoviruses) that encode genes for the regulation of

apoptosis. Nevertheless, cells infected with HIV have a prolonged survival time initiated by a variety of mechanisms including the modulation of Bcl-2, Fas, Fas-L, tumor necrosis factor α (TNF α) and caspase expression (Table 1).⁵⁴ HIV-1 is therefore not unique among viruses in regard to its ability to regulate apoptosis. HIV integrates into the host genome as part of its life cycle and, in general, integrated viruses that constitutively express viral gene products serve as good targets for the cellular immune response.^{3,4} HIV-induced apoptosis may therefore play an important role in the viral life cycle by limiting the host immune response to virus infection and thus facilitating viral persistence.⁵⁵ HIV infection has been reported to abnormally activate T cells and change the balance between T helper (Th)-1 and Th2 cells, possibly by increasing apoptosis and decreasing CD4⁺ T cells.⁵⁶ The modulation of apoptosis in HIV-infected and uninfected cells is complex. Figure 1 describes the overall integrated effect of various HIV gene products on apoptosis. Several viral proteins have been shown to effect apoptosis including Tat, Env, Nef, Vpr and Vpu (Table 1). The following sections address the specific actions of each of these proteins on the induction or inhibition of apoptosis in HIV-1 pathogenesis.

Tat

Tat is expressed early after HIV infection and stimulates viral gene expression by interacting with the TAR element found in HIV mRNA.^{57,58} Tat has been associated with several intracellular and extracellular proteins which suggests that Tat is capable of modulating various biological effects.⁵⁸ A soluble form of Tat (sTat) has been detected in HIV-1-infected patients and in the supernatant of Tat transfected cells, which can be taken up by neighboring cells.⁵⁹ Several reports indicate that Tat may effect B lymphocyte differentiation, growth of Kaposi sarcomas, the down-regulation of MHC class I and the induction and protection from apoptosis.^{60,65} Even though the role of direct cell killing of CD4⁺ T cells is a credible hypothesis for the T cell depletion seen in AIDS patients,¹⁰ apoptosis is rarely observed in HIV-1-infected cells. Co-culture experiments indicate that while uninfected T cells die by apoptosis, HIV-infected cells remain resistant and this resistance has been partially mapped to *tat*.^{55,66} Therefore, a dual role for Tat in regulation of apoptosis has been suggested: (1) Tat induces apoptosis of HIV uninfected cells via exogenous sTat by sensitizing bystander cells to Fas-mediated apoptosis; and (2) protecting HIV⁵⁵-infected cells from death by up-regulating T cell growth factors and anti-apoptotic proteins.

Support for the 'bystander'-induced apoptosis theory is supported by experiments examining the lymph nodes of SIV-infected macaques.⁵⁵ Apoptosis occurs in the lymph nodes of these animals primarily in neighboring, uninfected cells and not in the productively infected T cells.⁵⁴ Exogenous sTat interacts with cell surface receptors, possibly CD26 and integrin $\alpha 5\beta 1$, both of which can transduce signals leading to the down-regulation of Bcl-2 and induce apoptosis.^{60,67,68} Tat induces Fas/Fas-L-dependent activation of apoptosis in T cell death. These findings indicate that Tat, by initiating signals in T cells, either directly or indirectly induces apoptosis in uninfected cells.⁶⁷ The Tat-induced signaling pathway has yet to be elucidated, but may involve the activation of inositol triphosphate (IP3)-phospholipase C (PLC) pathway.⁶⁹ Several proteins, including p56^{lck}, CD45, and hematopoietic cell phosphatase (HCP) as well as the transcription factors NF- κ B and SP-1, have also been implicated in Tat-mediated apoptosis.⁶²

In contrast to sTat, endogenous Tat appears to protect T lymphocytes from apoptosis. However, the mechanism for this protection remains unknown. *In vitro* studies indicate that cells transfected with Tat expression plasmids were resistant to apoptosis.⁵⁵ Endogenous Tat may block apoptosis by up-regulation of IL-2 or the anti-apoptotic proteins *bcl-2/bcl-x*.⁷⁰ Interestingly, endogenous Tat protects cells from exogenous sTat-induced apoptosis.

Tat can have pronounced immunosuppressive effects. In mice transfected with Tat expression vectors and in seroconverted infected humans, Tat expression cripples the immune surveillance of the infected host.^{62-65, 71-74} This immunosuppression affects both T cells and macrophages and can be reversed with Fas/Fas-L antagonists.⁷⁴ These observations have immediate relevance to HIV vaccine development. Several vaccine approaches for limiting HIV infection are currently underway and many include a Tat component in the vaccine. Vaccines in clinical and pre-clinical trials have included: (1) Tat expression vectors (both soluble and endogenous forms of Tat);^{75, 76} (2) as part of a multiple DNA expression plasmids encoding for several HIV genes;⁷⁷ (3) in live attenuated HIV vaccines;⁷⁸⁻⁸³ and (4) in virus-like particles (VLPs) both in DNA expression vectors and as purified protein.⁸⁴ Therefore, a better comprehension of Tat-induced apoptosis and immunosuppressive mechanisms are important for new vaccine designs.⁸⁵ Interestingly, a recent study demonstrated that an oxidized, inactivated form of Tat does not produce the immunosuppressive effects associated with Tat and has been proposed for vaccinations.⁸⁶

Vpr

The 96 amino acid, 14 kDa accessory protein Vpr is highly conserved in HIV-1, HIV-2, and SIV and confers rapid growth advantage to viruses expressing this gene product.^{87, 88} Viruses expressing Vpr have faster growth rates than Vpr-deficient viruses. Also, the effects of Vpr are cell-type specific. Vpr augments the growth of HIV in macrophages, but does not enhance viral growth in primary T cells.⁸⁹ Vpr associates with the viral particle through the p6 molecule of Gag⁹⁰⁻⁹² and deletions or truncations of p6 prevent Vpr incorporation into the virion.⁹³ The incorporation of Vpr into progeny virions suggests that this protein participates in the early events of viral infection. Several functional roles have been demonstrated for Vpr during HIV replication. One of the more defined functions of Vpr is the nuclear translocation of the preintegration complex.^{91, 94-96} Vpr may enhance virus-specific functions such as reverse transcriptase by either stabilizing the RNA-DNA or DNA-DNA molecules, migration of the proviral DNA complex to the nucleus, or integration.⁹⁷ It has been shown that Vpr, along with the matrix p17 protein, acts to ensure the efficient nuclear import of the preintegration complex in non-dividing cells such as macrophages.⁸⁷

Besides the role in nuclear translocation, *in vitro* functions have been described for Vpr including the enhancement of viral gene transcription.⁹² Vpr is localized primarily in the nucleus. In dividing cells, Vpr has been shown to modestly *trans*-activate the HIV long terminal repeat (LTR) and heterologous viral promoters.^{87, 98} The exact mechanism by which Vpr increases protein synthesis is not clear,⁹⁹ however, it may be achieved by direct binding of Vpr to the transcription factors TFIIB and Sp1.^{99, 100}

In proliferating T cells and stimulated peripheral blood mononuclear cells (PBMCs), Vpr appears to be dispensable for the *in vitro* replication of HIV.¹⁰¹⁻¹⁰⁷ However, under certain conditions, Vpr contributes substantially to HIV-1 replication in these cells.⁹⁹ Vpr has been shown to induce cell cycle arrest of cells in the G2 phase of the cell cycle.^{103, 104, 108, 109} The Cdc2 kinase is an inactive form in G2-arrested cells. Recent reports suggest that Vpr disrupts the regulation of Cdc2 kinase, which leads to G2 arrest.^{104, 105, 109} Vpr interacts with highly conserved components of the cell cycle regulation pathways. Interestingly, others have reported that Vpr specifically binds to the highly conserved DNA repair enzyme uracil DNA glycosylase (UNG), that is involved in removing uracil from DNA.^{110, 112} However, the role of UNG-Vpr interactions and cell cycle arrest is unclear.

