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Review

Mechanisms of HIV-associated lymphocyte apoptosis: 2010

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The inevitable decline of CD4T cells in untreated infection with the Human immunodeficiency virus (HIV) is due in large part to apoptosis, one type of programmed cell death. There is accumulating evidence that the accelerated apoptosis of CD4T cells in HIV infection is multifactorial, with direct viral cytotoxicity, signaling events triggered by viral proteins and aberrant immune activation adding to normal immune defense mechanisms to contribute to this phenomenon. Current antiviral treatment strategies generally lead to reduced apoptosis, but this approach may come at the cost of preserving latent viral reservoirs. It is the purpose of this review to provide an update on the current understanding of the role and mechanisms of accelerated apoptosis of T cells in the immunopathogenesis of HIV infection, and to highlight potential ways in which this seemingly deleterious process could be harnessed to not just control, but treat HIV infection.
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The chronic gradual loss of CD4 + T cells in untreated human immunodeficiency virus (HIV) infection, and the consequent adverse effects on both innate and adaptive immunity, lead to the opportunistic infections and malignancies characteristic of acquired immunodeficiency syndrome (AIDS). Loss of a cell type can occur by one of the three mechanisms: (1) decreased production; (2) increased destruction; or (3) redistribution (Figure 1a). In viral infections, increased destruction can occur by direct cytotoxicity of infected cells, programmed cell death (either apoptotic or non-apoptotic) triggered in infected cells, or programmed cell death in uninfected, so called 'bystander', cells triggered by soluble or membrane-bound viral or host immune factors. In fact, all of these mechanisms likely contribute to HIV immunopathogenesis (Figure 1b); however, the relative contribution of each mechanism in clinical HIV infection remains unclear.

There is controversy regarding the effect of HIV infection on thymic output, because a reliable measure of thymic output is lacking, and a full discussion of the controversy is beyond the scope of this review. Early studies indicated decreased T-cell production in the thymus in HIV infection due to a combination of direct cytopathicity of HIV-infected thymocyte precursors and apoptosis of uninfected immature thymocytes. This manifests as thymic atrophy, decreased circulating naïve CD4T cells, and decreased T-cell receptor rearrangement excision circles (TRECs) in circulating T cells in HIV infection. TREC content is inversely correlated with HIV viral load, and after initiation of effective antiretroviral therapy returns to levels comparable with uninfected controls.[1] The usefulness of TREC content to quantify thymic output has been questioned, as mathematical models suggest that either division or death of naïve T cells would artificially lower measured TREC content in the absence of decreased thymic output. However, by examining the ratio of late TRECs to early TRECs in peripheral T cells as a marker of intrathymic proliferation, reduced intrathymic proliferation, and thus thymic output, is still evident in HIV infection compared with uninfected controls.[2] HIV also infects and induces apoptosis of CD34 + multipotent hematopoietic progenitor cells, thereby potentially decreasing progenitor cell input into the thymus.

Apoptosis of circulating CD4T cells has not been consistently found to correlate with HIV viral load.[3] This suggests several possibilities: (1) not all of the CD4T-cell apoptosis is driven by active viral replication or the immune response to such; or (2) the circulating CD4T cells, although the easiest to quantify, are not necessarily the most physiologically relevant compartment to gauge functional CD4T-cell loss. Both of these possibilities are supported by one early investigation of apoptosis in lymph nodes of HIV-infected persons that showed increased apoptosis compared with uninfected persons; and in that study, the majority of the apoptotic cells, defined by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining positivity, were not demonstrated to be infected.[4] However, some have questioned

