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Mechanisms of CD4 T-Cell Depletion Triggered by HIV-1 Viral Proteins

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Abstract

Infection with HIV-1 leads to progressive CD4 T-cell death, resulting in AIDS development. The mechanisms that trigger this CD4 T-cell death are still not fully understood, but a lot of data indicates that apoptosis plays a major role in this cell demise. Both infected and uninfected CD4 T-cells can die during HIV-1 infection by different cell-death pathways, but HIV-1-induced, bystander, CD4 T-cell killing is now recognized as central to immunodeficiency. The HIV-1 directly modulates CD4 T-cell death using multiple different strategies in which several viral proteins have an essential role. Recent data demonstrate that relationships can exist between the three main types of programmed cell death, i.e. apoptosis, autophagic programmed cell death, and necrosis-like programmed cell death. Almost nothing is currently known about the role of necrosis-like programmed cell death in CD4 T-cell death induced by the viral proteins, but a very recent study demonstrates that autophagy is needed to trigger apoptosis of bystander CD4 T-cells, further increasing the level of complexity of this pathology. This review presents an overview of the major types of programmed cell death and details the mechanisms by which the HIV-1 viral proteins control both infected and uninfected CD4 T-cell death. **(AIDS Reviews 2006;8:221-36)**

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Key words

HIV-1. Apoptosis. Autophagy. CD4 T-cells. Viral proteins.

Mechanisms of cell death

The mechanisms by which a cell is dying are multiple and complex. Programmed cell death (PCD) is generally defined as an active process that depends on the execution of a sequence of signaling events that eventually lead to cell demise. Recently, the Nomenclature Committee on Cell Death has proposed unified criteria for the definition of cell death, essentially based on cell-death morphologies[1]. The major mechanism-based PCD types are apoptosis (type I PCD), autophagic PCD (type II PCD) and necrosis-like PCD (type III PCD).

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Apoptosis

Cells that undergo apoptosis show rounding-up, reduction of cellular volume, phosphatidylserine (PS) exposure, chromatin condensation, fragmentation of the nucleus, and maintenance of an intact plasmic membrane until late stages of the process, leading to formation of membrane-bound fragments, called apoptotic bodies. These structures are taken up by other cells and degraded within phagosomes without eliciting inflammation.

Basically, the apoptotic mechanism involves activation of cysteine-dependent aspartate-specific proteases, called caspases. Two caspase-dependent apoptotic pathways are described, the extrinsic and intrinsic pathways, which differ in how the death signal is transduced.

The extrinsic pathway involves death receptors (DR) that belong to the tumor necrosis factor (TNF) family of receptors containing conserved intracellular death domains. Among the most well known DR are TNF-R1 (also called CD120a), Fas (CD95), DR4 (or TRAIL-R1 for TNF-related apoptosis-inducing ligand receptor-1) and DR5 (TRAIL-R2). These receptors share common signaling mechanisms, although each receptor is stimulated by its own specific ligand. Ligand binding to a DR triggers receptor homo-

trimerization, recruitment of cytoplasmic adaptor and effector proteins, formation of the death-inducing signaling complex[2,3], autoactivation of caspase-8 and then activation of effector caspases, essentially caspases-3, -6 and -7[4].

The intrinsic pathway is controlled by the balance between antiapoptotic (e.g. Bcl-2, Bcl-xL) and proapoptotic (e.g. Bax, Bak, Bad, Bid, Bim) members of the Bcl-2 protein family. In addition, posttranslational modifications of several members of the Bcl-2 protein family, such as Bcl-2, Bcl-xL, Bax and Bad, play a role in the regulation of this apoptotic pathway. Proapoptotic Bcl-2 members interact with a hydrophobic groove on antiapoptotic Bcl-2 family members to block their function. In addition, they directly impact on mitochondrial outer-membrane permeability through their oligomerization, resulting in the release of several proapoptotic proteins into the cytosol, including cytochrome c, apoptosis-inducing factor, endoG and HtrA2/Omi. It is worth noting that the exact mechanisms by which Bcl-2 proteins induce permeability of the outer mitochondrial membrane are controversial and still under investigation. The effector caspase-mediated cell death is then mainly initiated after formation of the active cytochrome c/Apaf-1/caspase-9 complex, called apoptosome. On the contrary, apoptosis-inducing factor, endoG and HtrA2/Omi can promote a caspase-independent death through mechanisms that are poorly defined.

In certain cell types, the proapoptotic Bcl-2 family member Bid is also involved in the extrinsic apoptotic pathway. Indeed, Bid can be cleaved by caspase-8, which in turn activates the Bcl-2 family member Bax that triggers the intrinsic pathway, making a connection between the two pathways of apoptosis[5,6]. In primary T-lymphocytes and myeloid cells, the initial concept in which the intrinsic and extrinsic apoptotic pathways are totally separated is still hotly debated. It is unclear whether this separation occurs within a given cell, or whether it is reflective of the different types of death-inducing stimuli used, or a combination of both parameters[7]. These two pathways of apoptosis are tightly regulated by inhibitor of apoptosis family proteins[8].

Nevertheless, mounting evidence indicates that a cell that has been treated with an apoptotic inducer can also initiate a suicide program without DNA fragmentation or caspase activation[9], and that beside apoptosis, other PCD mechanisms exist such as autophagic PCD and necrosis-like PCD.

Autophagic PCD

Macro-autophagy, herein referred to as autophagy, is a cellular mechanism essential for homeostasis. Autophagy, which literally means “to eat oneself”, is necessary for the lysosomal degradation and recycling of long-lived proteins and entire organelles[10,11]. This process has been observed in all eukaryotes, and autophagic cells are morphologically identical in yeasts, plants, and animals. It begins with an engulfment event of portions of the cytosol into a characteristic double-membrane vacuole, called the autophagosome. These autophagosomes fuse with lysosomes for the degradation of the sequestered material by lysosomal hydrolases, allowing the recycling of the degraded constituents[10-15]. The endosomal pathway can be connected to the autophagic process through formation of amphisomes, vacuoles formed after fusion of autophagosomes with late endosomes.

Autophagy is a highly regulated physiological process mediated by two conjugation systems related to ubiquitylation. Both systems depend on Atg (autophagy-related genes) proteins and their related signaling pathways[16,17]. Class III phosphatidylinositol-3 kinase (PI3K) is involved in the early stages of autophagic vesicle formation. This kinase controls the autophagic pathway through its association with Beclin-1[18,19].

Autophagy is involved in both survival and cell death. It is a cell-survival mechanism during nutrient starvation[20,21] to supply vital components until conditions improve, and during growth factor deprivation such as interleukin 3 (IL-3). However, several recent studies have shown that cell death can be blocked after knockdown of autophagy genes[22-24], indicating that autophagy plays a role in cell-death processes[25-27]. In addition, cell death with autophagic features can occur in cells lacking critical apoptosis executioners, and in this way, autophagy can compensate for defective apoptosis[28,29]. Autophagic cell death has also been described in cells after treatment with chemotherapeutic drugs[30-32].

Autophagic PCD is first defined by the presence of autophagosomes in the dying cells. These autophagic vacuoles can be observed by electron microscopy and by fluorescence microscopy after overexpression of the microtubule-associated protein 1 light chain 3 coupled to green fluorescent protein. Light chain 3 was the first protein identified on the autophagosome membranes[33]. This type of cell death occurs with very late, if any, chromatin condensation. No inflammatory response is triggered since the integrity of the plasma membrane is maintained. Importantly, exteriorization of PS occurs[34], indicating that PS exposure can be triggered by other PCD than apoptosis, and that autophagic cells are also recognized and engulfed by neighboring cells.

The mammalian serine/threonine protein kinase TOR (target of rapamycin) signaling pathway, activated by class I PI3K, is a key negative regulator of autophagy [35-37]. Thus, the phosphatase and tensin homolog, which acts antagonistically on PI3K, and rapamycin, the specific inhibitor of the mTOR pathway, activate autophagy.

As autophagy appears essential in the cell fate between life and death, it is not surprising that it has been implicated in numerous pathologies, including neurodegenerative diseases, infectious diseases, and cancer[27].

Necrosis-like PCD

Necrosis is usually defined as a type of cell death with mechanical rupture of the plasma membrane, allowing cytoplasmic swelling, dilatation of organelles such as mitochondria, endoplasmic reticulum (ER) and Golgi apparatus, and moderate chromatin

condensation. In contrast to apoptosis and autophagic PCD, necrosis-like PCD is associated with inflammation because cells dying by this process are phagocytosed after membrane lysis. Externalization of PS might occur before cell lysis, but is not a mark of necrosis. At a molecular level, necrosis is often associated with acute adenosine triphosphate (ATP) depletion that is thought to cause cell death[38,39]. Necrosis was first defined as an accidental, unregulated cell death after cellular injury, but an increasing number of reports indicate that cell death with necrotic features occurs under normal physiologic conditions and during development[40,41], and that specific mechanisms regulate this cell death. This form of regulated necrotic cell death has been defined as necrosis-like PCD to distinguish it from accidental necrosis, which is a passive process. However, necrosis is morphologically indistinguishable from necrosis-like PCD.

Depending on the type of cells, the availability of proteins involved in the death-inducing signaling complex formation and caspases, activation of DR, such as Fas and TNF-R1, can trigger necrosis-like PCD. Indeed, Fas-associated death domain and receptor-interacting protein 1, two adaptor proteins recruited by the DR, regulate necrosis-like PCD[42], and excess formation of reactive oxygen species (ROS) downstream Fas and TNF-R1 pathways are involved in necrosis-like PCD[43,44]. In addition to the role of ROS, ceramide is also involved in this type III PCD induction[45]. However, this form of PCD is especially triggered after blockade of the caspases, indicating that T-cells possess several cell-death pathways that can be activated sequentially or in parallel.

Table 1 summarizes the main characteristics of the three types of PCD.

Connections between these pathways

There is overlap between these cell-death mechanisms that can substitute for each other, indicating that a cell will take any available route to die when death is inevitable[34,46]. Indeed, molecular connections exist between the different cell-death pathways, and DR, mitochondria, lysosomes, and ER play key roles in these cross talks[47]. At a molecular level, ROS generation, ceramide production, proteins of the Bcl-2 family, heat-shock proteins, and enzymes (proteases, nucleases, phospholipases) are involved in both apoptosis, autophagic PCD, and necrosis-like PCD[23,34,45,48-51]. Thus, a new concept in cell-death regulation is emerging, in which different cell-death pathways share common mediators, and depending on the cell types and stimuli, the level and duration of cell-death inducer, the local environment (hypoxia, secretion of inflammatory cytokines, level of nutrients, etc.), a coordinated sequence of molecular events is activated, determining whether a cell dies by apoptosis, autophagic PCD and necrosis-like PCD, or presents a mixed type of PCD.

Mechanisms of CD4 T-cell depletion in HIV-1 infection

The HIV-1 infection usually leads to a progressive decline in the functionality and number of CD4 T-lymphocytes, resulting in AIDS development[52]. Despite intensive studies, several crucial questions remain to be addressed about the mechanisms through which HIV-1 infection induces CD4 T-cell death, and this subject is one of the most controversial issues in AIDS research.

First, HIV-1 infection results in high activation and turnover of immune cells, and thus accelerates both production and destruction of CD4 T-cells[52,53]. A strong immune response is *a priori* beneficial in controlling viral replication. However, independently of viral load, a chronic, heightened activation of the immune system may contribute in a direct manner to progressive CD4 T-cell depletion[54,55]. In addition, HIV-1 can interfere with T-cell renewal, both at the level of progenitors and thymic differentiation, preventing appropriate replacement of prematurely destroyed mature CD4 T-cells[56]. Thus, immune activation could drive the progression of HIV-1 disease by destabilizing or progressively changing the homeostatic states of resting T-cell populations.