The different Vpr functions have been attributed to different regions of the protein.^{88, 91} The amino terminus is involved in virion incorporation and the nuclear location of the protein. The carboxyl terminus of the protein is required to induce G2 arrest. Analysis of Vpr point mutants

demonstrates a correlation between the extent of G2 arrest and the levels of apoptosis.¹⁰⁶ Mutants that cause more cells to accumulate in G2 phase of the cell cycle show increased levels of apoptosis.¹⁰⁹ However, Vpr-induced apoptosis does not require maintenance of G2 arrest. Studies by Stewart *et al.*, indicate that treatment of Vpr-arrested cells with methylxanthine pentoxifyllin alleviates G2 arrest but does not reduce the levels of apoptosis.¹⁰⁶ The Cdc2 kinase remained hyperphosphorylated during apoptosis, indicating that Cdc2 kinase was not required for activation of the apoptotic pathways induced by Vpr.

In contrast, reports by Bartz *et al.*¹⁰⁴ show that Vpr induces cytostasis but not apoptosis. Infection of Jurkat T cells with virions expressing Vpr resulted in G2 arrest but not apoptosis.¹⁰⁴ These infected cells continued to accumulate at G2 over a 72 h period, however, no significant cell death was observed compared to Vpr mutant virions. The discrepancy between these two studies is mostly likely due to the post-infection time point at which the cells were monitored. Specifically, Bartz *et al.*, monitored infected cells for only 3 days, whereas Stewart *et al.*, observed that apoptosis increased over time and was usually maximal 4 days post-infection. Vpr increases apoptosis without addition of a second stimulus and Vpr-induced apoptosis has been shown to be independent of Cdc2 kinase activity.¹⁰⁶ Therefore, Vpr may contribute to the overall induction of cell death and contribute to CD4⁺ T cell decline in disease via G2 arrest and subsequent induction of apoptosis.

Env

Although the identification of specific coreceptors for HIV-1 infection represented an important step forward in our understanding of HIV-1 pathogenesis, information about how these receptors participate in the HIV replication cycle is just beginning to be understood. HIV infection requires binding of the gp120 subunit of Env to both CD4 receptor and one of a variety of chemokine receptors.^{5,113} The chemokine receptor CXCR4 is required for entry of T-tropic isolates of HIV (named X4 strains) and CCR5 was determined to be the major coreceptor for M-tropic strains of HIV (R5 strains).^{5,9,113} Even though these receptors are necessary for entry of HIV into cells, the importance of Env interaction with these receptors, resulting in signal transduction, is not totally understood. Activation of chemokine receptors by their cognate ligands or Env proteins results in prominent changes in cell migration and cell growth by stimulating various signal transduction pathways. Recent studies indicate that β -chemokines (which bind to CCR5) may exert indirect selective pressure *in vivo* in favor of the replication of X4 isolates that do not require CCR5 for entry.¹¹⁴ Interestingly, this effect is evident only at low inoculum of HIV.¹¹⁴ β -chemokine-mediated enhancement of X4 HIV replication can be detected at the early stages of HIV replication cycle and is dependent on signaling through G proteins. These findings provide insight regarding signals, generated from HIV Env receptors, which can enhance viral replication, possibly including signals that activate apoptosis. In that regard, Env from X4 strains of HIV can induce apoptosis *in vitro* in CD8⁺ T cells and neuronal cells.^{115,116} The signaling pathway induced by Env/CXCR4 interaction is complex, involving cross-talk with macrophages, and operates through the tumor-necrosis factor- α (TNF- α) receptor II (TNFRII).¹¹⁴ Signaling by CXCR4 induces the surface expression of TNF- α in macrophages and TNFRII in CD8⁺ T cells.¹¹⁷⁻¹¹⁹ Subsequently, contact between the macrophages and T cells triggers T cell death. Although a low percentage of CD8⁺ T cells were apoptotic in these studies, the loss of viable cells was high. Interestingly, this Env-induced death of CD8⁺ T cells through CXCR4 and TNF- α /TNFRII signaling resembles the Env-induced death of CD4⁺ T cells through CD4 and Fas/Fas-L receptor signaling.¹¹⁹ TNF- α /TNFRII and Fas/Fas-L belong to the same apoptosis-transducing pathway, which are involved in the physiological control of T cells.¹²⁰ Therefore, X4-Envs may induce premature cell death in both CD4⁺ and CD8⁺ T cell populations through subverting signaling pathways that normally down-regulate critical immune responses.

The CD4 receptor itself has been shown to transduce signals after stimulation with HIV Envs leading to apoptosis.¹²¹ One of the first studies to show the participation of apoptosis in CD4⁺ T cells infected with HIV-1 *in vitro* was performed by Laurent-Crawford *et al.*²⁵ This study demonstrated that apoptosis was initiated in HIV-infected cells prior to cell lysis and suggested that apoptosis is the direct cause of cell death by HIV infection.²⁵ Also, stable cell clones expressing Env showed the characteristic features of apoptosis (DNA fragmentation and nuclear chromosome condensation) and these effects were seen as early as 8 h after induction.⁸⁶

In addition to activating signaling by chemokine receptors, Env stimulates the induction of caspase-3 and caspase-6 in a CD4 receptor-dependent manner.^{85,122} The activation of these two caspases is most likely the molecular mechanism responsible for the major apoptotic activity induced by the HIV envelope, since this activity can be inhibited by use of soluble CD4 (sCD4).^{85,122} HIV Env also induces the phosphorylation of focal adhesion kinase (FAK) and the formation of this complex may contribute to the dysregulation of cellular activation and trafficking associated with HIV infection. FAK is cleaved in a caspase-3- and caspase-6-specific pattern and the cleavage of FAK by caspase-3 is associated with the early stages of apoptosis.^{85,122} Thus, the activation and subsequent cleavage of phosphorylated FAK may represent the critical early step in HIV-1 induced apoptosis.

Nef

Several biological activities have been attributed to the Nef protein of HIV, including the down-regulation of cell surface CD4,^{123,124} and MHC class I,^{122,123} enhancement of virion infectivity,¹²⁵⁻¹²⁷ increased activation of expressing cells,^{127,128} and enhancement of apoptosis.^{129,130} *In vitro*, several researchers have reported difficulties in establishing stable cell lines constitutively expressing Nef due to Nef toxicity when this protein is expressed in transfected cell lines.^{131,132}

The activation of apoptosis by Nef has recently been demonstrated by a variety of researchers. Okada *et al.*¹³³ demonstrated that extracellular Nef induced apoptosis in both lymphoid and myeloid cells through a Fas-independent pathway. However, a contradictory study indicated that Nef induced Fas-L expression, which triggered Fas-mediated T cell death. Xu *et al.*¹³⁴ showed that Nef and the ζ chain of the T cell receptor (TCR) were required and sufficient to upregulate Fas-L in T cells. Also lending support to this hypothesis, Fas-L activation in T cells by SIV depends on the expression of an intact Nef protein.¹³⁵ In addition, Nef from a highly pathogenic form of SIV (smpPBj14) alone can directly cause Fas-L up-regulation.¹³⁵

Possible intracellular targets, important for Nef-mediated apoptosis, have been identified. Nef associates with cellular serine/threonine kinases (possibly PAK), as well as *src*-like tyrosine kinases (Hck and Lck).¹³⁶⁻¹³⁸ Both of these types of kinases are important in T cell activation.^{136,139} Consistent with this observation, Fas-L is expressed following activation of the TCR leading to the phosphorylation of these kinases and expression is inhibited by cell treatment with immunosuppressive drugs.¹³⁹ Ras signaling has also been implicated in tyrosine kinase activation and hence both the Ras and Lck pathways may contribute to the up-regulation of Fas-L transcription.¹⁴⁰ Therefore, HIV infection may activate tyrosine kinases (via Nef) that converge in a common activation pathway leading to Fas/Fas-L interaction and the induction of apoptosis.¹²⁹ Other HIV-1 gene products, particularly Tat and Env, together with Nef may act in concert to induce Fas-L and to increase Fas-based cytotoxicity.^{19,141}

Vpu

One of the least understood accessory protein expressed by HIV is Vpu. Two separate functions have been ascribed to Vpu. One of these functions is the ability of this protein to enhance HIV

release from infected cells.^{26,142,143} This property is not restricted just to HIV, since Vpu can also enhance the release of virus particles generated from other retroviruses. A second function associated with Vpu is the disruption of Env-CD4 interactions in the endoplasmic reticulum.^{26,142} Vpu appears to interact with the cytoplasmic tail of CD4 and targets CD4 to proteosomes for degradation. These two functions can be segregated to different regions of the Vpu protein.^{26,142} The transmembrane region of Vpu is responsible for the increased viral release and the cytoplasmic tail is needed for CD4 degradation.^{26,142}