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Abbreviations: AICD, activation-induced cell death; AIDS, acquired immunodeficiency syndrome; DR, death receptor; HIV, human immunodeficiency virus; IFN, interferon; LTNP, long-term non-progressor; LTR, long terminal repeat; NK, natural killer; PBL, peripheral blood lymphocyte; PBMC, peripheral blood mononuclear cell; SIV, simian immunodeficiency virus; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TREC, T-cell receptor excision circle; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling
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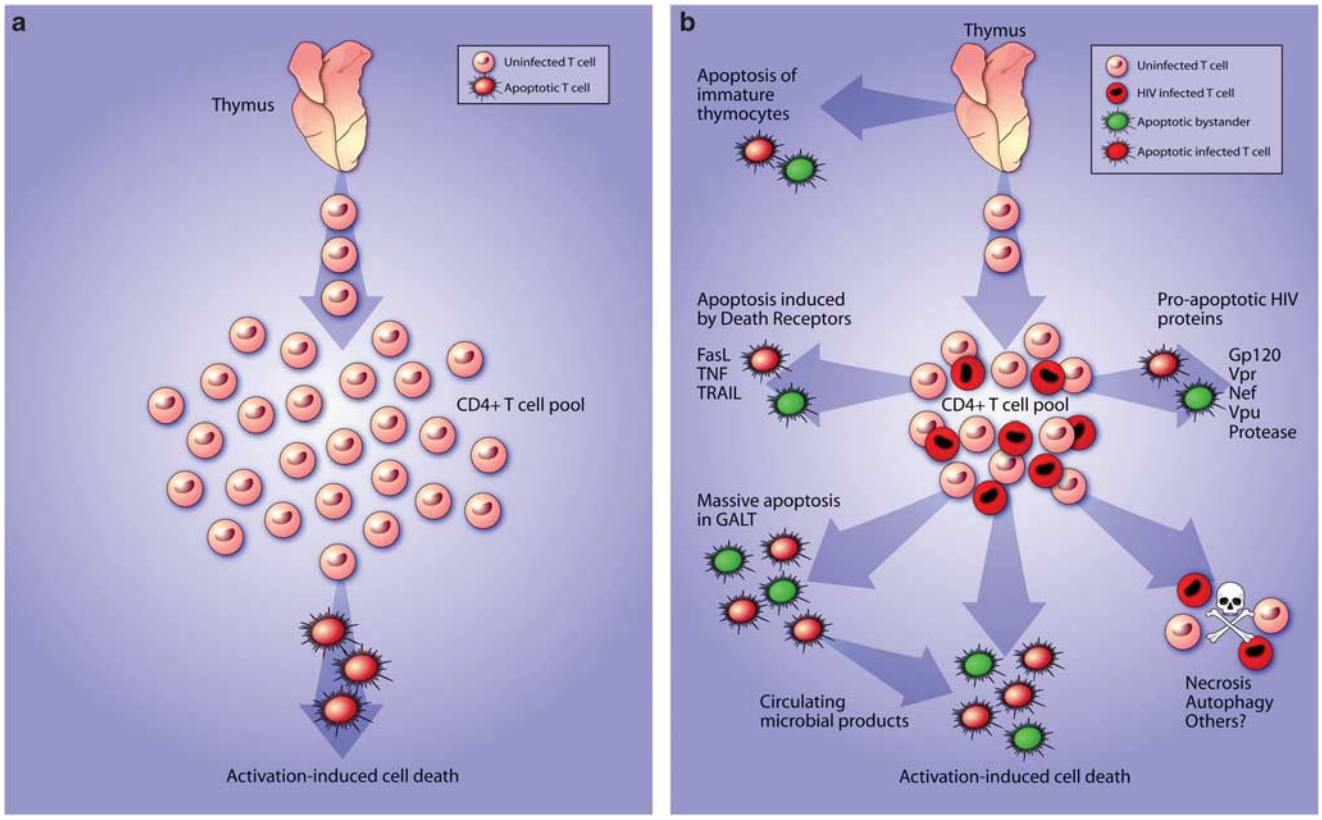

Figure 1 (**a**) Simplified diagram of normal CD4 + T-cell homeostasis. The peripheral CD4 + T-cell pool is maintained through a balance of thymopoiesis and activation-induced cell death. (**b**) Mechanisms of CD4 + T-cell death in untreated HIV infection. The peripheral CD4 + T-cell pool is depleted through decreased thymopoiesis and excessive apoptosis through multiple HIV viral-specific and non-specific mechanisms

the methods used to determine infection in that study. Specifically, infected cells were defined by *in situ* hybridization of riboprobes more than 2 kbp in length, which are likely to be degraded in TUNEL-positive cells. The accelerated apoptosis in HIV-infected lymph nodes affects all functional compartments of the node, and is not limited to just CD4T cells, but also affects CD8 + T cells and B cells, supporting the argument for both viral and non-viral factors in enhancing apoptosis. The importance of apoptosis of CD4T cells in gut-associated lymphoid tissue will be discussed below.

Redistribution, with CD4T-cell sequestration in secondary lymphoid organs, may play an important role in CD4T-cell decline in HIV infection. Resting CD4T cells, which are not permissive of HIV infection, exposed to HIV upregulate CD62L surface expression, potentially leading to accumulation in lymph nodes and exposure to proapoptotic signals.[5] CD4 + CD25 + FoxP3 + regulatory T cells when exposed to HIV upregulate CD62L, possibly contributing to immunosuppression by homing to lymph nodes. Increased splenic sequestration and apoptotic death of a subset of memory T cells expressing CCR6 has been demonstrated in progressive HIV disease.[6]

Correlations between Retroviral-induced Apoptosis and Immunodeficiency

In an attempt to discern the how and why of apoptosis in HIV disease, one must begin with the origin of the virus itself. HIV infection is the result of episodes of cross-species, zoonotic transmission of simian immunodeficiency viruses (SIVs) from African non-human primates. In natural infection of non-human primates, such as sooty mangabeys, with their species-specific strain of SIV, progressive CD4T-cell declines because of apoptosis, and manifestations of secondary immunodeficiency usually do not occur despite often robust and chronic viral replication.[7] However, non-human primate models of infection in which a strain of SIV is introduced into a non-natural host often result in CD4T-cell decline and AIDS-like manifestations. Recent evidence, though, suggests that SIVcpz infection of chimpanzees in the wild is indeed pathogenically associated with increased mortality and CD4T-cell loss, and may be an exception to this general rule. The role of apoptosis in SIV disease has been reviewed extensively elsewhere.[8] Pandrea *et al.* have offered a comprehensive model to explain why natural SIV infection does not generally lead to disease progression, whereas pathogenic HIV and SIV infections do. They propose that the presence of acute and chronic inflammatory states, loss of enteric CD4T cells, and increased T-cell apoptosis and proliferation that occur in pathogenic SIV infection are the determining factors in disease progression.[8] This model is supported by recent observations that pathogenic lentiviral infections are associated with chronic-sustained immune activation and expression of interferon (IFN)-responsive genes, including tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), whereas non-pathogenic infections