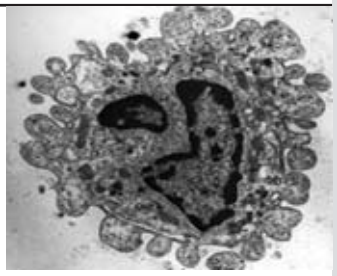
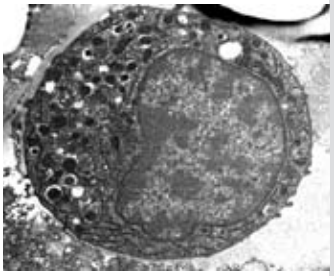
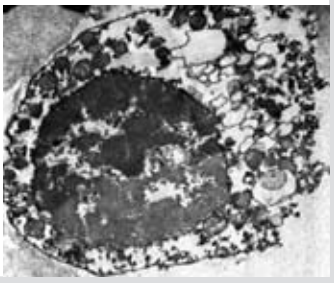
As early as 1991, CD4 T-cell apoptosis has been proposed as a mechanism responsible for T-cell depletion in patients infected with HIV-1[57,58], and an extensive body of literature since then has supported this hypothesis. In addition, there is a correlation between the extent of apoptosis and disease progression[59,60], and HAART is associated with a lower level of CD4 T-cell apoptosis in HIV-1-infected patients[61-63]. Recently, we have demonstrated that autophagy is involved in HIV-mediated CD4 T-cell death *in vitro*[64]. Furthermore, necrosis is also observed in peripheral blood CD4 T-cells after *in vitro* infection[65].

Importantly, in HIV-1-infected persons, both infected and uninfected cells undergo accelerated cell death *in vitro* and *in vivo*. In addition, the mechanisms leading to cell death are different, underlining the complexity of this pathogenesis. Thus, we differentiate in this review the cell death pathways induced in HIV-1-infected and uninfected CD4 T-cells.

HIV-1- infected CD4 T-cell death

During acute infection, characterized by rapid increase in viral load and CD4 T-cell depletion, the immune system is strongly stimulated, leading to CD8-mediated apoptosis, superantigen-induced cell death, and antibody dependent cellular cytotoxicity of infected CD4 T-cells[66]. The CD4 T-cell loss can also be due to direct destruction by the virus itself. Indeed, HIV-1 can actively kill the infected target cells by disruption of the cell membrane as HIV-1 buds from the surface[67], or by intracellular accumulation of hetero-disperse RNA and unintegrated DNA[68,69]. Virus replication in the cell can also trigger a cellular toxicity due to build up of unintegrated linear viral DNA[70,71], but recent data indicate that HIV-1 replication elicits little cytopathic effects *in vivo*[72]. However, a small fraction of infected CD4 T-cells do not die after HIV-1 infection, suggesting that resistance of apoptosis may be a mechanism for the development of HIV-1 reservoirs[73]. Indeed, viruses must avoid the rapid death of freshly infected cells, which is one of the most ancient antiviral defense mechanisms. To this aim,

Table 1. Principal characteristics of the three major types of programmed cell death

Type of Death	Principal characteristics	Electron Microscopy
Apoptosis (type I PCD)	– Chromatin condensation – DNA fragmentation – Cytoplasm condensation – Activation of caspases – PS exposure – No plasma membrane breakdown – Formation of apoptotic bodies – Phagocytosis of dying cell – No inflammation	
Autophagic PCD (type II PCD)	– Caspase-independent process – Formation of lysosome-derived cytosolic vacuoles containing organelles and cytoplasm – PS exposure – No plasma membrane breakdown – Phagocytosis of dying cell – No inflammation	
Necrosis-like PCD (type III PCD)	– Absence of chromatin condensation or chromatin clustering – Usually caspase-independent process – Externalization of PS might occur before lysis – Plasma membrane breakdown – Inflammation	

PCD: programmed cell death; PS phosphatidylserine.

HIV-1 has evolved multiple mechanisms to promote survival for long enough to ensure a productive infection, and several HIV-1 proteins can act in activating and/or inhibiting HIV-1-infected T-cell apoptosis. This point will be described later in this review. As a result, infected cells do not undergo apoptosis as readily as uninfected, bystander cells[74], even if infected cells have a shortened lifespan contributing to the overall CD4 T-cell demise[75-77].

Bystander CD4 T-cell death

The HIV-1-induced apoptosis of bystander, uninfected immune cells is likely the key to the depletion of T-lymphocytes observed in HIV-1-infected patients, since the vast majority of CD4 T-cells undergoing apoptosis in peripheral blood and lymph nodes of HIV-1 patients are uninfected[74,78]. Using several animal models, such as rhesus macaques infected by SIV or highly pathogenic SIV/HIV-1 chimeric viruses and human peripheral blood lymphocyte-transplanted nonobese diabetic/severe combined immunodeficient mice, massive apoptosis was predominantly observed in uninfected CD4 T-cells present in lymph nodes, thymus, or spleen[79-81].

Bystander CD4 T-cells can be killed by activation-induced cell death, which occurs as a result of repeated antigenic stimulation to limit lymphoproliferation[82]. This process is mediated by the interaction of the cell-death receptor Fas and its ligand (FasL), expressed either on the same cells or on neighboring activated T-cells. Kaplan and Sieg[83] have previously reviewed the role of this Fas/FasL apoptotic pathway in HIV-1 disease. The proportion of Fas-expressing T-cells in patients increases with disease progression, and peripheral blood CD4 T-lymphocytes from HIV-1-infected individuals undergo apoptosis in response to stimulation through Fas at a much higher frequency than from uninfected controls[84-90]. In the same way, high levels of Fas-susceptibility found in peripheral CD4 T-cells before HAART are significantly reduced after treatment, coinciding with a decrease in viral load and an increase in peripheral CD4 T-lymphocyte counts.

In addition, during HIV-1 infection, deregulation in cytokine production occurs, perturbing the immune response. Indeed, overproduction of type-2 cytokines (IL-4, IL-10) is known to increase susceptibility to activation-induced cell death, whereas type-1 cytokines (IL-12, IFNγ) may be protective[91]. Furthermore,

IFNα produced by HIV-1-infected dendritic cells contributes to CD4 T-cell apoptosis by the TRAIL/DR5 pathway[92].

Another mechanism controlling the death of activated T-cells is called activated T-cell autonomous death, which involves Bcl-2, Bim[93] and ROS, but acts independently of Fas and TNF[94]. Activated T-cell autonomous death, contrary to activation-induced cell death, is not affected by the lack of caspases[95] and is described as a necrotic pathway that seems to suffice for efficient primary T-cell demise[40]. However, this mechanism of cell death has not been studied during HIV-1 infection.

Besides the consequences of general activation of the immune system during HIV-1 infection, several HIV-1 proteins, such as envelope glycoproteins (Env), Tat, Vpr, Nef, Vpu, the protease and Vif are also involved in modulating CD4 T-cell death. No one has a full grasp of the real importance of this process *in vivo*, but cumulative data demonstrate a major role of Env in the cell death of uninfected lymphocytes[96-99]. Indeed, binding of HIV-1 Env gp120/gp41 to its receptors, CD4 and a coreceptor, constitutes the primary interface between viruses and T-cells and this event is likely able to modulate T-cell signaling.

HIV-1 proteins acting on cell-death pathways activated in both infected and bystander CD4 T-cells are presented hereafter and summarized in table 2.

Envelope proteins

The mature envelope glycoproteins (Env) are composed of gp120, the exterior envelope glycoprotein, and gp41, the transmembrane glycoprotein, assembled as trimer by noncovalent interactions. In most cases, to enter a target cell, HIV-1 must bind two molecules on the surface of target cells. The gp120 first interacts with CD4, which triggers conformational changes, leading to increased exposure of the gp120 V3 loop that is then able to bind to a coreceptor, mainly CCR5 and CXCR4[100-102]. These events trigger the formation of a coiled-coil structure in the gp41 ectodomain that places the hydrophobic aminoterminal region of gp41 in close proximity to the cellular membrane, thereby inducing cell fusion[103]. The HIV-1 infection of CD4 T-cells is favored by cell-to-cell contacts, through formation of the virologic synapse[104]. Contact of HIV-infected cells (which express Env) with uninfected CD4 T-cells induces a gp41-dependent hemi-fusion process in which a transfer of lipids from the membrane of Env-expressing cells to the target cells occurs. Complete cell-to-cell fusion can also be triggered, leading, eventually, to the formation of giant multinucleated cells, called syncytia[105].

Basically, R5 strains, which use CCR5 in addition to CD4 for entry, are responsible for primary infection. During the progression of the disease, X4 viruses, which use CXCR4 for entry, emerge and are associated with a rapid decline in the number of CD4 T-cells. These strains preferentially infect T-cells and induce membrane fusion between infected cells that express viral Env and uninfected target CD4 T-cells, leading to syncytium formation. Syncytia are not stable over an extended time-period[106-108] and are hardly detectable in infected individuals except in brain[109] and tonsils[110], but can amplify the global apoptotic signaling[111].

Role of Env in HIV-1-infected CD4 T-cell death

Syncytium formation leads to apoptosis mediated by the intrinsic mitochondrial pathway[112] and involves a precise sequence of events: (i) activation of the mammalian target of rapamycin (mTOR); (ii) mTOR-mediated phosphorylation of p53 on serine 15; (iii) p53-dependent upregulation of Bax expression; (iv) Bax-mediated permeability of mitochondrial membranes with reduction of the mitochondrial transmembrane potential and release of pro-apoptotic mitochondrial proteins such as apoptosis-inducing factor and cytochrome c; and (v) activation of caspase-3 and nuclear chromatin condensation[113,114].

This process may participate in the destruction of CD4 T-cells in lymphoid organs where contacts between HIV-1-infected cells and uninfected cells are numerous. It is also responsible for the death of uninfected CD4 T-cells, as indicated in the next paragraph.

Intracellular binding of CD4 and Env can also directly result in cell killing[115]. Furthermore, a recent study indicates that apoptosis of HIV-1-infected CD4 thymocytes is dependent on Env fusion as T20, a peptide that abolishes gp41 insertion into the target cell membrane, is capable of reducing this apoptosis[116].

Role of Env in bystander CD4 T-cell death

The mechanisms by which Env triggers bystander CD4 T-cell death are highly difficult to study because of the complexity and the multiplicity of the "contributing actors".

- Env activates different cell-death mechanisms depending on its presentation (i.e. soluble Env, Env expressed on virions or at the surface of infected cells).
- Env is composed of two glycoproteins, gp120 and gp41 that can trigger nonexclusive, different cell-death pathways.
- The gp120 sequentially binds to CD4 and a coreceptor, both capable of transducing functional responses such as proliferation, differentiation, chemotaxis, and proinflammatory cytokine secretion[117,118] in addition to cell death.
- The gp120 signaling through CCR5 or CXCR4 is different, even if several pathways can be identical such as phosphorylation of the tyrosine kinase Pyk2[119].
- Only about 15-30% of the CD4 T-lymphocytes express detectable levels of CCR5 on the cell surface, in contrast to CXCR4, which is expressed on nearly all of these T-cells[120,121]. This explains, at least in part, that X4 strains exert a profound cytopathic effect on a much wider range of target cells via their particular capacity to induce bystander cell death.

Table 2. Mechanisms by which different HIV-1 proteins modulate both infected and bystander CD4 T-cell death

HIV protein	Infected cells		Bystander cells	
	Proapoptotic	**Antiapoptotic**	**Proapoptotic**	**Anti-apoptotic**
Env	mTOR-mediated phosphorylation of p53, upregulation of Bax, release of cytochrome-c and AIF, and caspase-3 activation in syncytia	None	Upregulation of Fas/FasL Upregulation of TNF and TRAIL receptors Activation of autophagy ROS production, cytochrome-c release and activation of caspases-9 and -3	None
Tat	Upregulation of caspase-8 Deregulation of cytokine production Downregulation of Bcl-2	Upregulation of Bcl-2 and Bcl-xL Inhibition of p53 Inhibition of TRAIL-mediated apoptosis	Interaction with tubulin and Bim-mediated intrinsic pathway activation Upregulation of Fas/FasL Deregulation of p56lck, cyclin-dependent kinase and NFκB activation	None
Vpr	Inhibition of NFκB Activation of caspases-3 and -9 Cell cycle arrest in G2	Upregulation of Bcl-2 Downregulation of Bax Upregulation of survivin	Cytochrome-c release Caspase-independent pathway controlled by AIF Formation of cation-selective channels	None
Nef	Upregulation of Fas/ FasL Activation of caspase-3	Interaction and blockade of ASK-1 Bad phosphorylation Interaction and inhibition of p53 Downregulation of CD4 cell surface expression preventing Env-mediated cell death Downregulation of MHC class I preventing killing by CD8 T-cells	Disorganization of lipid bilayers	None
Vpu	Formation of cation-selective channels Enhanced sensibility to Fas apoptosis Inhibition of IκB degradation leading to inhibition of Bcl-xL expression and increase in caspase-3 expression level	Downregulation of CD4 cell surface expression preventing Env-mediated cell death Downregulation of MHC class I preventing killing by CD8 T-cells	None	None
PR	Cleavage of Bcl-2 Cleavage of procaspase-8, activation of Bid, release of cytochrome-c and activation of caspases-9 and -3	None	None	None
Vif	Cell cycle arrest in G2	None	None	None

mTOR: mammalian target of rapamycin; AIF: apoptosis-inducing factor; TNF: tumor necrosis factor: TRAIL: TNF-related apoptosis inducing ligand; ROS: reactive oxygen species; NFκB: nuclear factor kappa B; ASK: apoptosis signal regulating kinase; MHC: major histocompatibility complex.