Vpu has several structural similarities to the M2 protein of influenza virus.^{143,144} Both M2 and Vpu form cation-selective ion channels.¹⁴⁵⁻¹⁴⁷ The means by which Vpu ion channel activity might increase virus release is unknown. However, it is tempting to speculate that the ion channel properties of Vpu cause cells to be more responsive to apoptotic stimuli. Perturbations in potassium levels in neurons and decreased potassium levels in T cells directly cause apoptosis.¹⁴⁷⁻¹⁴⁹ Interestingly, both Bcl-2 and Bax also have ion channel properties and may enhance ion-channel formation.¹⁵⁰⁻¹⁵²

Vpu has a large effect on the susceptibility of cells to Fas-induced apoptosis. Deletion of Vpu from the HIV genome increases survival in response to Fas cross-linking in both T cell lines and freshly isolated PBLs. In addition, an enhanced death rate is observed in cells infected with clones of HIV expressing Vpu. This enhanced rate correlates with increased Fas-induced cell death. However, deleting Vpu does not completely eliminate the sensitivity of HIV-1-infected cells to Fas killing. Most likely, the increase in cell death can be attributed to other viral gene products including Env and Tat. Interestingly, there is no homologue of Vpu in SIV or HIV-2. Therefore, the lack of Vpu in these two lentiviruses may help to explain their reduced pathogenicity compared to HIV-1. Interesting results have been obtained from rhesus macaques infected with SHIV chimeras (proviruses with an HIV *env* region contained in an SIV genomic backbone), which used a Vpu gene containing a mutated start codon (ACG).¹⁵³ Several months after infection, these monkeys developed AIDS-like symptoms. The virus recovered from these animals had a Vpu start codon that had reverted to the wild-type ATG codon and therefore Vpu was expressed in these animals,¹⁵³ again demonstrating that Vpu expression is important for inducing immunodeficiency disease.

Summary

The regulation of apoptosis by HIV has profound implications for both the virus and the infected host cells. Induction of apoptosis has the potential of significantly influencing virus load and transmission rates as well as the response by the host immune system to the virus. HIV encodes a variety of gene products that can induce or inhibit apoptotic pathways (Figure 1). Apoptosis activation in bystander immune cells is the key to the depletion of T lymphocytes observed in HIV-1 infected patients. In a variety of model systems, viral genes can modulate apoptosis. Tat, Nef, and Vpu can protect infected cells from apoptotic stimuli. Env, Vpr, Vpu, and Tat can also induce apoptosis, particularly in non-infected cells. Understanding apoptotic mechanisms, induced by viral proteins, is important for the development of anti-HIV treatments. HIV-induced apoptosis may have detrimental effects on prophylactic administration of anti-viral drugs and vaccines. No vaccine capable of eliciting protective immunity to HIV infection has been formulated and HIV presents a formidable challenge to immune surveillance. The induction of apoptosis by HIV gene products, which could be expressed in a potential vaccine, may have adverse effects on the host. Therefore, a more thorough comprehension of the modulation of apoptosis by HIV proteins is critical for the development of an effective HIV vaccine and decreasing disease pathogenesis.

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References

1. United Nations AIDS Program. 1999. <http://www.us.unaids.org>
2. Maddon PJ, McDougal JS, Clapham PR, Dalgleish AG, Jamal S, Weiss RA, Axel R. HIV infection does not require endocytosis of its receptor, CD4. *Cell* 1988;54:865–874. [PubMed: 3261635]
3. Ward SG, Bacon K, Westwick J. *Immunity* 1998;9:1–11. [PubMed: 9697831]
4. Ross TM, Bieniasz PD, Cullen BR. Role of chemokine receptors in HIV-1 infection and pathogenesis. *Adv Vir Res* 1999;52:233–267.
5. Feng YBC, Kennedy PE, Berger EA. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 1996;272:872–877. [PubMed: 8629022]
6. Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR. Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 1996;381:661–666. [PubMed: 8649511]
7. Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, Wu L, Mackay CR, LaRosa G, Newman W, Gerard N, Gerard C, Sodroski J. The β -chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 1996;85:1135–1148. [PubMed: 8674119]
8. Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA, CC CKR5. A RANTES, MIP-1a, MIP-1b receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* 1996;272:1955–1958. [PubMed: 8658171]
9. Alkhatib G, Ahuja SS, Light D, Mummidi S, Berger EA, Ahuja SK. CC Chemokine receptor 5-mediated signaling and HIV-1 co-receptor activity share common structural determinants. *J Biol Chem* 1997;272:19771–19776. [PubMed: 9242636]
10. Hazenberg MD, Hamann D, Schuitemaker H, Miedema F. T cell depletion in HIV-1 infection: how CD4+ T cells go out of stock. *Nat Immunol* 2000;1:285–289. [PubMed: 11017098]
11. Alderson M, Tough T, Davis-Smith T, Braddy S, Falk B, Schooley K, Goodwin R, Smith C, Ramsdell F, Lynch D. Fas ligand mediates activation-induced cell death in human T lymphocytes. *J Exp Med* 1995;181:71–77. [PubMed: 7528780]
12. Brunner T, Mogiol R, Laface D, Yoo N, Mahboubi A, Echeverri F, Martin S, Force W, Lynch DL, Ware C, Green D. Cell autonomous Fas/Fas ligand interaction mediates activation-induced apoptosis in T-cell hybridomas. *Nature* 1995;373:441–444. [PubMed: 7530336]
13. Dhein J, Walczak H, Baumler C, Debatin K, Krammer P. Autocrine T-cell suicide mediated by APO-1 (Fas/CD95). *Nature* 1995;373:438–441. [PubMed: 7530335]
14. Ju S-T, Panka D, Cui H, Ettinger R, el-Khatib M, Sherr D, Stanger B, Marshak-Rothstein A. Fas/FasL interactions required for programmed cell death after T-cell activation. *Nature* 1995;373:444–448. [PubMed: 7530337]
15. Estaquier J, Tanaka M, Suda T, Nagata S, Goldstein P, Amiesen J. Fas-mediated apoptosis of CD4+ and CD8+ T cells from HIV-1 infected persons: differential in vitro preventive effect of cytokines and protease antagonists. *Blood* 1996;87:4959–4966. [PubMed: 8652808]
16. Baumler C, Bohler T, Herr I, Benner A, Krammer P, Debatin K. Activation of the CD95 (APO-1/Fas) system in T cells from HIV-1 infected children. *Blood* 1996;88:1741–1746. [PubMed: 8781430]
17. Sieg S, Smith D, Yildirim Z, Kaplan D. Fas ligand deficiency in HIV disease. *Proc Natl Acad Sci USA* 1997;94:5860–5865. [PubMed: 9159165]
18. Gehri R, Hahn S, Rothen M, Steuerwald M, Nuesch R, Erb P. The Fas receptor in HIV infection: expression of peripheral blood lymphocytes and role in the depletion of T cells. *AIDS* 1996;10:9–16. [PubMed: 8924258]
19. Badley A, Meclbinny J, Leibson P, Lynch D, Alderson M, Paya C. Upregulation of Fas ligand expression by HIV in human macrophages mediates apoptosis of uninfected T lymphocytes. *J Virol* 1996;70:199–206. [PubMed: 8523526]
20. Katsikis PD, Garcia-Ojeda ME, Terres-Roca JF, Tijoe IM, Smith CA, Herzenberg LA, Herzenberg LA. Interleukin-1B converting enzyme-like protease involvement in Fas-induced and activation

induced peripheral blood T cell apoptosis in HIV-1 infection. TNF-related apoptosis-inducing ligand can mediate activation-induced T cell death in HIV-1 infection. *J Exp Med* 1997;186:1365–1372. [PubMed: 9334376]