are not.[9] Further differences between HIV infection and non-pathogenic SIV infection, as they relate to mechanisms of CD4T-cell apoptosis, are discussed below.

It has long been recognized that some humans infected with HIV, much like non-human primates with natural SIV infection, do not develop progressive CD4T-cell decline and progressive disease despite ongoing viral replication, so called long-term non-progressors (LTNPs). Rates of *in vitro* spontaneous apoptosis of CD4T cells in LTNPs are less than in patients with progressive disease, and approximate those in uninfected controls. Inconsistent results, however, have been obtained with mitogen-induced apoptosis. Some clues to why there is decreased apoptosis in LTNPs compared with progressors include: decreased T-cell Fas sensitivity;[10] higher frequency of infection with virus with a Vpr R77Q mutation;[11] and decreased expression of IFNα, TRAIL, and death receptor 5 (DR5) in lymphoid tissues.[12]

It is possible that apoptosis affects the four subtypes of CD4T cells to different degrees in HIV infection, and that this differential susceptibility could contribute to the immunodeficiency associated with infection; however, this has not been definitively studied. It was recognized early that HIV infection *in vivo* is associated with an abnormal shift toward a predominately Th2 phenotype. Although both Th1 and Th2 cells are susceptible to Fas-mediating apoptosis,[13] one of the key players in HIV infection discussed below, Th1 cells when compared with Th2 cells, are more likely to be productively infected, and are more susceptible to activation-induced cell death.[13] On the other hand, CD4 + CD25 + FoxP3 + regulatory T cells, when exposed to HIV *in vitro*, do not undergo apoptosis.[14] Furthermore, in SIV-infected rhesus macaques, mucosal regulatory T cells have lower apoptosis-related gene expression than non-regulatory T cells and are spared from SIV-mediated cell death.[15] Circulating and mucosal Th17 cells are decreased in HIV-infected patients compared with uninfected controls,[16] whereas SIV-infected sooty mangabeys maintain normal levels of Th17 cells.[16] Notably, Th17 cells from HIV-infected patients are similarly susceptible to activation-induced cell death (AICD) as Th1 cells.[16] Despite these suggestive lines of evidence, no single study to date has compared markers of apoptosis across the four subtypes of CD4T cells in HIV-infected patients.

Mediators of Apoptosis in HIV Disease

Apoptosis may be stimulated by environmental stress, toxins, removal of growth factors, or by one of the three death-inducing ligands – tumor necrosis factor, FasL and TRAIL. The roles of each of these mediators in HIV disease, and their potential for targeted immunotherapy, will be discussed briefly below.

Fas/FasL. The role of Fas/FasL interactions in the immunopathology of HIV infection has been studied extensively and reviewed elsewhere.[17] In brief, both soluble and membrane-bound Fas and FasL levels are elevated in HIV-infected patients compared with uninfected patients and correlate with disease progression.[18] In HIV-infected patients, Fas expression is increased in CD4 and CD8-positive T cells and B cells, and FasL expression is increased on monocytes, macrophages and natural killer (NK) cells, both in the peripheral circulation and the lymph nodes.[19] Microarray analysis of gene expression in lymph nodes from HIV-infected patients confirms increased Fas/FasL.[20]

HIV-infected cells are more susceptible to Fas-mediated apoptosis *in vitro* compared with uninfected cells, but they do not make up the majority of apoptotic cells *in vivo*,[21] and the majority of circulating apoptotic peripheral blood mononuclear cells (PBMCs) from HIV-infected patients do not express Fas.[22] HIV-infected macrophages are able to induce apoptosis in T cells from HIV-infected donors, but not from HIV-uninfected donors, *in vitro* through Fas/FasL.[21] These observations contribute to the 'bystander effect' hypothesis that proposes that most of the apoptotic cell death occurring in HIV infection involves uninfected cells responding to infection in lymphoid tissues.

Interestingly, T cells from chimpanzees infected with HIV do not undergo apoptosis through Fas ligation.[23] However, cynomolgus monkeys infected with pathogenic SIV/HIV-C2/1 have increased expression of Fas on CD4 and CD8T cells, and FasL on T and B cells compared to before infection.[24] Non-progressing patients have significantly lower serum-soluble Fas concentrations, decreased lymphocyte expression of Fas and FasL, and decreased Fas-sensitivity[10] than progressing patients. How the HIV virus influences Fas/FasL expression will be discussed below. Inhibiting the Fas pathway with a blocking monoclonal antibody to FasL during the acute phase of SIV infection in macaques attenuated disease progression in one study.[25] There have been no human trials of Fas/FasL agonists or antagonists in the treatment of HIV infection to date because of significant toxicities in pre-clinical studies.