Here, we have summarized the apoptotic pathways activated by Env in bystander CD4 T-cells and described new data on Env-induced autophagic PCD.

Env binding to its receptors on target CD4 T-cells has been described to trigger activation of the extrinsic apoptotic pathway through Fas and other TNF-family receptors. Cross-ligation of CD4 molecules prior to T-cell-receptor stimulation triggers an upregulation of Fas on purified T-cells and expression of FasL upon antigen, mitogen, and CD3 stimulation, rendering the T-cells susceptible to Fas-mediated apoptosis[122]. It is quite likely that uninfected CD4 T-cells from HIV-1-infected patients are continuously undergoing CD4 cross-linking through interaction with virions or via Env expressed at the surface of infected cells.

In addition, even if bystander apoptosis is an important characteristic of X4 HIV-1 strains, mediated by binding of X4 Env to CXCR4 on CD4 T-lymphocytes, R5 Env binding to CCR5 expressed on uninfected, resting, primary CD4 T-cells has also been shown to trigger apoptosis via FasL upregulation and caspase-8 activation[123].

TNF[124-126] and TRAIL (DR4 and DR5) receptors[127-129] may also be involved in deregulated apoptosis during HIV-1 infection.

Besides the fact that CD4 is engaged in T-cell activation, direct cross-linking of CD4/HIV-1 gp120 complexes by antibodies was found to initiate T-cell apoptosis using *in vitro* cellular experiments from transgenic mice expressing human CD4 at the surface of lymphocytes[130,131].

However, numerous data demonstrate that the intrinsic pathway of apoptosis plays a major role in the cytotoxicity of X4 strains. Binding of HIV-1 Env to CXCR4 induces mitochondrial transmembrane depolarization, cytochrome-c release from the mitochondria to the cytosol, and activation of caspases-9 and -3. Of note, Env-induced apoptosis through CXCR4 is Fas independent[132-136].

There is still some controversy as to the conformation of gp120 needed to induce cell death. In a majority of cellular models, Env has to be expressed on cells to trigger bystander CD4 T-cell apoptosis, but recombinant gp120 alone or cross-linked with anti-gp120 antibodies was also shown to trigger CD4 T-cell death[132,137].

Direct implication of caspases in X4 Env-mediated CD4 T-cell death is still a subject of debate. Berndt, et al. described no involvement of known caspases in cross-linked recombinant gp120- and anti-CXCR4-induced apoptosis of human peripheral blood lymphocytes[132], and Vlahakis, et al. reported that CXCR4-dependent cell death is caspase independent on the basis of caspase inhibitors[138]. However, caspase-3 is cleaved in primary T-lymphocytes[136,137] following binding of HIV-1 Env.

The manner in which Env is presented, the cell population analyzed, and the nature of the receptor directly involved in this cell death could be responsible for the discrepancies between these reports. However, multiple experiments, using different cell lines, human primary T-cells, and human lymphoid cultures *ex vivo* support the view that Env interaction with CXCR4 on bystander CD4 T-cells triggers apoptosis. These results are consistent with observations made from AIDS patients, and explain the high CD4 T-cell depletion that occurs after X4 isolate emergence[139] and in poor immunologic X4-infected responders to HAART[140].

Furthermore, agents interfering with cell-to-cell fusion, such as the peptide T20 which abolishes a correct gp41 folding after gp120 binding to its receptor molecules and insertion of the gp41 fusion peptide into cell membrane[141], prevent cell death and T-cell depletion[142,143]. Blanco, et al. demonstrated that Env-induced cell death of single, bystander CD4 T-cells requires both gp120 and gp41 functions[144], and recent studies have shown that gp41-induced apoptosis is mediated by caspase-3-dependent mitochondrial depolarization and ROS production[145]. These results indicate that besides the role of gp120, gp41 could actively participate in the molecular events leading to Env-induced cell death.

Very recent data from our group demonstrate for the first time that HIV-infected cells induce Env-mediated autophagy in bystander CD4 T-lymphocytes. Indeed, independently of HIV-1 replication, HIV-1-infected cells that express Env induce autophagy and accumulation of Beclin-1 in bystander CD4 T-cells. Env-mediated autophagy is dependent on the presence of CXCR4, but is independent of CD4 signaling. Furthermore, this autophagic process is required to trigger CD4 T-cell apoptosis since blockade of autophagy at different steps, by either drugs or small interfering RNA specific for autophagic genes, totally inhibits apoptosis. In addition, CD4 T-cells still undergo Env-mediated cell death with autophagic features when apoptosis is inhibited. These results suggest that HIV-1-infected cells can induce autophagy in bystander CD4 T-lymphocytes through contact of Env, leading to apoptotic cell death, a mechanism most likely contributing to immunodeficiency. At present these data are the only ones demonstrating that an autophagic process can be involved in cell death during viral infection.

Until now, nothing is currently known about induction of necrosis-like PCD in bystander CD4 T-cells by Env, even if necrosis is observed *in vitro* during the late stages of Env-mediated apoptosis.

Tat

Trans-activating transcriptional activator (Tat) is an HIV-1, early expressed, regulatory protein that can be secreted by infected cells. Its size varies between 86 (9 kDa) and 101 (11 kDa) amino acids, and the latter is the predominant form. Tat is encoded by two exons and has six different regions, each having particular functional and biochemical characteristics[146]. It has been proposed that the region V (residues 60-72), a glutamine-rich region that forms an α-helix, is involved in Tat-mediated apoptosis of T-lymphocytes via mitochondria[147]. Tat stimulates

viral gene expression by recognizing and binding to an RNA stem-loop-bulge secondary structure called TAR (Tat-responsive element), present at the 5' extremities of HIV-1 mRNA[148]. The HIV-1 Tat undergoes transcriptional modifications such as acetylation[149], ubiquitination[150] and phosphorylation[151], which influence its functions. Furthermore, Tat is cleaved by the HIV-1 protease in the virion, but the role of this cleavage is still unclear[152]. Tat is an active player in HIV-1 infection due to its multiple interactions with different intracellular and extracellular proteins. It interferes with several cell-signaling pathways and has been described as a modulator of HIV-1-induced cell death in both infected and bystander CD4 T-cells.

Role of Tat in HIV-1-infected CD4 T-cell death

The effects of Tat expression in HIV-1-infected cell death are still controversial, underlining the complex and multiple roles of Tat in CD4 T-cell signaling pathways. Several studies have supported the hypothesis of a proapoptotic action of Tat. There is some evidence that endogenously expressed Tat can induce apoptosis and/or increase sensitivity to apoptosis in CD4 T-cells by upregulating caspase-8[153]. Another proposed mechanism by which Tat may induce apoptosis is the deregulation of the production of cytokines such as TGFβ (transforming growth factor-β), TNF and IL-2[154]. Apart from these proapoptotic functions, it has been observed that Tat can block apoptosis. Indeed, a number of reports have shown that Tat protects lymphoid cells from apoptosis[155-157]. Tat inhibits TRAIL-mediated apoptosis in CD4 lymphoblastoid T-cell lines[158] and the transcription of p53[159]. The expression level of Bcl-2 and Bax are controversial in Tat-transfected T-cells[160,161]. So far, those mechanisms are not yet completely elucidated and need further investigation.

All these results suggest that Tat plays a dual role in HIV-1-induced cell death, maybe depending on its concentration in the cell.

Role of Tat in bystander CD4 T-cell death

Regardless of its controversial role in infected cells, extracellular Tat has been shown to have rather devastating effects on uninfected bystander cells. Tat can be secreted from the HIV-1-infected cells through leaderless secretion pathway[162] in the absence of any cell lysis[163,164]. The neighboring uninfected cells efficiently take up this soluble form of Tat by clathrin-mediated endocytosis[165]. It can also interact with membrane cell receptors such as CD26[166] and integrin α5β1[167]. Once extracellular Tat enters the cell, it modulates the normal biologic host cell functions through its action on several signaling proteins such as p56lck[168], cyclin-dependent kinases[169], and NFκB transcription factor[170], leading directly or indirectly to apoptosis. Interaction of the central region of Tat with the αβ-tubulin dimer activates the intrinsic pathway of apoptosis in T-cells[171,172]. Furthermore, Bim plays a critical role in this Tat-induced apoptosis[172]. Tat is also able to directly trigger cytochrome-c release from mitochondria, which results in activation of apoptosis[171]. It can also indirectly enhance activation of apoptosis in uninfected T-cells by upregulating Fas/FasL[173]. It has also been shown that Tat upregulates TRAIL expression in macrophages, leading to increased production of soluble TRAIL that can then induce destruction of the bystander CD4 T-cells[174]. Recently it has been demonstrated that upregulation of FasL (CD178) and induction of apoptosis in the bystander cells depends on the C-terminal part of Tat[175]. Thus, Tat may induce bystander CD4 T-cell apoptosis directly via the intrinsic pathway and indirectly via the extrinsic one.

Vpr

Viral protein R (Vpr) is a highly conserved HIV-1 accessory viral protein with a molecular weight of 14 kDa. Vpr is abundantly associated with the virion through its interaction with the p6 domain of the Gag polyprotein precursor ($Pr55^{gag}$), underlining its role in the early steps of the viral infection[176]. Then, Vpr is expressed at the late stage of the viral infection and can also be released from HIV-1-infected cells[177]. Vpr has a nuclear localization in the cell, and its N-terminal domain is essential for this nuclear transport[178]. This viral protein has different functions including nuclear transport of the viral pre-integration complex[179], arrest of the cell cycle in G2 phase[180], enhancement of the viral gene transcription, and the HIV-1 long terminal repeat transactivation[181]. Besides all those activities, Vpr also acts as a modulator of apoptosis in different cell types, depending on its concentration in the cell.

Role of Vpr in HIV-1-infected CD4 T-cell death

The source of Vpr in infected cells could be either virion-associated Vpr or endogenously expressed Vpr. It has been shown that expression of endogenous HIV-1 Vpr results in the upregulation of Bcl-2 and downmodulation of Bax[182]. Further, survivin, a member of the inhibitor of apoptosis family, can be upregulated by Vpr, resulting in the inhibition of apoptosis[183]. Interestingly, these antiapoptotic effects have been described in cells expressing constitutive low levels of Vpr[182,184].

On the contrary, high levels of Vpr expression in the cells correlate with induction of apoptosis, suggesting that Vpr protects HIV-1-infected cells from apoptosis at the early stages of infection, while its expression at later stages of infection leads to a higher susceptibility to apoptosis. Some evidence indicates that Vpr inhibits activation of NFκB, very likely through upregulation of IκB, the inhibitor of NFκB[185]. Moreover, Vpr is able to induce apoptosis via activation of the caspases-3 and -9[186], and this phenomenon occurs independently of p53 in cell lines of various tissue origins[187]. There is still some controversy about a direct correlation between Vpr-induced G2 arrest and induction of apoptosis in CD4 T-cells. A very recent study has demonstrated that both Vpr and Vif are necessary for T-cell cytopathic effect and G2 cell cycle arrest[188]. Of note, it has been proposed that Vpr is packed in the

virion in significant quantities[181,189], and some observations raise the possibility that this virion-associated Vpr is sufficient for the induction of the G2 cell cycle arrest and cell death in CD4 T-cells[190].

These data indicate that Vpr has positive and negative effects on apoptosis in HIV-1-infected CD4 T-cells, depending on its level of expression and on the phase of HIV-1 infection. At the early stages of the infection, when the level of expression of Vpr is probably low, it plays a protective role by exerting antiapoptotic function, ensuring viral survival and dissemination. Ultimately, in the late stages when the level of expression Vpr gets high, it promotes apoptosis of the infected cell.