21. Katsikis PD, Garcia-Ojeda ME, Wunderlich ES, Smith C, Yagita H, Okumura K, Kayagaki N, Alderson M, Herzenberg LA, Herzenberg LA. Activation-induced peripheral blood T cell apoptosis is Fas independent in HIV-1 infected individuals. *Int Immunol* 1996;8:1311–1317. [PubMed: 8918700]
22. Ameisen JC. Setting death in motion. *Nature* 1998;395:117–119. [PubMed: 9744265]
23. Terai C, Kornbluth RS, Panza CD, Richman DD, Carson DA. Apoptosis as a mechanism of cell death in culture T lymphoblasts acutely infected with HIV-1. *J Clin Invest* 1991;87:1710–1715. [PubMed: 2022741]
24. Rapaport E, Casella CR, Ikle D, Mustafa F, Isaak D, Finkel TH. Mapping of HIV-1 determinants of apoptosis in infected T cells. *Virology* 1998;252:407–417. [PubMed: 9878620]
25. Laurent-Crawford AG, Krust B, Muller S, Riviere Y, Rey-Cuille MA, Bechet J-M, Montagnier L, Hovanessian AG. The cytopathic effect of HIV is associated with apoptosis. *Virology* 1991;185:829–839. [PubMed: 1683728]
26. Ameisen JC, Capron A. Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. *Immunol Today* 1991;12:71–77. [PubMed: 2059307]
27. Wyllie AH, Arends MJ, Morris RG, Walker SW, Evan G. The apoptosis endonuclease and its regulation. *Semin Immunol* 1992;4:389–397. [PubMed: 1337478]
28. Miller DK. The role of the caspase family of cysteine proteases in apoptosis. *Semin Immunol* 1997;9:982–988.
29. Cryns V.; Yuan J. The cutting edge: caspases in apoptosis and disease. In: Zakeri, Z.; Lockshin, R., editors. *When Cells Die: a Comprehensive Evaluation of Apoptosis and Programmed Cell Death*. 1. John Wiley; New York: 1998. p. 177-210.
30. Cryns V, Yuan J. Proteases to die for. *Genes Dev* 1998;12:1551–1570. [PubMed: 9620844]
31. Allen RT, Cluck MW, Agrawal DK. Mechanisms controlling cellular suicide: role of Bcl-2 and caspases. *Cell Mol Life Sci* 1998;54:427–445. [PubMed: 9645223]
32. Wilson KP, Black J-AF, Thompson JA, Kim EE, Griffith JP, Navia MA, Murcko MA, Chambers SP, Aldape RA, Raybuck SA, Livingston DJ. Structure and mechanisms of interleukin-1 β converting enzyme. *Nature* 1994;370:270–275. [PubMed: 8035875]
33. Black RA, Kronheim SR, Merriam JF, March CJ, Hopp TP. A pre-aspartate specific protease from human leukocytes that cleaves pro-interleukin-1 β . *J Biol Chem* 1989;264:5323–5326. [PubMed: 2784432]
34. Wang S, Miurya M, Zhu Y-K, Li E, Yuan J. Murine caspase-11, and ICE-interacting protease, is essential for the activation of ICE. *Cell* 1998;92:501–509. [PubMed: 9491891]
35. Alnemri ES, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. Human ICE/CED-3 protease nomenclature. *Cell* 1996;87:171. [PubMed: 8861900]
36. Croal DE, DeMartino GN. Calcium-activated neutral protease (calpain) system: structure, function, and regulation. *Physiol Rev* 1991;71:813–847. [PubMed: 2057527]
37. Teodoro JG, Branton PE. Regulation of apoptosis by viral gene products. *J Virol* 1997;71:1739–1746. [PubMed: 9032302]
38. Chinnaiyan AM, Hanna WL, Orth K, Duan H, Poirier GG, Froelich CJ, Dixit VM. Cytotoxic T cell derived granzyme B activate the apoptosis protease ICE-LAP3. *Curr Biol* 1996;6:897–899. [PubMed: 8805307]
39. Darmon AJ, Nicholson DW, Bleackley RC. Activation of the apoptotic protease CPP32 by cytotoxic T-cell derived granzyme B. *Nature* 1995;377:446–448. [PubMed: 7566124]
40. Quan LT, Tewari M, O'Rourke K, Dixit V, Smipas SJ, Poirier GG, Ray C, Pickup DJ, Salvesen GS. Proteolytic activation of the cell death protease Yama/CPP32 by granzyme B. *Proc Natl Acad Sci USA* 1996;93:1972–1976. [PubMed: 8700869]
41. Hacker G, Vaux DL. A sticky business. *Apoptosis*. *Curr Biol* 1995;5:622–624. [PubMed: 7552172]
42. Kelekar A, Thompson CB. Bcl-2 family proteins: the role of the BH3 domain in apoptosis. *Trends Cell Biol* 1998;8:324–330. [PubMed: 9704409]

43. Conradt B, Horvitz HR. C. Elegans protein EGL-1 is required for programmed cell death and interacts with the Bcl-2 like protein CED-9. *Cell* 1998;93:519–529. [PubMed: 9604928]
44. Adams JM, Cory S. The Bcl-2 protein family; arbiters of cell survival. *Science* 1988;281:1322–1326. [PubMed: 9735050]
45. Reed JC, Zha H, Aime-Sempe C, Takayama S, Wang HG. Structure-function analysis of Bcl-2 family protein: regulators of programmed cell death. *Adv Exp Med Biol* 1996;406:99–112. [PubMed: 8910675]
46. Hengartner MG, Horvitz HR. C. elegans cell survival gene ced-9 encodes a functional homologue of the mammalian proto-oncogene bcl-2. *Cell* 1994;67:665–676. [PubMed: 7907274]
47. Pan G, O'Rourke K, Dixit VM. Caspase-9, Bcl-x, and Apaf-1 form a ternary complex. *J Biol Chem* 1998;273:5841–5845. [PubMed: 9488720]
48. Hu Y, Benedict MA, Wu D, Inohara N, Nunez G. Bcl-SL interacts with Apaf-1 and inhibits Apaf-1 dependent caspase-9 activation. *Proc Natl Acad Sci USA* 1988;95:4386–4391. [PubMed: 9539746]
49. Ray CA, Black RA, Kronheim SR, Greenstreet TA, Sleath RR, Salvesen GS, Pickup DJ. Viral inhibition of inflammation: cowpox virus encodes an inhibitor of the interleukin-1 β -converting enzyme. *Cell* 1992;69:597–604. [PubMed: 1339309]
50. Clem RJ, Miller LK. Control of programmed cell death by the baculovirus genes p35 and Iap. *Mol Cell Biol* 1994;14:5212–5222. [PubMed: 8035800]
51. Bertin J, Mendrysa JM, LaCount DJ, Gaur S, Krebs IF, Armstrong RC, Tomaselli KJ, Friesen PD. Apoptotic suppression by baculovirus p35 involves cleavage by and inhibition of a virus-induced CED-3/ICE-like protease. *J Virol* 1996;70:6251–6259. [PubMed: 8709252]
52. Xue D, Horvitz HR. Inhibition of the *Caenorhabditis elegans* cell death protease CED-3 by a CED-3 cleavage site in baculovirus p35 protein. *Nature* 1995;377:248–251. [PubMed: 7675111]
53. Kolensitchenko V, King L, Rivo A, Tani Y, Korsmeyer SJ, Cohen DI. A major human immunodeficiency virus type 1-initiated killing pathway distinct from apoptosis. *J Virol* 1997;71:9253–9263.
54. Sandstrom PA, Pardi D, Goldsmith CS, Chengying D, Diamond AM, Folks TM. bc1–2 expression facilitates human immunodeficiency virus type-1 mediated cytopathic effects during acute spreading infections. *J Virol* 1996;70:4617–4622. [PubMed: 8676488]
55. Finkel TH, Tudor-Williams G, Banda NK, Cotten MF, Curiel T, Monks C, Baba TW, Ruprecht RM, Kupfer A. Apoptosis occurs predominately in bystander cells and not unproductively infected cells on HIV and SIV-infected lymphocytes. *Nature Med* 1995;1:129–134. [PubMed: 7585008]
56. Meyaard L, Otto SA, Keet IPM, Roughs MRL, Meiden F. Programmed cell death of T cells in human immunodeficiency virus infection, no correlation with progression to disease. *J Clin Invest* 1994;93:982–988. [PubMed: 8132784]
57. Jones KA. Tat and the HIV-1 promoter. *Curr Opin Cell Biol* 1993;5:461–468. [PubMed: 8352964]
58. Cullen BR. Human immunodeficiency virus as a prototypic complex retrovirus. *J Virol* 1991;65:1053–1056. [PubMed: 1995941]
59. Zagury D, Lachgar A, Chams V, Fall LS, Bernard J, Zagury J-F, Bizzini B, Gringeri A, Santagositno E, Rappaport J, Feldman M, Burny A, Gallo RC. Interferon α and Tat involvement in the immunosuppression of uninfected T cells and C-C chemokine decline in AIDS. *Proc Natl Acad Sci USA* 1998;95:3851–3856. [PubMed: 9520456]
60. Ensoli B, Bounaguro L, Barillari G, Fiorelli V, Gendelman R, Morgan RA, Wingfield P, Gallo RC. Release, uptake, and effects of extracellular HIV-1 Tat protein on cell growth and viral transactivation. *J Virol* 1993;67:277–287. [PubMed: 8416373]
61. Chirmule N, Kahn S, Than S, Phawa S. HIV tat induces functional unresponsiveness in T cells. *J Virol* 1995;69:492–498. [PubMed: 7983746]
62. Westendorp M, Shatrov V, Schulze-Osthoff K, Frank R, Kraft M, Los M, Krammer P, Droge W, Lehmann V. HIV-1 tat potentiates TNF-induced NF- κ B activation and cytotoxicity by altering the cellular redox state. *EMBO J* 1995;14:546–554. [PubMed: 7859743]
63. Viscidi RP, Mayu K, Lederman HM, Frankel AD. Inhibition of antigen-specific lymphocyte proliferation by tat protein of HIV-1. *Science* 1989;246:1606–1608. [PubMed: 2556795]
64. Li DJ, Friedman DJ, Wang C, Metelev V, Pardee AB. Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. *Science* 1995;268:429–431. [PubMed: 7716549]