Tumor necrosis factor-α. The important role of TNFα in the pathogenesis of HIV infection and its associated complications, particularly enhancing viral replication and mediating apoptosis of CD4T cells, has been studied extensively and recently reviewed elsewhere.[26] Table 1 summarizes the results of the prospective trials performed with the TNFα inhibitors, pentoxifylline, ketotifen, thalidomide and etanercept. Overall, no significant beneficial immunologic effect has been demonstrated with specific inhibition of TNFα; and several of the agents have significant adverse effects, including a paradoxical increase of the HIV viral load.

On the other hand, recombinant TNFα has been investigated in preclinical and phase I/II trials with the goal of clearing latently infected cells, but is unlikely to be a clinically useful option because of significant-related toxicities.[27]

TRAIL. TRAIL is a member of the TNF superfamily that has been implicated in mediating apoptosis of CD4T cells in HIV infection through its interactions with its death-inducing receptors, DR4 and DR5, on infected and uninfected T cells. HIV infection of CD4T cells results in increased expression of TRAIL and DR5 compared with uninfected cells.[28] HIV infection of dendritic cells and macrophages results in increased expression of TRAIL, which can then induce apoptosis in uninfected bystander T cells.[29]

HIV-infected patients have elevated serum levels of TRAIL[28] and increased expression of DR5 on circulating

Table 1 Studies on modulation of death-receptor-mediated apoptosis in HIV infection

Agent	Mechanism of action	Dose	Clinical outcomes assessed	Clinical effects
Clinical studies on TNF inhibitors				
Pentoxifylline	Antagonist – blocks TNFα-induced activation of NF-κB	400 mg TID orally for 8 weeks	TNF expression and HIV viral load	Decreased TNF expression; no effect on HIV replication
		400 mg TID orally for 12 weeks	TNF expression and HIV viral load	Decreased TNF and HIV viral load in AZT- and PTX-treated patients compared with either agent or alone
		800 mg TID orally for 8 weeks	TNF expression and HIV-viral load	Decreased TNF expression; no effect on HIV replication
		800 mg TID orally for 6 weeks	TNF expression; cellular immune responses; fever, weight, fatigue, and well-being.	No effects. Increased GI side effects.
		400 mg TID orally for 16 weeks	CD4 count; mitogen-stimulated cytokine production; HIV-viral load	Transient improvements in CD4 count, viral load, and cytokine production.
		1.5mg/min intravenously for 6 h	Dose tolerance and ex vivo LPS-induced TNF production	No effect on TNFα production at maximally tolerated dose
		400 mg TID orally for 24 weeks	Caspases 1 and 8 levels in blood	Decreased caspases 1 and 8 levels
		400 mg TID orally for 6–20 months	Symptoms and CD4 counts	Improved symptoms and weight; transient increase in CD4 count
Ketotifen	Antagonist – inhibits TNFα release from PBMCs	4 mg daily orally for 84 days	Body composition; TNFα release from PBMCs and serum concentrations	Transient weight gain; inhibited TNFα release from stimulated PBMCs but no difference in serum levels
Thalidomide	Antagonist – decreases TNFα expression	100 mg QID orally for 12 weeks	Weight gain, CD4 count, and viral load	Improved weight gain, no change in viral load, or CD4 count
		200 mg once daily orally for 4 weeks	Oral ulcer resolution, QOL, plasma TNFα, and TNFα receptors, HIV-viral load	Increased oral ulcer resolution; unexpected increases in plasma TNFα, soluble TNFα receptors, and HIV-viral load
		100 mg daily orally for 24 weeks	CD4 count, TNFα, and TNFα receptor levels	No significant clinical effects noted
		200 mg daily orally for 4 weeks	Immune activation, TNFα levels and HIV-viral load	No effect on TNFα; increase in HIV viral-load and immune activation
Etanercept	Antagonist – soluble p75 TNF receptor: Fc fusion protein	10 mg intravenous infusion once in combination with HAART and rIL2	HIV-viral load, serum levels of proinflammatory cytokines	No changes in already suppressed TNF and viral load; decrease in IL6 and CRP levels
		25 mg intravenously twice weekly for 4 weeks	Clinical response to antituberculous therapy, CD4 count, and viral load	Non-significant trend in improved responses to antituberculous therapy and improvements in CD4 count without change in HIV-viral load
Preclinical studies on fas inhibitors				
Monoclonal antibody to FasL	Antagonist – blocks Fas/FasL interaction	4mg/kg intravenously one week before, at the time of, and 1, 2, and 3 weeks after acute SIVmac infection	B and T-cell death, cytotoxic T lymphocyte and antibody responses, viral set point	Attenuated acute SIVmac disease and improved survival
In vitro studies on TRAIL				
Leucine-zipper recombinant human TRAIL	Agonist	1 μg/ml for 12 h	Viral RNA, proviral DNA, and p24 antigen production in PBMCs from HIV-infected patients treated *ex vivo*	Increased apoptosis and decreased viral RNA, proviral DNA, and p24 antigen production
Recombinant human TRAIL	Agonist	5 ng/mL	Recoverable virus from PBMCs from HIV-infected, -suppressed patients treated ex vivo with TRAIL	Decreased recoverable virus from latently infected PBMCs
Mapatumumab, Lexatumumab	Monoclonal agonistic antibodies to TRAIL receptors	3 μg/mL	Apoptosis of PBLs from HIV-infected patients treated *ex vivo*	No effect on apoptosis in *ex vivo* PBMCs from HIV-infected patients.
Monoclonal antibody to TRAIL	Antagonist	1 mg intraperitoneally 9 days after HIV infection	Apoptosis of CD4 T cells in human PBL-transplanted NOD-SCID	Decreased CD4 T-cell apoptosis.