Role of Vpr in bystander CD4 T-cell death

Vpr can be detected in the sera and in the cerebrospinal fluid of HIV-1-positive patients, which clearly shows that it can exist in a cell-free state[191]. It is still unclear whether infected cells release this extracellular Vpr, or if it is freed after breakdown of infected cells[191]. However, purified Vpr can enter into cells when added to the cell culture *in vitro*[192,193]. The existence of circulating extracellular Vpr and its ability to enter into host cells indicate that this viral protein can act on bystander cells. Indeed, it has been demonstrated that extracellular Vpr induces apoptosis via a direct effect on the mitochondrial permeability transition pore, leading to the loss of $\Delta\Psi$m (transmembrane potential) and release of cytochrome-c[194]. In addition, C-terminal peptides of Vpr containing the conserved sequence HFRIGCRHSRIG trigger a dramatic reduction of mitochondrial membrane potential, leading to CD4 T-cell death[195]. Furthermore, several studies suggest that Vpr is able to induce apoptosis through a caspase-independent pathway controlled by apoptosis-inducing factor, which translocates from the intermembrane space of the mitochondria to the nucleus where it exhibits apoptotic effects[196,197]. A very recent study has demonstrated that a chimeric peptide containing a mitochondrial membrane potential-inducing sequence derived from Vpr reaches mitochondria where it interacts with adenine nucleotide translocator and voltage-dependent anion channel and causes inner and outer mitochondrial membrane permeability[198].

These data indicate that Vpr is an important actor in bystander CD4 T-cell demise.

Nef

Negative factor (Nef) is a 27 kDa HIV-1 accessory protein expressed at a high level during the viral replication cycle. Nef is actually one of the earliest expressed HIV-1 proteins and some evidence suggests that its transcription takes place before viral integration[199]. Nef has been detected in HIV-1 particles[200] and is released in the extracellular medium since soluble Nef can be detected in the sera of HIV-1-positive patients[201]. In infected cells, Nef can be found in the perinuclear region and in the cytosol[202,203]. It also localizes at the cell membrane after its myristoylation. The crucial myristoylation motif is a highly conserved MGGxxS sequence at the N-terminal part of Nef, which is also essential for its functions[204]: modulation of several signal transduction pathways and cell activation[205]. Thus, this protein is essential for disease progression through its ability to interact with host-cell proteins.

Role of Nef in HIV-1-infected CD4 T-cell death

As with Vpr, this viral protein seems to act both in cell survival and cell death. Endogenous expression of Nef in infected cells is associated with an antiapoptotic effect through two mechanisms. First, Nef stops the death signals coming from the cell-surface death receptors TNF-R and Fas by binding and thus blocking the apoptosis signal-regulating kinase-1, a serine/threonine kinase[206]. On the other hand, Nef is able to inhibit the intrinsic apoptotic signals by interacting with the proapoptotic protein Bad[207]. By binding and activating the PI3K pathway, Nef also induces the phosphorylation of Bad, resulting in a pro-survival activity[208]. Furthermore, Nef can also interact with p53, leading to destabilization of the latter, thus inhibiting its proapoptotic activity[209].

However, some evidence suggests that Nef may have a proapoptotic action. Indeed, CD4 T-cells expressing Nef display a decrease in $\Delta\psi_m$, PS exposure and caspase-3 activation, and thus an increased level of apoptosis induction[210]. Moreover, endogenous expression of Nef upregulates Fas and induces expression of FasL, increasing cell sensibility to apoptosis[211]. Furthermore, it has been reported that Nef interacts with the ζ chain of the T-cell receptor (TCR), leading to stimulation of FasL expression on HIV-1 infected cells[210].

Among the Nef functions, one of the best characterized is its major impact on downregulation of several cell membrane molecules such as CD3[212], CD28[213], CD4[214], and MHC class I[215]. In particular, its ability to downregulate the cell-surface expression of CD4 and MHC class I leads to prevention of superinfection of the host cell[216] and to its evasion from the immune system[217], respectively. After Nef-mediated internalization of cell-surface CD4, this receptor is targeted to degradative compartments via the endosomal/lysosomal pathway[218-220].

Of note, cells expressing Nef show cytoplasmic accumulation of degradative vesicles containing lysosomal proteins (Lamp 2, cathepsin D and cationic ferritin)[221] suggesting a putative role of autophagy in this process.

Role of Nef in bystander CD4 T-cell death

Extracellular Nef induces apoptosis in CD4 T-cells through CXCR4 receptor[222], but the exact mechanism involved is still unknown.

The myristoylated N-terminal part of Nef has been found to disorganize lipid bilayers[223], to induce membrane disorder, and to have a cytotoxic activity in CD4 T-cells[224]. However, the mechanism of Nef secretion from to HIV-1-infected cells is still unclear and further insight into the extracellular functions of Nef is needed.

Vpu

Viral protein U (Vpu) is an accessory HIV-1 protein with an estimated molecular weight of 16 kDa, which has been detected in the Golgi and ER membranes. This membrane protein has an N-terminal transmembrane region and a C-terminal cytoplasmic tail, and is able to homo-oligomerize[225]. Vpu has two main functions in the cell: it promotes the budding of the virions from the plasma membrane[226], thus contributing to the viral spread, and it induces the degradation of CD4 in the ER by the proteasome[227]. This accessory protein is involved in the late stages of viral replication and has not been detected in cell-free virions[228].

Role of Vpu in HIV-1-infected CD4 T-cell death

Vpu can form cation-selective ion channels in artificial lipid bilayers in amphibian oocytes and E. coli, inducing conductance with no discrimination of Na+ over K+ [229,230]. This modification of the membrane permeability may induce cell sensibility to apoptotic stimuli as perturbation of K+ levels in T-cells has been shown to cause apoptosis[231]. On the other hand, expression of Vpu in HIV-1-infected T-cells renders those cells more sensitive to Fas-induced apoptosis[232]. Furthermore, it has been demonstrated that Vpu inhibits the degradation of IκB, resulting in its accumulation and inactivation of NFκB. That leads to caspase-dependent apoptosis by suppressing the NFκB-dependent expression of the anti-apoptotic factor Bcl-xL[233]. However, recent results indicate that the effect of Vpu on direct cell killing in HIV-1-infected cells is not substantial, especially at low levels of infection[188].

In contrast to those proapoptotic effects, Vpu has been shown to enhance HIV-1 release from infected cells, avoiding accumulation of viral proteins like Env on the cell surface, and consequently reducing the syncytium-induced cytopathic effects[234]. Furthermore, by modulating CD4 cell-surface expression, Vpu may prevent superinfection of the cell, and more importantly Env-mediated apoptosis[235]. Indeed, the transmembrane region of Vpu is necessary for enhancement of virus particle release from the cell surface, while its C-terminal part and the phosphorylation of two serine residues (52 and 56)[236] are responsible for the reduction of CD4 levels by trapping it in the ER. It has been shown that CD4 and Vpu interact physically in the ER, and that Vpu is necessary, but not sufficient to induce CD4 degradation[237]. Vpu also down-regulates the MHC-I molecule in the host cell[238], allowing the infected cells to escape detection and killing by cytotoxic T-cells.

Role of Vpu in bystander CD4 T-cell death

So far there is no direct proof that Vpu can induce cell death in the bystander cells.

Protease

The HIV-1 protease (PR) is an 11 kDa regulatory protein that belongs to the family of aspartic proteases containing two copies of the triplet Asp-Thr-Glu[239]. It is produced as a monomer, but is fully active as a homodimer[240]. Dimerization occurs when the concentration of PR is sufficiently high[241].

One of the major functions of PR is the cleavage of viral protein precursors during the late stages of the viral assembly process, a function essential for the maturation of infectious virions[242]. PR has also been reported to participate in HIV-1-induced cell death.

Role of protease in HIV-1-infected CD4 T-cell death

Besides the cleavage of viral polyproteins, it has been shown that PR can also cleave several host cell proteins. Indeed, it has been demonstrated that recombinant HIV-1 PR cleaves *in vitro* the cytoskeleton proteins vimentin, desmin, glial fibrillary acidic protein[243], and the translational initiation factor eIF4GI[244]. Actin, troponin C, Alzheimer amyloid precursor protein and pro-IL-1β can also be PR substrates *in vitro*[245]. Interestingly, there is some evidence that the viral PR is active in the cytoplasmic fraction of acutely infected cells before virus budding, raising the possibility that PR may play a role in virus-induced cytotoxicity in hydrolyzing physiologically important host cellular proteins[241].

Importantly, HIV-1 PR cleaves Bcl-2, and this cleavage correlates with a decrease in the intracellular concentration of glutathione and oxidative stress-promoted activation of the DNA-binding property of NFκB[246]. Further evidence for the influence of PR on cell death is provided by the observation of its ability to directly cleave procaspase-8, which in turn activates Bid, inducing mitochondrial release of cytochrome-c and activation of caspases-9 and -3[247].

Another interesting point is the fact that specific inhibition of HIV-1 PR by synthetic inhibitors prevents virus-induced T-cell death[248]. Indeed, protease inhibitor (PI)-based therapy results in improved CD4 T-cell counts and reduced T-cell apoptosis in HIV-1-infected patients[249]. In addition, PI may possess intrinsic antiapoptotic properties. For instance, it has been shown that PI treatment of T-cells *in vitro* reduces susceptibility to apoptosis[250,251]. Several mechanisms have been proposed to explain this antiapoptotic effect: (i) decrease in expression of apoptosis regulatory molecules like caspase-1[251]; (ii) inhibition of calpains[252]; (iii) alteration of proliferative responses and inhibition of lymphocyte cell cycle entry[253]; and (iv) direct inhibitory effect on mitochondrial membrane permeability[254]. However, further research into the mechanisms of action of PI is needed.

Role of protease in bystander CD4 T-cell death

So far, there is no evidence that PR can induce cell death in the bystander cells.

Vif

Viral infectivity factor (Vif) is a basic 23 kDa HIV-1 accessory protein. It is synthesized late in the viral infection in a Rev-dependent manner[255]. In the cell, Vif has been detected in the nucleus[256], the cytosol, and is associated to cell membrane[257]. However, Vif

does not appear to be incorporated in the virion[258]. This viral protein has several key functions in the infection: it is necessary for the production of virions by CD4 T-cells[259], and it enhances virion infectivity[260] and efficient viral transmission[261]. It also protects HIV-1 from the antiviral activity of several members of the APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide like) family of cytidine deaminases (APOBEC3G and APOBEC3F) by inhibiting their incorporation into the virions[262,263]. Moreover, Vif targets APOBEC3G[264] and APOBEC3F[265,266] for ubiquitination followed by proteasome degradation, thus preventing the editing and degradation of viral DNA in infected CD4 T-cells.

Role of Vif in HIV-1-infected CD4 T-cell death

The role of Vif in the viral pathogenesis is still controversial. Recent evidence shows that inactivation of both Vif and Vpr leads to a decrease in infected CD4 T-cell death, which indicates that together these proteins play an important cytopathic effect[188]. Alternatively, Vif contributes to the cell cycle G2 arrest in HIV-1-infected primary human CD4 T-cells, and the deletion of both Vpr and Vif abolishes completely this arrest[267].

Role of Vif in bystander CD4 T-cell death

A possible contribution of Vif to the viral pathogenic effect triggered in bystander cells has not been determined until now.

Conclusion

The HIV-1 uses multiple strategies to manipulate different cell-death mechanisms to its own advantage. Structural, regulatory and accessory HIV-1 proteins, which are produced throughout the viral life cycle, are major actors of this strategy in both inducing and/or inhibiting CD4 T-cell death, depending on their level of expression, localization, biochemical characteristics, presence in the extracellular medium, and status of the target cells. Even though contradictory results exist, the overall data suggest that the same viral proteins are able to trigger survival of HIV-1-infected cells for long enough to ensure a productive infection and death of bystander CD4 T-lymphocytes, which cannot be productively infected by HIV-1.

On the other hand, the different forms of PCD are strongly connected and can substitute each other. This fact suggests that apoptosis is not the only PCD pathway targeted by HIV-1 viral proteins. First evidence of this new concept came from the demonstration that Env triggers autophagy, and then apoptosis, in bystander CD4 T-cells. Further studies are needed to better characterize HIV-1-induced cell-death pathways and the role of HIV-1 proteins in their modulation to develop more effective antiviral therapies.