65. Purvis SF, Jacobberger JW, Sramkoski RM, Patki AH, Lederman MM. HIV-1 tat protein induces apoptosis and death in Jurkat T-cells. *AIDS Res Hum Retroviruses* 1995;11:443–450. [PubMed: 7632460]
66. Ho D, Neumann AU, Perlson AS, Chen W, Leonard JM, Markovitz M. Rapid turnover of plasma viruses and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;373:123–126. [PubMed: 7816094]
67. Guntheil WMS, Flentke GR, Sanford DG, Munoz E, Huber BT, Bachovchin WW. HIV-1 Tat binds to dipeptidyl aminopeptidase IV (CD26): a possible mechanism for Tat's immunosuppressive activity. *Proc Natl Acad Sci USA* 1994;91:6594–6598. [PubMed: 7912830]
68. Shasty K, Marin MC, Nehete PN, McConnel K, El-Naggar AK, McDonnell TJ. Expression of HIV-1 tat results in down-regulation of bcl-2 and induction of apoptosis in hematopoietic cells. *Oncogene* 1996;13:487–493. [PubMed: 8760290]
69. Zauli G, Previati M, Caramelli E, Bassinin A, Falcieri E, Givellini D, Berolaso L, Bosco D, Robot L, Capitani S. Exogenous HIV-1 Tat protein selectively stimulates a phosphoinositide-specific phospholipase C nuclear pathway in the Jurkat T cell line. *Eur J Immunol* 1995;25:2695–2700. [PubMed: 7589147]
70. Reinhold D, Wrenger S, Bank U, Buhling F, Hoffmann T, Neubert K, Kraft M, Frank R, Ansorge S. CD26 mediates the action of HIV-1 Tat protein on DNA synthesis and cytokine production in U937 cells. *Immunobiology* 1996;195:119–128. [PubMed: 8852605]
71. Wang Z, Morris GF, Reed JC, Kelly GD, Morris CB. Activation of Bcl-2 promoter-directed gene expression by the human immunodeficiency virus type-1 Tat protein. *Virology* 1999;257:502–510. [PubMed: 10329560]
72. Zagury JF, Chams V, Lachgar A, Caragno M, Rappaport J, Bizzini B, Burny A. *Cell Pharmacol AIDS Sci* 1996;3:123–128.
73. Zagury D. A naturally unbalanced combat. *Nature Med* 1997;3:156–157. [PubMed: 9018232]
74. Wu MX, Scholossman SF. Decreased ability of HIV-1 Tat protein-treated accessory cells to organize cellular clusters is associated with partial activation of T cells. *Proc Natl Acad Sci USA* 1997;94:13832–13837. [PubMed: 9391113]
75. Cafaro A, Caputo A, Fracasso C, Maggiorella MT, Goletti D, Baroncelli S, Pace M, Sernicola L, Koanga-Mogtomo ML, Betti M, Borsetti A, Belli R, Akerblom L, Corrias F, Butto S, Heeney J, Verani P, Titti F, Ensoli B. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. *Nature Med* 1999;5:643–650. [PubMed: 10371502]
76. Osterhaus AD, van Baalen CA, Gruters RA, Shcutten M, Siebelink CH, Hulskotte E, Tijhaar EJ, Randall RE, van Amerongen G, Fluechouse A. Vaccination with Rev and Tat against AIDS. *Vaccine* 1999;17:2713–2714. [PubMed: 10418922]
77. Robinson HL, Montefiori DC, Johnson RP, Manson KH, Kalish ML, Lifson JD, Rizvi TA, Lu S, Hu S-L, Mazzara GP, Panicali DL, Herndon JG, Glickman R, Candido MA, Lydy SL, Wyand MS, McClure HM. Immunodeficiency virus vaccine: neutralizing antibody-independent containment of serial challenges by subunit immunization. *Nature Med* 1999;5:526–534. [PubMed: 10229229]
78. Gundlach BR, Lewis MG, Sopper S, Schnell T, Sodroski J, Stahl-Hennig C, Uberla K. Related evidence for recombination of live, attenuated immunodeficiency virus vaccine with challenge virus to a more virulent strain. *J Virol* 2000;74:3537–3542. [PubMed: 10729127]
79. Sawai ET, Hamza MS, Ye M, Shaw KE, Luciw PA. Pathogenic conversion of live attenuated simian immunodeficiency virus vaccines is associated with expression of truncated Nef. *J Virol* 2000;74:2038–2045. [PubMed: 10644378]
80. Ui M, Kuwata T, Igarashi T, Miyazaki Y, Tamaru K, Shimada T, Nakamura M, Uesaka H, Yamamoto H, Hayami M. Protective immunity of gene-deleted SHIVs having an HIV-1 Env against challenge infection with a gene-intact SHIV. *J Med Primatol* 1999;28:242–248. [PubMed: 10593491]
81. Nixon DF, Donahoe SM, Kakimoto WM, Samuel RV, Metzner KJ, Gettie A, Hanke T, Marx PA, Connor RI. Simian immunodeficiency virus-specific cytotoxic T lymphocytes and protection against challenge in rhesus macaques immunized with a live attenuated simian immunodeficiency virus vaccine. *Virology* 2000;266:203–210. [PubMed: 10612675]
82. Gauduin MC, Glickman RL, Ahmad S, Yilma T, Johnson RP. Immunization with live attenuated simian immunodeficiency virus induces strong type 1 T helper responses and beta-chemokine production. *Proc Natl Acad Sci USA* 1999;96:14031–14036. [PubMed: 10570193]