Abbreviations: AZT, azidothymidine; CRP, C-reactive protein; GI, gastrointestinal; HIV, human immunodeficiency virus; NOD-SCID, non-obese-severe-combined immunodeficiency mice; PBLs, peripheral blood lymphocytes; PBMCs, peripheral blood mononuclear cells; PTX, pentoxifylline; QID, four times daily; QOL, quality of life; SIV, simian immunodeficiency virus; TID, three times daily; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand

PBMCs compared with uninfected patients. Plasmacytoid dendritic cells from HIV-infected, viremic patients express TRAIL and are able to induce apoptosis in uninfected but not infected CD4T lymphocytes. HIV-infected patients also demonstrate elevated TRAIL and DR5 in lymphoid tissues.[30] Initiation of HAART in infected patients decreases serum TRAIL levels, as well as TRAIL and DR5 expression on circulating CD4T cells.[28,30] However, in lymphoid tissues, infected patients on HAART exhibit decreased TRAIL expression, but not DR5 expression, compared with untreated infected patients.[30] In a mouse model of HIV infection using human peripheral blood lymphocyte (PBL)-transplanted non-obese diabetic severe-combined immunodeficiency mice, treatment with neutralizing anti-TRAIL monoclonal antibody decreased CD4T-cell apoptosis compared with untreated infected animals.[31]

Treatment of HIV-infected PBLs and monocyte-derived macrophages with recombinant TRAIL results in decreased HIV burden compared with control-treated cells *in vitro*, suggesting this might be an approach for therapy.[32] Recombinant human TRAIL and agonistic monoclonal antibodies to DR4 and DR5 are currently in phases I and II clinical trials for cancer chemotherapy where induction of apoptosis in malignant cells is the goal of the therapy. A theoretical concern of TRAIL agonist therapy in HIV disease is that although HIV-infected cells may be killed by such therapy, uninfected bystander T cells may also undergo apoptosis, thereby, worsening immune suppression. However, treatment of PBLs from HIV-infected patients *in vitro* with recombinant human TRAIL decreases recoverable virus without a detectable change in quantity or function of the lymphocytes.[33]

Chronic immunologic stimulation in HIV disease. After responding to any neoantigen, contraction of previously expanding immune cell populations is necessary; one mechanism that is used is the induction of apoptosis of activated immune cells – activation induced cell death (AICD). Widespread and chronic activation of the immune system during HIV infection is characterized by generalized lymphadenopathy, increased circulating levels of B lymphocytes, activated T lymphocytes, NK cells, antigen-presenting cells, hypergammaglobulinemia and other serum markers of immune activation in HIV-infected patients compared with uninfected patients. The contribution of chronic immunologic stimulation to HIV pathogenesis is supported by observations of circulating activated (HLA-DR$+$) monocytes and activated (CD38$+$) CD8T cells correlating with CD4T-cell loss and disease progression.[34] Chronic immunologic stimulation leads to AICD in CD4T cells via both death receptor (Fas)-dependent and -independent mechanisms. However, the increased AICD seen in HIV disease is not limited to CD4T cells, as it is hypothesized to contribute to CD8T-cell exhaustion as well, a process that may be associated with the expression of programmed death-1 (PD-1) on activated CD8T cells in HIV-infected patients.[35]

The sources of chronic immunologic stimulation in HIV are multiple and can include persistent viral replication, effects of circulating HIV proteins and virus like particles, opportunistic infections, and reactivation of other latent viral infections. Also, absolute counts of regulatory T cells decrease over time in progressive HIV infection, and this correlates with immune activation.[36] Recent attention has focused on the depletion of CD4T cells in the gastrointestinal tract, leading to increased microbial translocation and circulation of microbial cellular components, including lipopolysaccharide and bacterial DNA, the levels of which correlate with HIV disease progression.[37] Systemic exposure to microbial by-products could lead to activation through Toll-like receptor signaling, thereby promoting AICD and contributing to the loss of CD4T cells outside of the GI tract.[38] Systemic microbial by-products may also inhibit T-cell expansion and function by upregulating PD-1 expression and IL-10 production by monocytes.[39]

Pharmacological attempts at modulating immune activation in HIV disease with the goal of increasing CD4T cells counts with corticosteroids, cyclosporine, cyclooxygenase 2 inhibitors, mycophenolate mofetil and chloroquine have either been unsuccessful or demonstrated only modest benefits in short-term surrogate endpoints.