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References

1. Kroemer G, El-Deiry W, Golstein P, et al. Classification of cell death: recommendations of the Nomenclature Committee on Cell Death. Cell Death Differ 2005;12(Suppl 2):1463-7.
2. Krammer P. CD95's deadly mission in the immune system (in process citation). Nature 2000;407:789-95.
3. Peter M, Krammer P. The CD95(APO-1/Fas) DISC and beyond. Cell Death Differ 2003;10:26-35.
4. Kaufmann S, Hengartner M. Programmed cell death: alive and well in the new millennium. Trends Cell Biol 2001;11:526-34.
5. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 1998; 94:481-90.
6. Scaffidi C, Fulda S, Srinivasan A, et al. Two CD95 (APO-1/Fas) signaling pathways. Embo J 1998;17:1675-87.
7. Green D. Overview: apoptotic signaling pathways in the immune system. Immunol Rev 2003;193:5-9.
8. Deveraux Q, Reed J. IAP family proteins–suppressors of apoptosis. Genes Dev 1999;13:239-52.
9. Chipuk J, Green D. Do inducers of apoptosis trigger caspase-independent cell death? Nat Rev Mol Cell Biol 2005;6:268-75.
10. Wang C, Klionsky D. The molecular mechanism of autophagy. Mol Med 2003;9:65-76.
11. Yorimitsu T, Klionsky D. Autophagy: molecular machinery for self-eating. Cell Death Differ 2005;12(Suppl 2):1542-52.
12. Kelekar A. Autophagy. Ann N Y Acad Sci 2005;1066:259-71.
13. Klionsky D. The molecular machinery of autophagy: unanswered questions. J Cell Sci 2005;118:7-18.
14. Klionsky D. Autophagy. Curr Biol 2005;15:R282-3.
15. Marino G, Lopez-Otin C. Autophagy: molecular mechanisms, physiological functions and relevance in human pathology. Cell Mol Life Sci 2004;61:1439-54.
16. Mizushima N, Sugita H, Yoshimori T, Ohsumi Y. A new protein conjugation system in human. The counterpart of the yeast Apg12p conjugation system essential for autophagy. J Biol Chem 1998; 273:33889-92.
17. Ohsumi Y. Molecular dissection of autophagy: two ubiquitin-like systems. Nat Rev Mol Cell Biol 2001;2:211-6.
18. Kihara A, Kabeya Y, Ohsumi Y, Yoshimori T. Beclin-phosphatidylinositol 3-kinase complex functions at the trans-Golgi network. EMBO Rep 2001;2:330-5.
19. Liang X, Jackson S, Seaman M, et al. Induction of autophagy and inhibition of tumorigenesis by Beclin 1. Nature 1999;402:672-6.
20. Kuma A, Hatano M, Matsui M, et al. The role of autophagy during the early neonatal starvation period. Nature 2004;432:1032-6.
21. Lum J, Bauer D, Kong M, et al. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005;120:237-48.
22. Pattingre S, Tassa A, Qu X, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005;122:927-39.
23. Shimizu S, Kanaseki T, Mizushima N, et al. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004;6:1221-8.
24. Yue Z, Jin S, Yang C, Levine A, Heintz N. Beclin 1, an autophagy gene essential for early embryonic development, is a haplo-insufficient tumor suppressor. Proc Natl Acad Sci USA 2003;100:15077-82.
25. Baehrecke E. Autophagic programmed cell death in drosophila. Cell Death Differ 2003;10:940-5.
26. Debnath J, Baehrecke E, Kroemer G. Does autophagy contribute to cell death? Autophagy 2005;1:e10-8.
27. Levine B, Yuan J. Autophagy in cell death: an innocent convict? J Clin Invest 2005;115:2679-88.
28. Bursch W, Ellinger A, Kienzl H, et al. Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy. Carcinogenesis 1996;17:1595-607.

29. Janicke R, Ng P, Sprengart M, Porter A. Caspase-3 is required for α-fodrin cleavage but dispensable for cleavage of other death substrates in apoptosis. J Biol Chem 1998;273:15540-5.
30. Kanzawa T, Germano I, Komata T, Ito H, Kondo Y, Kondo S. Role of autophagy in temozolomide-induced cytotoxicity for malignant glioma cells. Cell Death Differ 2004;11:448-57.
31. Opipari A, Tan L, Boitano A, Sorenson D, Aurora A, Liu J. Resveratrol-induced autophagocytosis in ovarian cancer cells. Cancer Res 2004;64:696-703.
32. Shao Y, Gao Z, Marks P, Jiang X. Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. Proc Natl Acad Sci USA 2004;101:18030-5.
33. Kabeya Y, Mizushima N, Ueno T, et al. LC3, a mammalian homolog of yeast Apg8p, is localized in autophagosome membranes after processing. Embo J 2000;19:5720-8.
34. Lockshin R, Zakeri Z. Apoptosis, autophagy, and more. Int J Biochem Cell Biol 2004;36:2405-19.
35. Kamada Y, Sekito T, Ohsumi Y. Autophagy in yeast: a TOR-mediated response to nutrient starvation. Curr Top Microbiol Immunol 2004;279:73-84.
36. Nair U, Klionsky D. Molecular mechanisms and regulation of specific and nonspecific autophagy pathways in yeast. J Biol Chem 2005;280:41785-8.
37. Yang Y, Liang Z, Gu Z, Qin Z. Molecular mechanism and regulation of autophagy. Acta Pharmacol Sin 2005;26:1421-34.
38. Eguchi Y, Shimizu S, Tsujimoto Y. Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res 1997;57: 1835-40.
39. Leist M, Single B, Castoldi A, Kuhnle S, Nicotera P. Intracellular ATP concentration: a switch in the decision between apoptosis and necrosis. J Exp Med 1997;185:1481-6.
40. Jaattela M, Tschopp J. Caspase-independent cell death in T-lymphocytes. Nat Immunol 2003;4:416-23.
41. Yuan J, Lipinski M, Degterev A. Diversity in the mechanisms of neuronal cell death. Neuron 2003;40:401-13.
42. Holler N, Zaru R, Micheau O, et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 2000;1:489-95.
43. Goossens V, De Vos K, Vercammen D, et al. Redox regulation of TNF signaling. Biofactors 1999;10:145-56.
44. Vercammen D, Brouckaert G, Denecker G, et al. Dual signaling of the Fas receptor: initiation of both apoptotic and necrotic cell death pathways. J Exp Med 1998;188:919-30.
45. Hetz C, Torres V, Quest A. Beyond apoptosis: non-apoptotic cell death in physiology and disease. Biochem Cell Biol 2005;83: 579-88.
46. Golstein P, Kroemer G. Redundant cell death mechanisms as relics and backups. Cell Death Differ 2005;12(Suppl 2):1490-6.
47. Broker L, Kruyt F, Giaccone G. Cell death independent of caspases: a review. Clin Cancer Res 2005;11:3155-62.
48. Proskuryakov S, Gabai V, Konoplyannikov A. Necrosis is an active and controlled form of programmed cell death. Biochemistry (Mosc) 2002;67:387-408.
49. Proskuryakov S, Konoplyannikov A, Gabai V. Necrosis: a specific form of programmed cell death? Exp Cell Res 2003;283:1-16.
50. Tsujimoto Y, Shimizu S. Another way to die: autophagic programmed cell death. Cell Death Differ 2005;12(Suppl 2):1528-34.
51. Nelson D, White E. Exploiting different ways to die. Genes Dev 2004;18:1223-6.
52. Fauci A. HIV: infectivity and mechanisms of pathogenesis. Science 1988;239:617-22.
53. McCune J. The dynamics of CD4+ T-cell depletion in HIV disease. Nature 2001;410:974-9.
54. Deeks S, Walker B. The immune response to AIDS virus infection: good, bad, or both? J Clin Invest 2004;113:808-10.
55. Grossman Z, Meier-Schellersheim M, Sousa A, Victorino R, Paul W. CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? Nat Med 2002;8:319-23.
56. Douek D, Picker L, Koup R. T-cell dynamics in HIV-1 infection. Annu Rev Immunol 2003;21:265-304.
57. Ameisen J, Capron A. Cell dysfunction and depletion in AIDS: the programmed cell death hypotheses. Immunol Today 1991;12:102-5.
58. Terai C, Kornbluth R, Pauza C, Richman D, Carson D. Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1. J Clin Invest 1991;87:1710-5.
59. Cotton M, Ikle D, Rapaport E, et al. Apoptosis of CD4+ and CD8+ T-cells isolated immediately *ex vivo* correlates with disease severity in HIV-1 infection. Pediatr Res 1997;42:656-64.
60. Gougeon M, Lecoeur H, Dulioust A, et al. Programmed cell death in peripheral lymphocytes from HIV-infected persons: increased susceptibility to apoptosis of CD4 and CD8 T-cells correlates with lymphocyte activation and with disease progression. J Immunol 1996;156:3509-20.
61. Badley A, Dockrell D, Algeciras A, et al. *In vivo* analysis of Fas/FasL interactions in HIV-infected patients. J Clin Invest 1998;102:79-87.
62. Badley A, Parato K, Cameron D, et al. Dynamic correlation of apoptosis and immune activation during treatment of HIV infection. Cell Death Differ 1999;6:420-32.
63. Bohler T, Walcher J, Holzl-Wenig G, et al. Early effects of antiretroviral combination therapy on activation, apoptosis and regeneration of T-cells in HIV-1-infected children and adolescents. Aids 1999; 13:779-89.
64. Espert L, Denizot M, Grimaldi M, et al. Autophagy is involved in T-cell death after binding of HIV-1 envelope proteins to CXCR4. J Clin Invest 2006;116:2161-72.
65. Lenardo M, Angleman S, Bounkeua V, et al. Cytopathic killing of peripheral blood CD4+ T-lymphocytes by HIV-1 appears necrotic rather than apoptotic and does not require Env. J Virol 2002; 76:5082-93.
66. Gougeon M. To kill or be killed: how HIV exhausts the immune system. Cell Death Differ 2005;12(Suppl 1):845-54.
67. Leonard R, Zagury D, Desportes I, Bernard J, Zagury J, Gallo R. Cytopathic effect of HIV in T4 cells is linked to the last stage of virus infection. Proc Natl Acad Sci USA 1988;85:3570-4.
68. Koga Y, Lindstrom E, Fenyo E, Wigzell H, Mak T. High levels of hetero-disperse RNA accumulate in T-cells infected with HIV and in normal thymocytes. Proc Natl Acad Sci USA 1988;85:4521-5.
69. Pauza C, Galindo J, Richman D. Reinfection results in accumulation of unintegrated viral DNA in cytopathic and persistent HIV-1 infection of CEM cells. J Exp Med 1990;172:1035-42.
70. Levy J. Pathogenesis of HIV infection. Microbiol Rev 1993;57:183-289.
71. Shaw G, Hahn B, Arya S, Groopman J, Gallo R, Wong-Staal F. Molecular characterization of human T-cell leukemia (lymphotropic) virus type III in AIDS. Science 1984;226:1165-71.
72. Funk G, Oxenius A, Fischer M, et al. HIV replication elicits little cytopathic effects in vivo: analysis of surrogate markers for virus production, cytotoxic T-cell response and infected cell death. J Med Virol 2006;78:1141-6.
73. Lum J, Badley A. Resistance to apoptosis: mechanism for the development of HIV reservoirs. Curr HIV Res 2003;1:261-74.
74. Finkel T, Tudor-Williams G, Banda N, et al. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med 1995;1:129-34.
75. Ho D, Neumann A, Perelson A, Chen W, Leonard J, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995;373:123-6.
76. Perelson A, Neumann A, Markowitz M, Leonard J, Ho D. HIV-1 dynamics *in vivo*: virion clearance rate, infected cell life-span, and viral generation time. Science 1996;271:1582-6.
77. Wei X, Ghosh S, Taylor M, et al. Viral dynamics in HIV-1 infection. Nature 1995;373:117-22.
78. Debatin K, Fahrig-Faissner A, Enenkel-Stoodt S, Kreuz W, Benner A, Krammer PH. High expression of APO-1 (CD95) on T-lymphocytes from HIV-1-infected children. Blood 1994;83:3101-3.
79. Igarashi T, Brown C, Byrum R, et al. Rapid and irreversible CD4+ T-cell depletion induced by the highly pathogenic SHIV(DH12R) is systemic and synchronous. J Virol 2002;76:379-91.
80. Miura Y, Misawa N, Maeda N, et al. Critical contribution of TTRAIL to apoptosis of human CD4+ T-cells in HIV-1-infected hu-PBL-NOD-SCID mice. J Exp Med 2001;193:651-60.