83. Wyand MS, Manson K, Montefiori DC, Lifson JD, Johnson RP, Desrosiers RC. Protection by live, attenuated simian immunodeficiency virus against heterologous challenge. *J Virol* 1999;73:8356–8363. [PubMed: 10482586]
84. Kraiselburd EN, Torres JV. Properties of virus-like particles produced by SIV-chronically infected human cell clones. *Cell Mol Biol* 1995;41(Suppl 1):S41–52. [PubMed: 8574147]
85. Cicala C, Arthos J, Rubbert A, Selig S, Wildt K, Cohen OJ, Fauci AS. HIV-1 envelope induces activation of caspase-3 and cleavage of focal adhesion kinase in primary human CD4(+) T cells. *Proc Natl Acad Sci USA* 2000;97:1178–1183. [PubMed: 10655504]
86. Cohen SS, Li C, Ding L, Cao Y, Pardee AB, Shevach EM, Cohen DI. Pronounced acute immunosuppression in vivo mediated by HIV Tat challenge. *Proc Natl Acad Sci USA* 1999;96:10842–10847. [PubMed: 10485913]
87. Cohen EA, Terwilliger EF, Jalinoos Y, Proulx J, Sodroski JG, Haseltine WA. Identification of HIV-1 Vpr product and function. *J Acquir Immune Defic Syndr* 1990;1:11–18. [PubMed: 2136707]
88. Ogawa K, Shibata R, Kiyomasu T, Higuchi I, Kishida Y, Ishimoto A, Adachi A. Mutational analysis of the human immunodeficiency virus vpr open reading frame. *J Virol* 1989;63:4110–4114. [PubMed: 2474678]
89. Balliet JW, Kolson DL, Eiger G, Kim FM, McGann KA, Srinivasan A, Collman R. Distinct effects in primary macrophages and lymphocytes of the human immunodeficiency virus type 1 accessory genes vpr, vpu, and nef: mutational analysis of a primary HIV-1 isolate. *Virology* 1994;200:623–631. [PubMed: 8178448]
90. Lu YL, Bennett RP, Wills JW, Gorelick R, Ratner L. A leucine triplet repeat sequence (LXX)₄ in p6gag is important for vpr incorporation into human immunodeficiency virus type 1 particles. *J Virol* 1995;69:6873–6879. [PubMed: 7474102]
91. Mahalingam S, MacDonald B, Ugen KE, Ayyavoo V, Agadjanyan MG, Williams WV, Weiner DB. In vitro and in vivo tumor growth suppression by HIV-1 Vpr. *DNA Cell Biol* 1997;16:137–143. [PubMed: 9052734]
92. Yao X-J, Subbramanian RA, Rougeau N, Boisvert F, Bergeron D, Cohen EA. Mutagenic analysis of human immunodeficiency virus type 1 Vpr: role of a predicted N-terminal alpha-helical structure in vpr nuclear localization and virion incorporation. *J Virol* 1995;69:7032–7044. [PubMed: 7474123]
93. Paxton W, Connor RI, Landau NR. Incorporation of Vpr into human immunodeficiency virus type 1 virions: requirement for the p6 region of gag and mutational analysis. *J Virol* 1993;67:7229–7237. [PubMed: 8230445]
94. Vodicka MA, Koepf DM, Silver PA, Emerman M. HIV-1 Vpr interacts with the nuclear transport pathway to promote macrophage infection. *Genes Dev* 1998;12:175–185. [PubMed: 9436978]
95. Gallay P, Hope T, Chin D, Trono D. HIV-1 infection of nondividing cells throughout the recognition of integrase by the importin/karyopherin pathway. *Proc Natl Acad Sci USA* 1997;94:9825–9830. [PubMed: 9275210]
96. DiMarzio P, Choe S, Ebright M, Knoblauch R, Landau NR. Mutational analysis of cell cycle arrest, nuclear localization, and virion packaging of human immunodeficiency virus type 1 Vpr. *J Virol* 1995;69:7909–7916. [PubMed: 7494303]
97. Heinzinger NK, Bukrinsky MI, Haggerty SA, Ragland AM, Kewalramani V, Lee M-A, Gendelman HE, Ratner L, Stevenson M, Emerman M. The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proc Natl Acad Sci USA* 1994;91:7311–7315. [PubMed: 8041786]
98. Wang L, Mukherjee S, Jia F, Narayan O, Zhao LJ. Interaction of virion protein Vpr of human immunodeficiency virus type 1 with cellular transcription factor Sp1 and transactivation of viral long terminal repeat. *J Biol Chem* 1995;270:25564–25569. [PubMed: 7592727]
99. Connor RI, Chen BK, Choe S, Landau NR. 1995; Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology* 1995;206:935–944. [PubMed: 7531918]
100. Agostini I, Navarro JM, Rey F, Bouhamdan M, Spire B, Vigne R, Sire J. The human immunodeficiency virus type 1 Vpr transactivator: cooperation with promoter-bound activator domains and binding to TFIIB. *J Mol Biol* 1996;261:599–606. [PubMed: 8800208]

101. Stivahtis GL, Soares MA, Vodicka MA, Hahn BH, Emerman M. Conservation and host specificity of Vpr-mediated cell cycle arrest suggest a fundamental role in primate lentivirus evolution and biology. *J Virol* 1997;71:4331–4338. [PubMed: 9151821]
102. Re F, Braaten D, Franke EK, Luban J. Human immunodeficiency virus type 1 Vpr arrests the cell cycle in G2 by inhibiting the activation of p34cdc2-cyclin B. *J Virol* 1995;69:6859–6864. [PubMed: 7474100]
103. He J, Choe S, Walker R, Di Marzio P, Morgan DO, Landau NR. Human immunodeficiency virus type 1 viral protein R (Vpr) arrests cells in the G2 phase of the cell cycle by inhibiting p34cdc2 activity. *J Virol* 1995;69:6705–6711. [PubMed: 7474080]
104. Bartz SR, Rogel ME, Emerman M. Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control. *J Virol* 1996;70:2324–2331. [PubMed: 8642659]
105. Hattori N, Michaels F, Fargnoli K, Marcon L, Gallo RC, Franchini G. The human immunodeficiency virus type 2 vpr gene is essential for productive infection of human macrophages. *Proc Natl Acad Sci USA* 1990;87:8080–8084. [PubMed: 2236020]
106. Stewart SA, Poon B, Jowett JBM, Chen IS. Human immunodeficiency virus type 1 Vpr induces apoptosis following cell cycle arrest. *J Virol* 1997;71:5579–5592. [PubMed: 9188632]
107. Jowett JBM, Planeles V, Poon B, Shah NP, Chen ML, Chen ISY. The human immunodeficiency virus type 1 vpr gene arrests infected T-cells in the G2+M phase of the cell cycle. *J Virol* 1995;69:6304–6313. [PubMed: 7666531]
108. Levy DN, Fernandes LS, Williams WV, Weiner DB. Induction of cell differentiation by human immunodeficiency virus 1 vpr. *Cell* 1993;72:541–550. [PubMed: 8440020]
109. Rogel ME, Wu LI, Emerman M. The human immunodeficiency virus type 1 vpr gene prevents cell proliferation during chronic infection. *J Virol* 1995;69:882–888. [PubMed: 7815556]
110. Mansky LM, Preveral S, Selig L, Benarous R, Benichou S. The interaction of vpr with uracil DNA glycosylase modulates the human immunodeficiency virus type 1 in vivo mutation rate. *J Virol* 2000;74:7039–7047. [PubMed: 10888643]
111. Selig L, Benichou S, Rogel ME, Wu LI, Vodicka MA, Sire J, Benarous R, Emerman M. Uracil DNA glycosylase specifically interacts with Vpr of both human immunodeficiency virus type 1 and simian immunodeficiency virus of sooty mangabeys, but binding does not correlate with cell cycle arrest. *J Virol* 1997;71:4842–4846. [PubMed: 9151883]
112. Bouhamdan M, Benichou S, Rey F, Navarro JM, Agostini I, Spire B, Camonis J, Slupphaug G, Vigne R, Benarous R, Sire J. Human immunodeficiency virus type 1 Vpr protein binds to the uracil DNA glycosylase DNA repair enzyme. *J Virol* 1996;70:697–704. [PubMed: 8551605]
113. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 1995;270:1811–1815. [PubMed: 8525373]
114. Savill J. Apoptosis. Phagocytic docking without shocking. *Nature* 1998;392:442–443. [PubMed: 9548247]
115. Kinter A, Catanzaro A, Monaco J, Ruiz M, Justement J, Moir S, Arthos J, Oliva A, Ehler L, Mizell S, Jackson R, Ostrowski M, Hoxie J, Offord R, Fauci AS. CC-chemokines enhance the replication of T-tropic strains of HIV-1 in CD4⁺ T cells: role of signal transduction. *Proc Natl Acad Sci USA* 1998;95:11880–11885. [PubMed: 9751759]
116. Herbein G, Mahlknecht U, Batliwalla F, Gregersen P, Pappas T, Butler J, O'Brien WA, Verdin E. Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature* 1998;10:189–194. [PubMed: 9744279]
117. Herbein G, Van Lint C, Lovett JL, Verdin E. Distinct mechanisms trigger apoptosis in human immunodeficiency virus type 1-infected and in uninfected bystander T lymphocytes. *J Virol* 1998;72:660–670. [PubMed: 9420271]
118. Hesselgesser J, Taub D, Baskar P, Greenberg M, Hoxie J, Kolson DL, Horuk R. Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1 alpha is mediated by the chemokine receptor CXCR4. *Curr Biol* 1998;8:595–598. [PubMed: 9601645]
119. Algeciras A, Cockrell D, Lynch D, Paya C. CD4 regulates susceptibility to Fas ligand- and tumor necrosis factor-mediated apoptosis. *J Exp Med* 1998;187:711–720. [PubMed: 9480981]