HIV proteins and apoptosis. Many of the proteins that are encoded by the HIV genome, including gp120, Tat, Nef, Vpr, Vpu and HIV protease, have been found to have pro- and/or antiapoptotic qualities (listed in Table 2 and depicted in Figure 2). Each of these proteins will be reviewed below. It is important to note that the actual *in vivo* concentrations of these proteins seen in HIV infection are largely unknown, and that *in vitro* experiments involving overexpression of a particular protein, or exogenous treatment with high protein concentrations, may not be truly reflective of *in vivo* effects.

Gp120 and apoptosis. Gp120 is the glycoprotein expressed on the HIV envelope that binds to the CD4 receptor and either CXCR4 or CCR5 coreceptors facilitating viral attachment and, along with Gp41, entry into the cell. Both membrane-bound and soluble gp120 binding to CD4 leads to apoptosis of infected and uninfected CD4T cells. However, ligation with CD4 is not required, and of the coreceptors, gp120 signaling through CXCR4 is a more potent apoptotic stimulus than CCR5. Several mechanisms have been proposed for gp120's proapoptotic effect, including through upregulation of Fas, FasL, and TNFα expression;[40] molecular mimicry with Fas;[41] upregulation of TRAIL receptors DR4 and DR5;[42] induction of cell cycle arrest at the G2 phase;[43] generation of reactive oxygen intermediates;[44] reduced expression of Bcl-2;[45] phosphorylation of mTOR and p53;[46] increased expression of the proapoptotic protein PUMA;[47] and activation of p38.[48] Membrane-bound Gp120 may also induce apoptosis through syncytia formation, although the role of syncytia formation in *in vivo* infection is controversial.

Although it is currently unclear which of these potential mechanisms predominates in *in vivo* HIV infection, it is clear that Gp120 is pluripotent, able to induce apoptosis in other types of cells, including CD8T cells, neurons, human vascular endothelial cells, cardiomyocytes, proximal renal tubular cells, hepatocytes, oral keratinocytes, lung endothelial cells, breast cancer cells, osteoblasts, and prostate cancer cells.

Tat and apoptosis. The HIV-1 transactivator protein, Tat, that promotes HIV-LTR (long terminal repeat) transcription

Table 2 HIV encoded proteins and their reported pro- and antiapoptotic impact

Protein	Pro- or antiapoptotic	Reported mechanisms
Gp120	Proapoptotic	Molecular mimicry with Fas Upregulation of Fas, FasL, and TNFα expression G2 cell cycle arrest Generation of reactive oxygen species Downregulation of Bcl-2 expression Phosphorylation of mTOR and p53 Upregulation of PUMA expression Upregulation of TRAIL-R1 and -R2 Induction of syncytia formation Activation of p38
Tat	Proapoptotic	Upregulation of FasL expression Upregulation of Bax expression Upregulation of caspase 8 expression Microtubule alteration Oxidative stress Upregulation of RCAS-1 expression
	Antiapoptotic	Decreased susceptibility to TNFα and Fas Upregulation of Bcl-2 expression Decreased susceptibility to TRAIL Downregulation of caspase 10 expression Upregulation of c-FLIP expression
Vpu	Proapoptotic	Increased susceptibility to Fas Inhibition of NF-κB
Nef	Proapoptotic	Upregulation of Fas and FasL expression Downregulation of Bcl-2 and Bcl-XL expression Lysosomal permeabilization and Cathepsin-D release Upregulation of PD-1
	Anti-apoptotic	Inhibition of ASK-1 Inhibition of Bad Inhibition of p53
Vpr	Pro-apoptotic	Binding to ANT/VDAC leading to mitochondrial depolarization Binding to Bax leading to mitochondrial depolarization
	Anti-apoptotic	Suppression of NF-κB proinflammatory cytokine production Upregulation of Bcl-2 and downregulation of Bax expression
Protease	Pro-Apoptotic	Cleavage of Bcl-2 Cleavage of Caspase 8 creating pro-apoptotic Casp8p41

has pleiotropic effects on apoptosis of CD4T cells. Tat is produced early in the life cycle of the virus, but also is secreted by infected cells and taken up by uninfected T cells via clathrin-mediated endocytosis.[49] Both pro- and antiapoptotic effects have been demonstrated *in vitro,* depending upon cell lines used, use of endogenous expression vectors or exogenous administration, dose of Tat administered, whether the cell is infected or not, and oxygen level. Treatment of uninfected Jurkat T-cell lines with low doses (pM) of Tat results in apoptotic resistance to TNF, Fas[50] and TRAIL,[51] decreased expression of caspase 10,[52] and increased expression of Bcl-2 and c-FLIP[52] compared with untreated cells. However, treatment of uninfected T-cell lines and PBMCs with higher doses (nM-mM) of Tat can increase FasL,[53] caspase 8,[54] Bax[55] and RCAS-1[56]