81. Monceaux V, Estaquier J, Fevrier M, et al. Extensive apoptosis in lymphoid organs during primary SIV infection predicts rapid progression towards AIDS. Aids 2003;17:1585-96.
82. Hanabuchi S, Koyanagi M, Kawasaki A, et al. Fas and its ligand in a general mechanism of T-cell-mediated cytotoxicity. Proc Natl Acad Sci USA 1994;91:4930-4.
83. Kaplan D, Sieg S. Role of the Fas/Fas ligand apoptotic pathway in HIV-1 disease. J Virol 1998;72:6279-82.
84. Aries S, Schaaf B, Muller C, Dennin R, Dalhoff K. Fas (CD95) expression on CD4+ T-cells from HIV-infected patients increases with disease progression. J Mol Med 1995;73:591-3.
85. 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;88:1741-6.
86. Estaquier J, Idziorek T, Zou W, et al. T helper type 1/T helper type 2 cytokines and T-cell death: preventive effect of IL-12 on activation-induced and CD95 (FAS/APO-1)-mediated apoptosis of CD4+ T-cells from HIV-infected persons. J Exp Med 1995;182:1759-67.
87. Gehri R, Hahn S, Rothen M, Steuerwald M, Nuesch R, Erb P. The Fas receptor in HIV infection: expression on peripheral blood lymphocytes and role in the depletion of T-cells. Aids 1996;10:9-16.
88. Hosaka N, Oyaizu N, Kaplan M, Yagita H, Pahwa S. Membrane and soluble forms of Fas (CD95) and Fas ligand in peripheral blood mononuclear cells and in plasma from HIV-infected persons. J Infect Dis 1998;178:1030-9.
89. Katsikis P, Wunderlich E, Smith C, Herzenberg L, Herzenberg L. Fas antigen stimulation induces marked apoptosis of T-lymphocytes in HIV-infected individuals. J Exp Med 1995;181:2029-36.
90. Silvestris F, Cafforio P, Frassanito M, et al. Overexpression of Fas antigen on T-cells in advanced HIV-1 infection: differential ligation constantly induces apoptosis. Aids 1996;10:131-41.
91. Alimonti J, Ball T, Fowke K. Mechanisms of CD4+ T-lymphocyte cell death in HIV infection and AIDS. J Gen Virol 2003;84:1649-61.
92. Herbeuval J, Grivel J, Boasso A, et al. CD4+ T-cell death induced by infectious and noninfectious HIV-1: role of type 1 IFN-dependent, TRAIL/DR5-mediated apoptosis. Blood 2005;106:3524-31.
93. O'Connor L, Strasser A, O'Reilly L, et al. Bim: a novel member of the Bcl-2 family that promotes apoptosis. Embo J 1998;17:384-95.
94. Hildeman D, Mitchell T, Teague T, et al. Reactive oxygen species regulate activation-induced T-cell apoptosis. Immunity 1999; 10:735-44.
95. Ferraro-Peyret C, Quemeneur L, Flacher M, Revillard J, Genestier L. Caspase-independent phosphatidylserine exposure during apoptosis of primary T-lymphocytes. J Immunol 2002;169:4805-10.
96. Heinkelein M, Sopper S, Jassoy C. Contact of HIV-1-infected and uninfected CD4+ T-lymphocytes is highly cytolytic for both cells. J Virol 1995;69:6925-31.
97. Laurent-Crawford A, Krust B, Riviere Y, et al. Membrane expression of HIV Env triggers apoptosis in CD4 cells. AIDS Res Hum Retroviruses 1993;9:761-73.
98. Ohnimus H, Heinkelein M, Jassoy C. Apoptotic cell death upon contact of CD4+ T-lymphocytes with HIV glycoprotein-expressing cells is mediated by caspases but bypasses CD95 (Fas/Apo-1) and TNF receptor 1. J Immunol 1997;159:5246-52.
99. Roshal M, Zhu Y, Planelles V. Apoptosis in AIDS. Apoptosis 2001; 6:103-16.
100. Alkhatib G, Combadiere C, Broder C, et al. CC-CKR5: a RANTES, MIP-1α, MIP-1β receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 1996;272:1955-8.
101. Bleul C, Farzan M, Choe H, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTRE/fusin and blocks HIV-1 entry. Nature 1996;382:829-33.
102. Feng Y, Broder C, Kennedy P, Berger E. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science 1996;272:872-7.
103. Weissenhorn W, Dessen A, Harrison S, Skehel J, Wiley D. Atomic structure of the ectodomain from HIV-1 gp41 [see comments]. Nature 1997;387:426-30.
104. Piguet V, Sattentau Q. Dangerous liaisons at the virologic synapse. J Clin Invest 2004;114:605-10.
105. Munoz-Barroso I, Durell S, Sakaguchi K, Appella E, Blumenthal R. Dilation of the HIV-1 Env fusion pore revealed by the inhibitory action of a synthetic peptide from gp41. J Cell Biol 1998; 140:315-23.
106. Lifson J, Feinberg M, Reyes G, et al. Induction of CD4-dependent cell fusion by the HTLV-III/LAV Env. Nature 1986;323:725-8.
107. Sodroski J, Goh W, Rosen C, Campbell K, Haseltine W. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. Nature 1986;322:470-4.
108. Yoffe B, Lewis D, Petrie B, Noonan C, Melnick J, Hollinger F. Fusion as a mediator of cytolysis in mixtures of uninfected CD4+ lymphocytes and cells infected by HIV. Proc Natl Acad Sci USA 1987; 84:1429-33.
109. Navia B, Cho E, Petito C, Price R. The AIDS dementia complex: II. Neuropathology. Ann Neurol 1986;19:525-35.
110. Frankel S, Wenig B, Burke A, et al. Replication of HIV-1 in dendritic cell-derived syncytia at the mucosal surface of the adenoid. Science 1996;272:115-7.
111. Scheller C, Jassoy C. Syncytium formation amplifies apoptotic signals: a new view on apoptosis in HIV infection *in vitro*. Virology 2001;282:48-55.
112. Roumier T, Castedo M, Perfettini J, et al. Mitochondrion-dependent caspase activation by the HIV-1 envelope. Biochem Pharmacol 2003;66:1321-9.
113. Castedo M, Roumier T, Blanco J, et al. Sequential involvement of Cdk1, mTOR and p53 in apoptosis induced by the HIV-1 envelope. Embo J 2002;21:4070-80.
114. Ferri K, Jacotot E, Blanco J, et al. Apoptosis control in syncytia induced by the HIV-1-Env complex. Role of mitochondria and caspases. J Exp Med 2000;192:1081-92.
115. Hoxie J, Alpers J, Rackowski J, et al. Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. Science 1986; 234:1123-7.
116. Meissner E, Zhang L, Jiang S, Su L. Fusion-induced apoptosis contributes to thymocyte depletion by a pathogenic HIV-1 envelope in the human thymus. J Virol 2006;80:11019-30.
117. Misse D, Cerutti M, Noraz N, et al. A CD4-independent interaction of HIV-1 gp120 with CXCR4 induces their co-internalization, cell signaling, and T-cell chemotaxis. Blood 1999;93:2454-62.
118. Weissman D, Rabin R, Arthos J, et al. Macrophage-tropic HIV and SIV Env induce a signal through the CCR5 chemokine receptor. Nature 1997;389:981-5.
119. Davis C, Dikic I, Unutmaz D, et al. Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5. J Exp Med 1997;186:1793-8.
120. Bleul C, Wu L, Hoxie J, Springer T, MacKay C. The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T-lymphocytes. Proc Natl Acad Sci USA 1997; 94:1925-30.
121. Grivel J, Margolis L. CCR5- and CXCR4-tropic HIV-1 are equally cytopathic for their T-cell targets in human lymphoid tissue. Nat Med 1999;5:344-6.
122. Tateyama M, Oyaizu N, McCloskey T, Than S, Pahwa S. CD4 T-lymphocytes are primed to express Fas ligand by CD4 cross-linking and to contribute to CD8 T-cell apoptosis via Fas/FasL death signaling pathway. Blood 2000;96:195-202.
123. Algeciras-Schimnich A, Vlahakis S, Villasis-Keever A, et al. CCR5 mediates Fas- and caspase-8 dependent apoptosis of both uninfected and HIV infected primary human CD4 T-cells. Aids 2002; 16:1467-78.
124. Algeciras A, Dockrell D, Lynch D, Paya C. CD4 regulates susceptibility to Fas ligand- and TNF-mediated apoptosis. J Exp Med 1998;187:711-20.
125. Badley A, Dockrell D, Paya C. Apoptosis in AIDS. Adv Pharmacol 1997;41:271-94.
126. Katsikis P, Garcia-Ojeda M, Torres-Roca J, et al. IL-1β converting enzyme-like protease involvement in Fas-induced peripheral blood T-cell apoptosis in HIV infection. TRAIL can mediate activation-induced T-cell death in HIV infection. J Exp Med 1997;186:1365-72.
127. De Oliveira Pinto L, Garcia S, Lecoeur H, Rapp C, Gougeon M. Increased sensitivity of T-lymphocytes to TNF-R1- and TNF-R2-