120. Zheng L, Fisher G, Miller RE, Peschon J, Lynch DH, Lenardo MJ. Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature* 1995;377:348–351. [PubMed: 7566090]
121. Rosok JE. Correlates of apoptosis of CD4+ and CD8+ T cells in tonsillar tissue in HIV type 1 infection. *AIDS Res Human Retro* 1998;14:1635–1643.
122. Cicala C, Arthos J, Ruiz M, Vaccarezza M, Rubbert A, Riva A, Wildt K, Cohen O, Fauci AS. Induction of phosphorylation and intracellular association of CC chemokine receptor 5 and focal adhesion kinase in primary human CD4+ T cells by macrophage-tropic HIV envelope. *J Immunol* 1999;163:420–426. [PubMed: 10384144]
123. Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T-lymphocytes. *Nature* 1998;391:397–401. [PubMed: 9450757]
124. Lu X, Yu H, Liu S, Brodsky FM, Peterlin M. Interactions between HIV-1 Nef and vascular ATPase facilitate the internalization of CD4. *Immunity* 1998;8:647–656. [PubMed: 9620685]
125. Lama J, Mangasarian A, Trono D. Cell-surface expression of CD4 reduces HIV-1 infectivity by blocking Env incorporation in a Nef-dependent manner. *Cur Biol* 1999;9:622–631.
126. Ross TM, Oran AE, Cullen BR. Inhibition of HIV-1 progeny virion release by cell-surface CD4 is relieved by expression of the viral Nef protein. *Curr Biol* 1999;9:613–621. [PubMed: 10375525]
127. Aiken C, Konner J, Landau NR, Lenburg ME, Trono D. Nef induces CD4 endocytosis: requirement for a critical dileucine motif in the membrane-proximal CD4 cytoplasmic domain. *Cell* 1994;76:853–864. [PubMed: 8124721]
128. Alexander L, Du Z, Rosenzweig M, Jung JU, Desrosiers RC. A role for natural simian immunodeficiency virus and human immunodeficiency virus type 1 nef alleles in lymphocyte activation. *J Virol* 1997;71:6094–6099. [PubMed: 9223503]
129. Hua J, Blair W, Truant R, Cullen BR. Identification of regions in HIV-1 Nef required for efficient downregulation of cell surface CD4. *Virology* 1997;231:231–238. [PubMed: 9168885]
130. Garcia JV, Miller AD. Serine phosphorylation-independent downregulation of cell-surface CD4 by Nef. *Nature* 1991;350:1561–1568.
131. Murphy KM, Sweet RT, Ross IL, Hume DA. Effects of the tat and nef gene products of human immunodeficiency virus type 1 (HIV-1) on transcription controlled by the HIV-1 LTR and on cell growth in macrophages. *J Virol* 1993;67:6959–6964.
132. Baur AS, Sawai ET, Dazin P, Fantl WJ, Cheng-Mayer C. HIV-1 Nef leads to inhibition or activation of T cells depending on its intracellular localization. *Immunity* 1994;1:373–384. [PubMed: 7882168]
133. Okada H, Morikawa S, Tashiro M. HIV-1 Nef binding protein expressed on the surface of murine blood cells. *Med Microbiol Immunol* 1998;186:201–207. [PubMed: 9574903]
134. Xu X-N, Laffert B, Screaton GR, Kraft M, Wolf D, Kolanus W, Mongkolsapay J, McMichael AJ, Baur AS. Induction of Fas ligand expression by HIV involves the interaction of Nef with T cell receptor z chain. *J Exp Med* 1999;189:1489–1496. [PubMed: 10224289]
135. Hodge S, Novembre FJ, Whetter L, Gelbard HA, Dewhurst S. Induction of fas ligand expression by an acutely lethal simian immunodeficiency virus, SIVsmmPBj14. *Virology* 1998;252:354–363. [PubMed: 9878614]
136. Collette Y, Duarte H, Benziene A, Ramosmorales F, Benarous R, Harris M, Olive D. Physical and functional interaction of Nef with lck-HIV-1 Nef-induced T-cell signaling defects. *J Biol Chem* 1996;271:6333–6341. [PubMed: 8626429]
137. Greeway A, Azad A, McPhee D. Human immunodeficiency virus type 1 Nef protein inhibits activation pathways in peripheral blood mononuclear cells and T-cell lines. *J Virol* 1995;69:1842–1850. [PubMed: 7853525]
138. Sawai ET, Baur A, Struble H, Peterlin BM, Levy JA, Cheng-Mayer C. Human immunodeficiency virus type 1 Nef associates with cellular serine kinase in T lymphocytes. *Proc Natl Acad Sci USA* 1994;91:1539–1543. [PubMed: 8108442]
139. Anel A, Buferne M, Boyer C, Schitt VA, Golstein P. T cell receptor-induced Fas ligand expression in cytotoxic T lymphocyte clones is blocked by protein tyrosine kinase inhibitors and cyclosporin A. *Eur J Immunol* 1994;24:2469–2474. [PubMed: 7523141]

140. Hodge DR, Dunn KJ, Pei GK, Chakrabarty MK, Heidecker G, Lautenberger JA, Samuel KP. Binding of c-Raf1 kinase to a conserved acidic sequence within the carboxyl-terminal region of the HIV-1 Nef protein. *J Biol Chem* 1998;273:15727–15733. [PubMed: 9624170]
141. Van AL, D'Souza SC. Rho GTPases and signaling networks. *Genes Dev* 1997;11:2295–2322. [PubMed: 9308960]
142. Margottin F, Bour SP, Durand H, Selig L, Benichou S, Richard V. A novel human WD protein, hBrCP, that interact with HIV-1 Vpu connects CD4 to the ER degradation pathway throughout an F-box motif. *Mol Cell* 1998;1:565–574. [PubMed: 9660940]
143. Bour S, Strebel K. The human immunodeficiency virus (HIV) type 2 envelope protein is a functional complement to HIV type 1 Vpu that enhances particle release of heterologous retroviruses. *J Virol* 1996;70:8285–8300. [PubMed: 8970948]
144. Strebel K, Kimball T, Maldarelli F, Martin MA. A novel gene of HIV-1 vpu, and its 16 kilodalton product. *Science* 1988;241:1221–1223. [PubMed: 3261888]
145. Edwart GD, Sutherland T, Gage PW, Cox GB. The Vpu protein of human immunodeficiency virus type 1 forms ion cation-selective ion channels. *J Virol* 1996;70:7108–7115. [PubMed: 8794357]
146. Schubert U, Ferer-Montiel AV, Oblatt-Montal M, Henklien P, Strebel K, Montal M. Identification of an ion channel activity of the Vpu transmembrane domain and its involvement in the regulation of virus release from HIV-1 infected cells. *FEBS Lett* 1996;398:12–18. [PubMed: 8946945]
147. Bortner CD, Hughers FM, Cidlowski JA. A primary role for K⁺ and Na⁺ efflux in the activation of apoptosis. *J Biol Chem* 1997;272:32436–32442. [PubMed: 9405453]
148. D'Mello SR, Aglieco R, Roberts MR, Brodezt K, Haycock JW. A DEVD-inhibited caspase other than CPP32 is involved in the commitment of cerebellar granule neurons to apoptosis induced by K⁺ deprivation. *J Neurosci* 1998;70:1809–1818.
149. Szabo I, Bulbins E, Apfel H, Zhang X, Barth P, Busch AE, Schlottman K, Pongs O, Lang F. Tyrosine phosphorylation-dependent suppression of a voltage-gated K⁺ channel in T-lymphocytes upon Fas stimulation. *J Biol Chem* 1996;271:20465–20469. [PubMed: 8702786]
150. Minn AJ, Velez SL, Schendel SL, Linag H, Muchmore SW, Feisk SW, Fill M, Thmposon CB. Bcl-XL forms an ion channel in synthetic lipid membranes. *Nature* 1997;385:353–357. [PubMed: 9002522]
151. Schendel S, Xie Z, Montal MO, Matsuyman S, Montal M, Reed JC. Channel formation by antiapoptotic protein Bcl-2. *Proc Natl Acad Sci USA* 1997;94:5113–5118. [PubMed: 9144199]
152. Antonasson R, Conti F, Clavatta A, Montessuit S, Lewis S, Marinou I, Barnasconi I, Bernard A, Mermod J-J, Mazzei G, Maundrell K, Gambale F, Sadoui R, Martinou J-C. Inhibition of Bax channel-forming activity by Bcl-2. *Science* 1997;277:370–372. [PubMed: 9219694]
153. Stephens EB, Mukherjee S, Sahni M, Zhuge W, Raghavan R, Singh DK, Leung K, Atkinson B, Li A, Joag SV, Liu ZQ, Narayan O. A cell-free stock of simian-human immunodeficiency virus that causes AIDS in pig-tailed macaques has limited number of amino acid substitutions in both SIVmac and HIV-1 regions of the genome and has altered cytotropism. *Virology* 1997;231:313–321. [PubMed: 9168893]