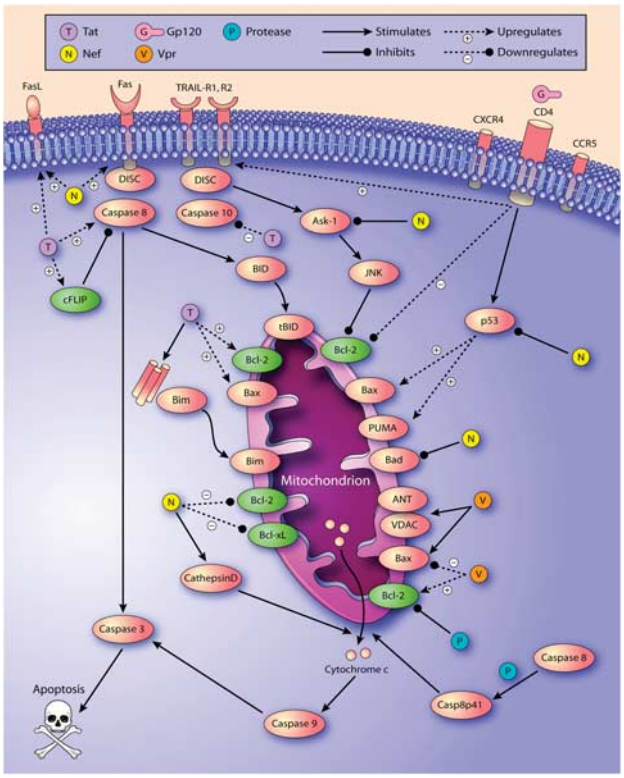

Figure 2 This figure depicts select interactions of HIV proteins with the mitochondrial pathway of apoptosis demonstrated in *in vitro* studies, demonstrating the both complexity and duplicity of these pathways. Which of these potential mechanisms occurs *in vivo*, and the relative importance, though, is less clear

expression, and cause oxidative stress[57] compared with untreated cells. Tat can also bind to tubulin, resulting in microtubule alteration and Bim-mediated mitochondria-dependent apoptosis.[58]

The role of Tat in inducing or inhibiting CD4T-cell apoptosis *in vivo* is unclear. Tat is present in concentrations in the serum of HIV-infected patients approximating the *in vitro* antiapoptotic doses.[59] On the other hand, HIV infection of human monocytes and macrophages, or treatment with exogenous Tat, results in upregulation of TRAIL expression in these cells, which can then induce apoptosis in uninfected bystander T cells.[60] Interestingly, chimpanzee T cells treated with exogenous Tat are resistant to Tat-mediated apoptosis,[61] and macrophages from chimpanzees, sooty mangabeys and African green monkeys do not upregulate TRAIL expression in response to Tat.[62]

Vpu and apoptosis. Vpu is an HIV-encoded accessory protein that downregulates the CD4 receptor, thereby preventing superinfection of infected cells and allowing efficient budding of newly produced virus. Vpu may also play a significant role in CD4T-cell apoptosis in HIV infection. *In vitro* overexpression of Vpu in Jurkat T cells increases susceptibility to Fas-mediated apoptosis.[63] This may be because expression of Vpu in HIV-infected or -transfected cells inhibits NF-κB-mediated expression of antiapoptotic genes.[64] Deletion of Vpu from an HIV NL4-3 proviral construct significantly decreases CD4T-cell depletion in *ex vivo*-infected human lymphoid tissue compared with

the wild-type parent virus.[65] Interestingly, in the SHIV/pig-tailed macaque model of HIV infection, Vpu proteins from different HIV-1 subtypes are associated with different rates of CD4T-cell loss over time, arguing for a pathogenic effect *in vivo*.[66]

Nef and apoptosis. Nef is a multifunctional HIV-encoded protein expressed early in the life cycle of the virus, responsible for downregulating CD4 receptor and MHC-I expression as well as enhancing viral replication. Nef-expressing T cells demonstrate upregulated Fas and FasL,[67] decreased Bcl-2 and Bcl-XL expression,[68] increased PD-1 expression,[69] and undergo apoptosis by both caspase-dependent or -independent mechanisms. Endogenous Nef produced in infected cells can cause lysosomal permeabilization, with release of cathepsin-D into the cytosol and consequent outer mitochondrial membrane rupture.[70] Nef is also secreted from HIV-infected cells via exosomes.[71] Exogenous administration of Nef to uninfected CD4T cells results in Fas-independent apoptosis, possibly by associating directly with the T-cell receptor, CXCR4 and SDF-1α[72] to induce apoptosis through unknown mechanisms.