mediated apoptosis in HIV infection: relation to expression of Bcl-2 and active caspase-8 and caspase-3. Blood 2002;99:1666-75.
128. Jeremias I, Herr I, Boehler T, Debatin K. TRAIL/Apo-2-ligand-induced apoptosis in human T-cells. Eur J Immunol 1998;28:143-52.
129. Herbeuval J, Shearer G. HIV-1 immunopathogenesis: How good interferon turns bad. Clin Immunol 2006 [Epub ahead of print].
130. Finco O, Nuti S, De Magistris M, et al. Induction of CD4+ T-cell depletion in mice doubly transgenic for HIV gp120 and human CD4. Eur J Immunol 1997;27:1319-24.
131. Kang Y, Melo E, Scott D. An ongoing immune response to HIV Env gp120 in human CD4- transgenic mice contributes to T-cell decline upon intravenous administration of gp120. Eur J Immunol 1998; 28:2253-64.
132. Berndt C, Mopps B, Angermuller S, Gierschik P, Krammer P. CXCR4 and CD4 mediate a rapid CD95-independent cell death in CD4+ T-cells. Proc Natl Acad Sci USA 1998;95:12556-61.
133. Blanco J, Jacotot E, Cabrera C, et al. The implication of the chemokine receptor CXCR4 in HIV-1 Env-induced apoptosis is independent of the G protein-mediated signaling. AIDS 1999;13:909-17.
134. Herbein G, VanLint C, Lovett J, Verdin E. Distinct mechanisms trigger apoptosis in HIV-1-infected and in uninfected bystander T-lymphocytes. J Virol 1998;72:660-70.
135. Noraz N, Gozlan J, Corbeil J, Brunner T, Spector S. HIV-induced apoptosis of activated primary CD4+ T-lymphocytes is not mediated by Fas-Fas ligand. AIDS 1997;11:1671-80.
136. Roggero R, Robert-Hebmann V, Harrington S, et al. Binding of HIV-1 gp120 to CXCR4 induces mitochondrial transmembrane depolarization and cytochrome c-mediated apoptosis independently of Fas signaling. J Virol 2001;75:7637-50.
137. Cicala C, Arthos J, Rubbert A, et al. HIV-1 Env induces activation of caspase-3 and cleavage of focal adhesion kinase in primary human CD4+ T-cells. Proc Natl Acad Sci USA 2000;97:1178-83.
138. Vlahakis S, Algeciras-Schimnich A, Bou G, et al. Chemokine-receptor activation by Env determines the mechanism of death in HIV-infected and uninfected T-lymphocytes. J Clin Invest 2001; 107:207-15.
139. Jekle A, Keppler O, De Clercq E, Schols D, Weinstein M, Goldsmith M. *In vivo* evolution of HIV-1 toward increased pathogenicity through CXCR4-mediated killing of uninfected CD4 T-cells. J Virol 2003; 77:5846-54.
140. Delobel P, Nugeyre M, Cazabat M, et al. Naive T-cell depletion related to infection by X4 HIV-1 in poor immunologic responders to HAART. J Virol 2006;80:10229-36.
141. Wild C, Shugars D, Greenwell T, McDanal C, Matthews T. Peptides corresponding to a predictive α-helical domain of HIV-1 gp41 are potent inhibitors of virus infection. Proc Natl Acad Sci USA 1994; 91:9770-4.
142. LaBonte J, Madani N, Sodroski J. Cytolysis by CCR5-using HIV-1 Env is dependent on membrane fusion and can be inhibited by high levels of CD4 expression. J Virol 2003;77:6645-59.
143. Stocker H, Scheller C, Jassoy C. Destruction of primary CD4+ T-cells by cell-cell interaction in HIV-1 infection *in vitro*. J Gen Virol 2000;81:1907-11.
144. Blanco J, Barretina J, Ferri K, et al. Cell-surface-expressed HIV-1 Env induces the death of CD4 T-cells during GP41-mediated hemifusion-like events. Virology 2003;305:318-29.
145. Garg H, Blumenthal R. HIV gp41-induced apoptosis is mediated by caspase-3-dependent mitochondrial depolarization, which is inhibited by HIV PI nelfinavir. J Leukoc Biol 2006;79:351-62.
146. Kuppuswamy M, Subramanian T, Srinivasan A, Chinnadurai G. Multiple functional domains of Tat, the trans-activator of HIV-1, defined by mutational analysis. Nucleic Acids Res 1989;17:3551-61.
147. Campbell G, Pasquier E, Watkins J, et al. The glutamine-rich region of the HIV-1 Tat protein is involved in T-cell apoptosis. J Biol Chem 2004;279:48197-204.
148. Jones K. Tat and the HIV-1 promoter. Curr Opin Cell Biol 1993; 5:461-8.
149. Hetzer C, Dormeyer W, Schnolzer M, Ott M. Decoding Tat: the biology of HIV Tat posttranslational modifications. Microbes Infect 2005; 7:1364-9.
150. Bres V, Kiernan R, Linares L, et al. A non-proteolytic role for ubiquitin in Tat-mediated transactivation of the HIV-1 promoter. Nat Cell Biol 2003;5:754-61.
151. Ammosova T, Berro R, Jerebtsova M, et al. Phosphorylation of HIV-1 Tat by CDK2 in HIV-1 transcription. Retrovirology 2006;3:78.
152. Apolloni A, Hooker C, Mak J, Harrich D. HIV-1 protease regulation of Tat activity is essential for efficient reverse transcription and replication. J Virol 2003;77:9912-21.
153. Bartz S, Emerman M. HIV-1 Tat induces apoptosis and increases sensitivity to apoptotic signals by upregulating FLICE/caspase-8. J Virol 1999;73:1956-63.
154. Westendorp M, Li-Weber M, Frank R, Krammer P. HIV-1 Tat upregulates IL-2 secretion in activated T-cells. J Virol 1994; 68: 4177-85.
155. Zauli G, Gibellini D, Milani D, et al. HIV-1 Tat protein protects lymphoid, epithelial, and neuronal cell lines from death by apoptosis. Cancer Res 1993;53:4481-5.
156. Finkel T, Tudor-Williams G, Banda N, et al. Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes. Nat Med 1995;1:129-34.
157. Gibellini D, Caputo A, Celeghini C, et al. Tat-expressing Jurkat cells show an increased resistance to different apoptotic stimuli, including acute HIV-1 infection. Br J Haematol 1995;89:24-33.
158. Gibellini D, Re M, Ponti C, et al. HIV-1 Tat protects CD4+ Jurkat T-lymphoblastoid cells from apoptosis mediated by TRAIL. Cell Immunol 2001;207:89-99.
159. Li C, Wang C, Friedman D, Pardee A. Reciprocal modulations between p53 and Tat of HIV-1. Proc Natl Acad Sci USA 1995;92: 5461-4.
160. Macho A, Calzado M, Jimenez-Reina L, Ceballos E, Leon J, Munoz E. Susceptibility of HIV-1-TAT transfected cells to undergo apoptosis. Biochemical mechanisms. Oncogene 1999;18:7543-51.
161. Sastry K, Marin M, Nehete P, McConnell K, el-Naggar A, McDonnell T. Expression of HIV-I Tat results in downregulation of bcl-2 and induction of apoptosis in hematopoietic cells. Oncogene 1996; 13: 487-93.
162. Chang H, Samaniego F, Nair B, Buonaguro L, Ensoli B. HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. AIDS 1997;11:1421-31.
163. Ensoli B, Buonaguro L, Barillari G, et al. Release, uptake, and effects of extracellular HIV-1 Tat protein on cell growth and viral transactivation. J Virol 1993;67:277-87.
164. Ensoli B, Barillari G, Salahuddin S, Gallo R, Wong-Staal F. Tat protein of HIV-1 stimulates growth of cells derived from Kaposi's sarcoma lesions of AIDS patients. Nature 1990;345:84-6.
165. Vendeville A, Rayne F, Bonhoure A, Bettache N, Montcourrier P, Beaumelle B. HIV-1 Tat enters T-cells using coated pits before translocating from acidified endosomes and eliciting biological responses. Mol Biol Cell 2004;15:2347-60.
166. Gutheil WG, Subramanyam M, Flentke GR, et al. HIV-1 Tat binds to dipeptidyl aminopeptidase IV (CD26): a possible mechanism for Tat's immunosuppressive activity. Proc Natl Acad Sci USA 1994; 91:6594-8.
167. Ensoli B, Buonaguro L, Barillari G, et al. Release, uptake, and effects of extracellular HIV-1 Tat protein on cell growth and viral transactivation. J Virol 1993;67:277-87.
168. Manna S, Aggarwal B. Differential requirement for p56lck in HIV-tat versus TNF-induced cellular responses: effects on NFκB, activator protein-1, c-Jun N-terminal kinase, and apoptosis. J Immunol 2000; 164:5156-66.
169. Li C, Friedman D, Wang C, Metelev V, Pardee A. Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. Science 1995;268:429-31.
170. Demarchi F, d'Adda di Fagagna F, Falaschi A, Giacca M. Activation of transcription factor NFκB by the Tat protein of HIV-1. J Virol 1996;70:4427-37.
171. De Mareuil J, Carre M, Barbier P, et al. HIV-1 Tat protein enhances microtubule polymerization. Retrovirology 2005;2:5.
172. Chen D, Wang M, Zhou S, Zhou Q. HIV-1 Tat targets microtubules to induce apoptosis, a process promoted by the pro-apoptotic Bcl-2 relative Bim. Embo J 2002;21:6801-10.

173. Li-Weber M, Laur O, Dern K, Krammer P. T-cell activation-induced and HIV Tat-enhanced CD95(APO-1/Fas) ligand transcription involves NFκB. Eur J Immunol 2000;30:661-70.
174. Zhang M, Li X, Pang X, et al. Identification of a potential HIV-induced source of bystander-mediated apoptosis in T-cells: upregulation of trail in primary human macrophages by HIV-1 Tat. J Biomed Sci 2001;8:290-6.
175. Campbell G, Watkins J, Esquieu D, Pasquier E, Loret E, Spector S. The C terminus of HIV-1 Tat modulates the extent of CD178-mediated apoptosis of T-cells. J Biol Chem 2005;280: 38376-82.
176. Checroune F, Yao X, Gottlinger H, Bergeron D, Cohen E. Incorporation of Vpr into HIV-1: role of conserved regions within the P6 domain of Pr55gag. J Acquir Immune Defic Syndr Hum Retrovirol 1995;10:1-7.
177. Le Rouzic E, Benichou S. The Vpr protein from HIV-1: distinct roles along the viral life cycle. Retrovirology 2005;2:11.
178. Mahalingam S, Ayyavoo V, Patel M, Kieber-Emmons T, Weiner D. Nuclear import, virion incorporation, and cell cycle arrest/differentiation are mediated by distinct functional domains of HIV-1 Vpr. J Virol 1997;71:6339-47.
179. Heinzinger N, Bukinsky M, Haggerty S, et al. The Vpr protein of HIV-1 influences nuclear localization of viral nucleic acids in nondividing host cells. Proc Natl Acad Sci USA 1994;91:7311-5.
180. Jowett J, Planelles V, Poon B, Shah N, Chen M, Chen I. The HIV-1 vpr gene arrests infected T-cells in the G2 + M phase of the cell cycle. J Virol 1995;69:6304-13.
181. Cohen E, Dehni G, Sodroski J, Haseltine W. HIV Vpr product is a virion-associated regulatory protein. J Virol 1990;64:3097-9.
182. Conti L, Rainaldi G, Matarrese P, et al. The HIV-1 Vpr protein acts as a negative regulator of apoptosis in a human lymphoblastoid T-cell line: possible implications for the pathogenesis of AIDS. J Exp Med 1998;187:403-13.
183. Zhu Y, Roshal M, Li F, Blackett J, Planelles V. Upregulation of survivin by HIV-1 Vpr. Apoptosis 2003;8:71-9.
184. Fukumori T, Akari H, Iida S, et al. The HIV-1 Vpr displays strong anti-apoptotic activity. FEBS Lett 1998;432:17-20.
185. Ayyavoo V, Mahboubi A, Mahalingam S, et al. HIV-1 Vpr suppresses immune activation and apoptosis through regulation of NFκB. Nat Med 1997;3:1117-23.
186. Muthumani K, Hwang D, Desai B, et al. HIV-1 Vpr induces apoptosis through caspase-9 in T-cells and PBMC. J Biol Chem 2002; 277:37820-31.
187. Shostak L, Ludlow J, Fisk J, et al. Roles of p53 and caspases in the induction of cell cycle arrest and apoptosis by HIV-1 Vpr. Exp Cell Res 1999;251:156-65.
188. Sakai K, Dimas J, Lenardo M. The Vif and Vpr accessory proteins independently cause HIV-1-induced T-cell cytopathicity and cell cycle arrest. Proc Natl Acad Sci USA 2006;103:3369-74.
189. Yu X, Matsuda M, Essex M, Lee T. Open reading frame Vpr of SIV encodes a virion-associated protein. J Virol 1990;64:5688-93.
190. Hrimech M, Yao X, Bachand F, Rougeau N, Cohen E. HIV-1 Vpr functions as an immediate-early protein during HIV-1 infection. J Virol 1999;73:4101-9.
191. Levy D, Refaeli Y, MacGregor R, Weiner D. Serum Vpr regulates productive infection and latency of HIV-1. Proc Natl Acad Sci USA 1994;91:10873-7.
192. Huang M, Weeks O, Zhao L, Saltarelli M, Bond V. Effects of extracellular HIV-1 Vpr protein in primary rat cortical cell cultures. J Neurovirol 2000;6:202-20.
193. Tungaturthi P, Sawaya B, Singh S, et al. Role of HIV-1 Vpr in AIDS pathogenesis: relevance and implications of intra-virion, intracellular and free Vpr. Biomed Pharmacother 2003;57:20-4.
194. Jacotot E, Ravagnan L, Loeffler M, et al. The HIV-1 Vpr induces apoptosis via a direct effect on the mitochondrial permeability transition pore. J Exp Med 2000;191:33-46.
195. Arunagiri C, Macreadie I, Hewish D, Azad A. A C-terminal domain of HIV-1 accessory protein Vpr is involved in penetration, mitochondrial dysfunction and apoptosis of human CD4+ lymphocytes. Apoptosis 1997;2:69-76.
196. Lorenzo H, Susin S, Penninger J, Kroemer G. Apoptosis inducing factor: a phylogenetically old, caspase-independent effector of cell death. Cell Death Differ 1999;6:516-24.
197. Roumier T, Vieira H, Castedo M, et al. The C-terminal moiety of HIV-1 Vpr induces cell death via a caspase-independent mitochondrial pathway. Cell Death Differ 2002;9:1212-9.
198. Borgne-Sanchez A, Dupont S, Langonne A, et al. Targeted Vpr-derived peptides reach mitochondria to induce apoptosis of α(V)β(3)-expressing endothelial cells. Cell Death Differ 2006 [Epub ahead of print].
199. Wu Y, Marsh J. Selective transcription and modulation of resting T-cell activity by preintegrated HIV-DNA. Science 2001;293:1503-6.
200. Kotov A, Zhou J, Flicker P, Aiken C. Association of Nef with the HIV-1 core. J Virol 1999;73:8824-30.
201. Fujii Y, Otake K, Tashiro M, Adachi A. Soluble Nef antigen of HIV-1 is cytotoxic for human CD4+ T-cells. FEBS Lett 1996;393:93-6.
202. Greenberg M, Bronson S, Lock M, Neumann M, Pavlakis G, Skowronski J. Co-localization of HIV-1 Nef with the AP-2 adaptor protein complex correlates with Nef-induced CD4 downregulation. Embo J 1997;16:6964-76.
203. Kaminchik J, Margalit R, Yaish S, et al. Cellular distribution of HIV-1 Nef protein: identification of domains in Nef required for association with membrane and detergent-insoluble cellular matrix. AIDS Res Hum Retroviruses 1994;10:1003-10.
204. Das S, Jameel S. Biology of the HIV Nef protein. Indian J Med Res 2005;121:315-32.
205. Simmons A, Aluvihare V, McMichael A. Nef triggers a transcriptional program in T-cells imitating single-signal T-cell activation and inducing HIV virulence mediators. Immunity 2001;14:763-77.
206. Geleziunas R, Xu W, Takeda K, Ichijo H, Greene W. HIV-1 Nef inhibits ASK1-dependent death signaling providing a potential mechanism for protecting the infected host cell. Nature 2001;410:834-8.
207. Gross A, McDonnell J, Korsmeyer S. BCL-2 family members and the mitochondria in apoptosis. Genes Dev 1999;13:1899-911.
208. Wolf D, Witte V, Laffert B, et al. HIV-1 Nef associated PAK and PI3-kinases stimulate Akt-independent Bad-phosphorylation to induce anti-apoptotic signals. Nat Med 2001;7:1217-24.
209. Greenway A, McPhee D, Allen K, et al. HIV-1 Nef binds to tumor suppressor p53 and protects cells against p53-mediated apoptosis. J Virol 2002;76:2692-702.
210. Xu X, Laffert B, Screaton G, et al. Induction of FasL expression by HIV involves the interaction of Nef with the T-cell receptor zeta chain. J Exp Med 1999;189:1489-96.
211. Zauli G, Gibellini D, Secchiero P, et al. HIV-1 Nef protein sensitizes CD4+ T-lymphoid cells to apoptosis via functional upregulation of the CD95/CD95 ligand pathway. Blood 1999;93:1000-10.
212. Bell I, Ashman C, Maughan J, Hooker E, Cook F, Reinhart T. Association of SIV Nef with the T-cell receptor (TCR) zeta chain leads to TCR down-modulation. J Gen Virol 1998;79:2717-27.
213. Swigut T, Shohdy N, Skowronski J. Mechanism for down-regulation of CD28 by Nef. Embo J 2001;20:1593-604.
214. Aiken C, Konner J, Landau N, Lenburg M, Trono D. Nef induces CD4 endocytosis: requirement for a critical dileucine motif in the membrane-proximal CD4 cytoplasmic domain. Cell 1994;76:853-64.
215. Schwartz O, Marechal V, Le Gall S, Lemonnier F, Heard J. Endocytosis of MHC class I molecules is induced by the HIV-1 Nef protein. Nat Med 1996;2:338-42.
216. Benson R, Sanfridson A, Ottinger J, Doyle C, Cullen B. Downregulation of cell-surface CD4 expression by SIV Nef prevents viral superinfection. J Exp Med 1993;177:1561-6.
217. Collins K, Chen B, Kalams S, Walker B, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T-lymphocytes. Nature 1998;391:397-401.
218. Luo T, Fredericksen B, Hasumi K, Endo A, Garcia J. HIV-1 Nef-induced CD4 cell surface downregulation is inhibited by ikarugamycin. J Virol 2001;75:2488-92.
219. Luo T, Anderson S, Garcia J. Inhibition of Nef- and phorbol ester-induced CD4 degradation by macrolide antibiotics. J Virol 1996; 70:1527-34.