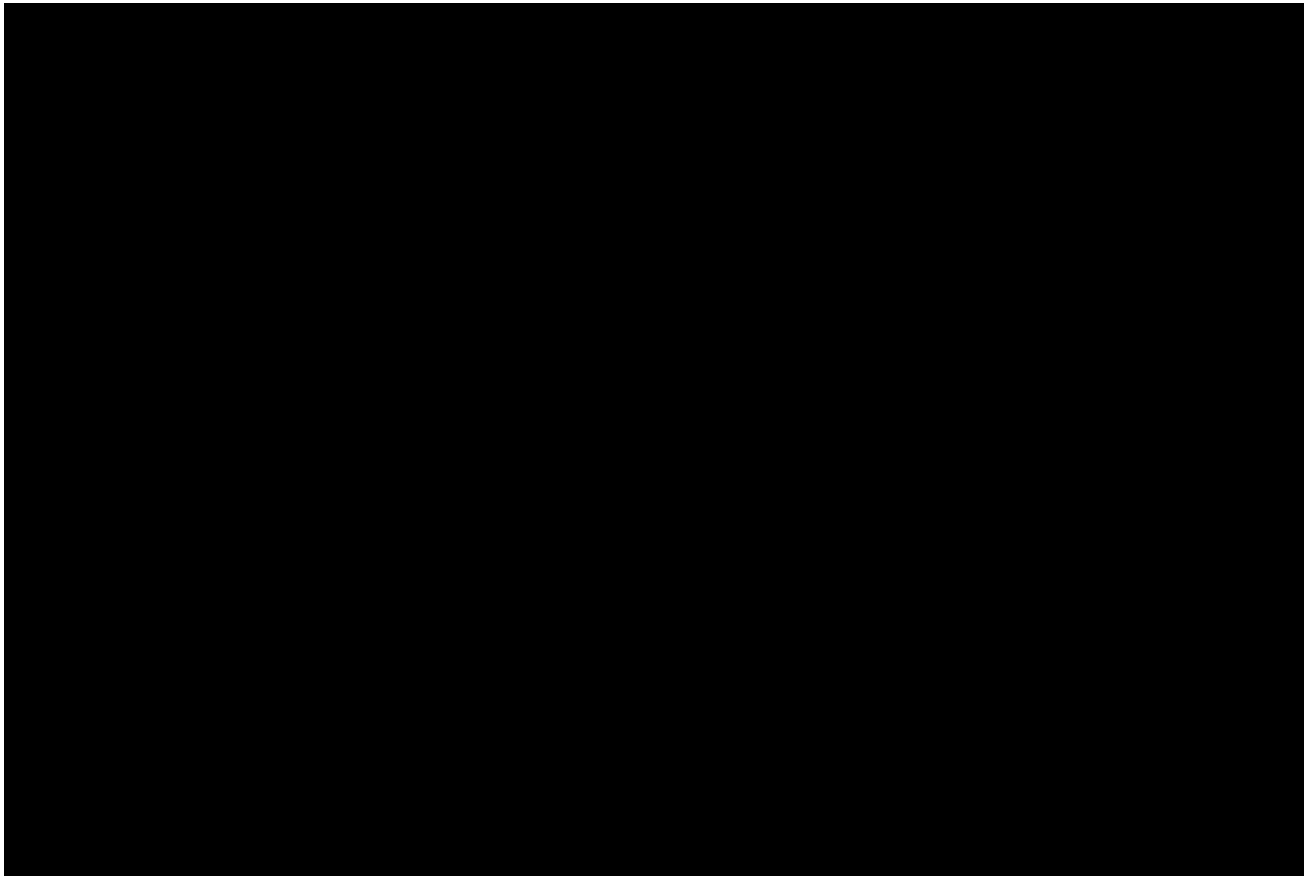


Figure 1.

Model describing mechanisms of HIV modulation of apoptosis. HIV virions bind to cell surface CD4 and a coreceptor (primarily CCR5 or CXCR4) via Env initiating fusion with the cell membrane and releasing the virus-containing capsid into the cell cytoplasm. After reverse transcription process, the provirus is translocated to the nucleus and integrates into the host genome. Transcription of viral mRNA leads to the production of viral gene products. (1) Nef is expressed early in the life cycle and associates with the cell membrane where it interacts with a variety of proteins including p56lck. Nef interaction with the ζ chain of the TCR results in the up-regulation of Fas-L expression. (2) Fas-L expression can protect infected cells from cytotoxic T cell lymphocyte (CTL) killing by destroying immune cells expressing Fas. (3) Nef can down-regulate the expression of cell surface receptors CD4 and MHC class I and (4) protect the infected cells from recognition by CTLs. (5) Vpu can also prevent the cell surface expression of CD4 and therefore also protect infected cells from killing by CTLs. (6) In contrast to Nef and Vpu, Vpr expression can induce apoptosis by the induction of cell arrest at the G₂ phase of the cell cycle in HIV-infected cells. (7) Tat is among the first viral gene products to be expressed after proviral integration. Tat increases the expression of IL-2 (a T cell growth factor) and Bcl-2 (an anti-apoptotic protein), both of which protect infected cells from the induction of apoptosis. (8) However, expression of an exogenous, soluble form of Tat (sTat) induces apoptosis in many bystander cells. Interacting with cell surface receptors on T cells and macrophages, sTat can induce bystander cells to undergo apoptosis by initiating the expression of Fas, Fas-L, and TNF- α . In addition, sTat can down-regulate the expression of Bcl-2. Both TNF- α /TNFRII and Fas/Fas-L interactions can induce apoptosis in T cells by activation of caspases and the upregulation of Fas and Fas-L expression. (9) Progeny virion release can also induce apoptosis in bystander cells. The interaction of Env with cell receptors

initiates CD4 and CXCR4 cell signaling pathways and subsequently activates various caspases leading to cell death.

Table 1

HIV gene products involved in modulation of apoptosis

<i>HIV protein</i>	<i>Mechanisms of action</i>	<i>Effect on apoptosis</i>
Env	Signal activation of CD4	Enhancement
	Signal activation of chemokine receptor	Enhancement
	Syncytium formation	Enhancement
Nef	Activation of Fas-L expression	Enhancement
	Down-regulation of CD4 and MHC I	Inhibition
Tat	Signal transduction in Fas-dependent manner	Enhancement
	Activation of cyclin-dependent kinases	Enhancement
	Increases in Bcl-2 expression	Inhibition
Vpr	Increases in IL-2 expression	Inhibition
	Induction of cell cycle G ₂ arrest	Enhancement
Vpu	Activation of IκB	Inhibition
	Increases in ion channel activity	Enhancement
	Interactions with Fas	Enhancement
	Down-regulation of CD4	Inhibition