However, not all *in vitro* effects of Nef are proapoptotic. Nef can directly interact with and inhibit the proapoptotic serine/threonine kinase ASK-1[73] as well as p53,[74] and can lead to inhibitory phosphorylation of the proapoptotic protein Bad by p21-activated kinase.[75] Nef also inhibits apoptosis in HIV-infected monocyte-derived macrophages through phosphorylation of Bad.[76]

An overall *in vivo* proapoptotic effect of Nef, though, is suggested by animal models of HIV. Treatment of mice with Nef-derived peptides leads to increased CD4T-cell apoptosis compared with untreated mice,[77] and transgenic mice that express human CD4 and HIV proteins develop an AIDS-like illness that is dependent on Nef.[78] SIV Nef, on the other hand, increases Bcl-2 expression in transfected Jurkat cells compared with non-transfected cells, and inhibits cell cycle progression and Fas-mediated apoptosis.[79] In non-pathogenic SIV infection, Nef may function to downmodulate the TCR to prevent activation-induced cell death.[80]

Vpr and apoptosis. HIV Vpr is an accessory, virion-associated protein with many functions, including induction of G(2)/M cell cycle arrest upon infection of the cell. The mechanisms of G(2) arrest by Vpr, induction of apoptosis and contribution to the immunopathogenesis of HIV infection have been reviewed extensively recently.[81] Briefly, Vpr's *in vitro* pleiotropic effects on apoptosis are species, cell type, and concentration dependent, and vary based on HIV subtype and whether the TCR has been activated or not.[82] Vpr expressed in low levels early after infection is antiapoptotic via suppression of NF-κB-dependent proinflammatory cytokine production,[82] as well as upregulation of Bcl-2 and downregulation of Bax.[83] However, later, after G(2) arrest, Vpr can induce apoptosis by binding to either Bax or ANT and VDAC in the mitochondrial membrane, causing release of cytochrome *c* and activation of caspases 9 and 3.[84] Vpr expression in CD4T cells also results in increased expression of NKG2D ligands, rendering infected CD4T cells susceptible to NK-cell-mediated killing.[85]

The contribution of Vpr to CD4T-cell loss *in vivo* was supported early by the demonstration of extracellular Vpr in serum from HIV-infected patients. Mice transgenic for the HIV-1 *Vpr* gene show enhanced CD4T-cell apoptosis compared with wild-type mice. Also, the R77Q polymorphism in Vpr, which is associated with decreased apoptotic-inducing ability *in vitro*, is overrepresented in LTNPs compared with typical progressors.[11] *Ex vivo* infection of human lymphoid tissue with R5-tropic HIV with directed mutation at R77Q exhibits decreased CD4T-cell apoptosis compared with wild-type virus.[86] The proapoptotic potential of HIV-1 Vpr is being exploited in preclinical studies on various types of cancer.

HIV protease and apoptosis. In the life cycle of the virus, the HIV protease cleaves the Gag/Pol polyprotein into functional subunits for production, maturation and budding of new virions. *In vitro* expression models demonstrate that HIV protease also has the ability to cleave several cellular targets to induce apoptosis, including Bcl-2.[87] Our lab has demonstrated that the HIV protease is also able to cleave procaspase 8 to generate a proapoptotic cleavage fragment 41 kDa in size – Casp8p41 – both *in vitro* and *in vivo*.[88] Casp8p41 is able to induce apoptosis in infected CD4T cells via a mitochondrial dependent pathway,[89] although the exact target on the mitochondria for its effect has yet to be identified. T cells expressing a procaspase 8 engineered to be resistant to HIV protease cleavage are resistant to apoptosis upon infection with HIV, suggesting that this mechanism is necessary for apoptosis of HIV-infected cells.[90]

Future Directions and Unanswered Questions

Many fundamental questions remain regarding apoptosis in the immunopathogenesis of HIV infection. Does apoptosis occur chiefly in infected cells or uninfected bystander cells in clinical HIV infection? Answering this question is of paramount importance if one is to either pharmacologically enhance or inhibit apoptosis. It is likely that apoptosis is occurring to some degree in both cellular populations, and thus further research is needed to find heretofore undiscovered regulators of apoptosis that are altered in productively and latently HIV-infected cells compared with uninfected cells that could serve as novel targets for intervention. Of the many mechanisms of HIV-induced apoptosis demonstrated in *in vitro* and *in vivo* models, which ones actually exist and are clinically relevant in human infection? If one attempts to inhibit one particular apoptotic pathway in bystander cells, will that drive the emergence of an alternative pathway to the same end? Are there clinically relevant biomarkers of ongoing-apoptotic activity, viral apoptosis-inducing ability, or host apoptosis susceptibility, which can be used to predict disease progression, response to antiviral therapy, or development of antiviral resistance? As effective preventive strategies, either with vaccination or microbicide, are lacking, viral-specific targets for development of new classes of antiviral therapy are dwindling, and drug resistance is rising; there is urgent need to address these fundamental issues in HIV pathogenesis.

Conflict Of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Cell Death and Disease website (http://www.nature.com/cddis)