220. Rhee S, Marsh J. HIV-1 Nef activity in murine T-cells. CD4 modulation and positive enhancement. J Immunol 1994;152:5128-34.
221. Sanfridson A, Hester S, Doyle C. Nef proteins encoded by HIV and SIV induce the accumulation of endosomes and lysosomes in human T-cells. Proc Natl Acad Sci USA 1997;94:873-8.
222. James C, Huang M, Khan M, Garcia-Barrio M, Powell M, Bond V. Extracellular Nef protein targets CD4+ T-cells for apoptosis by interacting with CXCR4 surface receptors. J Virol 2004;78: 3099-109.
223. Curtain C, Separovic F, Rivett D, et al. Fusogenic activity of aminoterminal region of HIV-1 Nef protein. AIDS Res Hum Retroviruses 1994;10:1231-40.
224. Curtain C, Lowe M, Arunagiri C, Mobley P, Macreadie I, Azad A. Cytotoxic activity of the aminoterminal region of HIV-1 Nef protein. AIDS Res Hum Retroviruses 1997;13:1213-20.
225. Maldarelli F, Chen MY, Willey R, Strebel K. HIV-1 Vpu protein is an oligomeric type I integral membrane protein. J Virol 1993; 67:5056-61.
226. Bour S, Schubert U, Peden K, Strebel K. The Env of HIV-2 enhances viral particle release: a Vpu-like factor? J Virol 1996; 70: 820-9.
227. Schubert U, Anton L, Bacik I, et al. CD4 glycoprotein degradation induced by HIV-1 Vpu protein requires the function of proteasomes and the ubiquitin-conjugating pathway. J Virol 1998;72:2280-8.
228. Strebel K, Klimkait T, Maldarelli F, Martin M. Molecular and biochemical analyses of HIV-1 Vpu protein. J Virol 1989;63:3784-91.
229. Schubert U, Ferrer-Montiel A, Oblatt-Montal M, Henklein 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;398:12-8.
230. Ewart G, Sutherland T, Gage P, Cox GB. The Vpu protein of HIV-1 forms cation-selective ion channels. J Virol 1996;70:7108-15.
231. Szabo I, Gulbins E, Apfel H, et al. Tyrosine phosphorylation-dependent suppression of a voltage-gated K+ channel in T-lymphocytes upon Fas stimulation. J Biol Chem 1996;271:20465-9.
232. Casella C, Rapaport E, Finkel T. Vpu increases susceptibility of HIV-1-infected cells to Fas killing. J Virol 1999;73:92-100.
233. Akari H, Bour S, Kao S, Adachi A, Strebel K. The HIV-1 accessory protein Vpu induces apoptosis by suppressing the NFκB-dependent expression of antiapoptotic factors. J Exp Med 2001;194: 1299-312.
234. Yao X, Garzon S, Boisvert F, Haseltine W, Cohen E. The effect of Vpu on HIV-1-induced syncytia formation. J Acquir Immune Defic Syndr 1993;6:135-41.
235. Willey R, Maldarelli F, Martin M, Strebel K. HIV-1 Vpu protein regulates the formation of intracellular gp160-CD4 complexes. J Virol 1992;66:226-34.
236. Schubert U, Henklein P, Boldyreff B, Wingender E, Strebel K, Porstmann T. HIV-1 encoded Vpu protein is phosphorylated by casein kinase-2 at positions Ser52 and Ser56 within a predicted α-helix-turn-α-helix-motif. J Mol Biol 1994;236:16-25.
237. Bour S, Schubert U, Strebel K. HIV-1 Vpu protein specifically binds to the cytoplasmic domain of CD4: implications for the mechanism of degradation. J Virol 1995;69:1510-20.
238. Piguet V, Schwartz O, Le Gall S, Trono D. The downregulation of CD4 and MHC-I by primate lentiviruses: a paradigm for the modulation of cell surface receptors. Immunol Rev 1999;168:51-63.
239. Navia M, Fitzgerald P, McKeever B, et al. Three-dimensional structure of aspartyl protease from HIV-1. Nature 1989;337:615-20.
240. Konvalinka J, Litterst M, Welker R, et al. An active-site mutation in the HIV-1 protease (PR) causes reduced PR activity and loss of PR-mediated cytotoxicity without apparent effect on virus maturation and infectivity. J Virol 1995;69:7180-6.
241. Kaplan A, Swanstrom R. HIV-1 Gag proteins are processed in two cellular compartments. Proc Natl Acad Sci USA 1991;88:4528-32.
242. Ashorn P, McQuade T, Thaisrivongs S, Tomasselli A, Tarpley W, Moss B. An inhibitor of the protease blocks maturation of HIV and SIV and spread of infection. Proc Natl Acad Sci USA 1990; 87:7472-6.
243. Shoeman R, Honer B, Stoller T, et al. HIV-1 protease cleaves the intermediate filament proteins vimentin, desmin, and glial fibrillary acidic protein. Proc Natl Acad Sci USA 1990;87:6336-40.
244. Ventoso I, Blanco R, Perales C, Carrasco L. HIV-1 protease cleaves eukaryotic initiation factor 4G and inhibits cap-dependent translation. Proc Natl Acad Sci USA 2001;98:12966-71.
245. Tomasselli A, Hui J, Adams L, et al. Actin, troponin C, Alzheimer amyloid precursor protein and pro-IL-1β as substrates of the protease from HIV. J Biol Chem 1991;266:14548-53.
246. Strack P, Frey M, Rizzo C, et al. Apoptosis mediated by HIV protease is preceded by cleavage of Bcl-2. Proc Natl Acad Sci USA 1996;93:9571-6.
247. Nie Z, Phenix B, Lum J, et al. HIV-1 protease processes procaspase 8 to cause mitochondrial release of cytochrome c, caspase cleavage and nuclear fragmentation. Cell Death Differ 2002;9:1172-84.
248. Ventoso I, Navarro J, Munoz MA, Carrasco L. Involvement of HIV-1 protease in virus-induced cell killing. Antiviral Res 2005;66:47-55.
249. Phenix B, Angel J, Mandy F, et al. Decreased HIV-associated T-cell apoptosis by HIV protease inhibitors. AIDS Res Hum Retroviruses 2000;16:559-67.
250. Weichold F, Bryant J, Pati S, Barabitskaya O, Gallo R, Reitz M. HIV-1 PI ritonavir modulates susceptibility to apoptosis of uninfected T-cells. J Hum Virol 1999;2:261-9.
251. Sloand E, Kumar P, Kim S, Chaudhuri A, Weichold F, Young N. HIV-1 PI modulates activation of peripheral blood CD4+ T-cells and decreases their susceptibility to apoptosis *in vitro* and *in vivo*. Blood 1999;94:1021-7.
252. Wan W, DePetrillo P. Ritonavir inhibition of calcium-activated neutral proteases. Biochem Pharmacol 2002;63:1481-4.
253. Andre P, Groettrup M, Klenerman P, et al. An inhibitor of HIV-1 protease modulates proteasome activity, antigen presentation, and T-cell responses. Proc Natl Acad Sci USA 1998;95:13120-4.
254. Phenix B, Lum J, Nie Z, Sanchez-Dardon J, Badley A. Antiapoptotic mechanism of HIV PI: preventing mitochondrial transmembrane potential loss. Blood 2001;98:1078-85.
255. Garrett E, Tiley L, Cullen B. Rev activates expression of HIV-1 vif and vpr gene products. J Virol 1991;65:1653-7.
256. Farrow M, Somasundaran M, Zhang C, Gabuzda D, Sullivan J, Greenough T. Nuclear localization of HIV-1 Vif isolated from a long-term asymptomatic individual and potential role in virus attenuation. AIDS Res Hum Retroviruses 2005;21:565-74.
257. Goncalves J, Jallepalli P, Gabuzda D. Sub-cellular localization of the Vif protein of HIV-1. J Virol 1994;68:704-12.
258. Dettenhofer M, Yu X. Highly purified HIV-1 reveals a virtual absence of Vif in virions. J Virol 1999;73:1460-7.
259. Madani N, Kabat D. An endogenous inhibitor of HIV in human lymphocytes is overcome by the viral Vif protein. J Virol 1998; 72:10251-5.
260. Wang W, Chen M, Chuang C, Jeang K, Huang L. Molecular biology of HIV-1. J Microbiol Immunol Infect 2000;33:131-40.
261. Hoglund S, Ohagen A, Lawrence K, Gabuzda D. Role of Vif during packing of the core of HIV-1. Virology 1994;201:349-55.
262. Mariani R, Chen D, Schrofelbauer B, et al. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 2003;114:21-31.
263. Tian C, Yu X, Zhang W, Wang T, Xu R, Yu X. Differential requirement for conserved tryptophans in HIV-1 Vif for the selective suppression of APOBEC3G and APOBEC3F. J Virol 2006;80:3112-5.
264. Sheehy A, Gaddis N, Malim M. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat Med 2003;9:1404-7.
265. Liu B, Sarkis P, Luo K, Yu Y, Yu X. Regulation of Apobec3F and HIV-1 Vif by Vif-Cul5-ElonB/C E3 ubiquitin ligase. J Virol 2005; 79:9579-87.
266. Wiegand H, Doehle B, Bogerd H, Cullen B. A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. Embo J 2004;23:2451-8.
267. Wang J, Shackelford J, Casella C, et al. The Vif accessory protein alters the cell cycle of HIV-1 infected cells. Virology 2006 (in press).