

Review

Mechanisms of apoptosis induction by the HIV-1 envelope

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Abstract

The envelope glycoprotein complex (Env) of human immunodeficiency virus-1 (HIV-1) can induce apoptosis by a cornucopia of distinct mechanisms. A soluble Env derivative, gp120, can kill cells through signals that are transmitted by chemokine receptors such as CXCR4. Cell surface-bound Env (gp120/gp41), as present on the plasma membrane of HIV-1-infected cells, can kill uninfected bystander cells expressing CD4 and CXCR4 (or similar chemokine receptors, depending on the Env variant) by at least three different mechanisms. First, a transient interaction involving the exchange of lipids between the two interacting cells ('the kiss of death') may lead to the selective death of single CD4-expressing target cells. Second, fusion of the interacting cells may lead to the formation of syncytia which then succumb to apoptosis in a complex pathway involving the activation of several kinases (cyclin-dependent kinase-1, Cdk1; checkpoint kinase-2, Chk2; mammalian target of rapamycin, mTOR; p38 mitogen-activated protein kinase, p38 MAPK; inhibitor of NF- κ B kinase, IKK), as well as the activation of several transcription factors (NF- κ B, p53), finally resulting in the activation of the mitochondrial pathway of apoptosis. Third, if the Env-expressing cell is at an early stage of imminent apoptosis, its fusion with a CD4-expressing target cell can precipitate the death of both cells, through a process that may be considered as contagious apoptosis and which does not involve Cdk1, mTOR, p38 nor p53, yet does involve mitochondria. Activation of some of the above-mentioned lethal signal transducers have been detected in patients' tissues, suggesting that HIV-1 may indeed trigger apoptosis through molecules whose implication in Env-induced killing has initially been discovered *in vitro*.

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Abbreviations: AIDS, acquired immunodeficiency syndrome; Cdk1, cyclin-dependent kinase-1; Cyt *c*, cytochrome *c*; Env, envelope glycoprotein complex; HIV-1, human immunodeficiency virus-1; mTOR, mammalian target of rapamycin; p38T180/Y182P, p38 with phosphorylated threonine 180 and tyrosine 182; p53S15P, p53 with phosphorylated serine 15; p53S46P, p53 with phosphorylated serine 46; siRNA, small interfering RNA; Z-VAD.fmk, *N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone

Introduction

Viral infection can actively elicit apoptosis, in particular at late stages of the viral life cycle when viral spreading and/or subversion of the host's immune system can help the virus to colonize its host. In accord with this general rule, the acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus-1 (HIV-1) involves the apoptotic destruction of infected cells ('direct killing') and of noninfected cells many of which are immunologically relevant ('bystander killing').^{1–3} Without doubt, HIV-1 can induce apoptosis through a cornucopia of different mechanisms, including effects that are non-cell-autonomous and can only be studied *in vivo*, using material from human subjects or HIV/SIV-infected primates.² Thus, HIV-1 has been postulated to subvert the trophic interactions among different immune cells, be they contact-dependent or be they dictated by cytokines. In addition, HIV-1 and HIV-1 products can also kill cells *in vitro*, in a variety of model systems that can easily be explored.

HIV-1 encodes for several apoptogenic proteins including envelope glycoprotein complex (Env), Vpr, Tat, Nef, and Nfu,^{1,2,4} as well as for at least one antiapoptotic protein, a putative glutathion peroxidase.^{5,6} Clinical case reports⁷ and epidemiological data⁸ that await confirmation suggest that loss-of-function mutations affecting the apoptogenic action of Vpr^{9,10} are more frequent in viral isolates from long-term nonprogressors (untreated patients with diagnosed HIV-1 infection for > 10 years, with CD4 T cell count of > 500 Cells/mm³) than in patients developing AIDS. Conversely, mutations that truncate the putative glutathion peroxidase gene tend to be more frequent in AIDS patients than in long-term nonprogressors (Cohen *et al.*, 2004), again underscoring the notion that a higher apoptogenic (or a lower antiapoptotic) potential of HIV-1 accelerates AIDS pathogenesis.

In viral infection assays involving wild-type HIV-1, the apoptogenic effect of a clinically important HIV-1-encoded protein such as Vpr⁹ is not observable due to the intrinsically high apoptosis-inducing effect of the HIV-1-encoded envelope glycoprotein complex (Env). Thus, the apoptogenic effect of

Vpr is only revealed when using pseudotyped viruses, that is genetically modified HIV-1 strains in which the endogenous Env gene has been replaced by nonapoptogenic Env proteins from other viruses.^{8,11} This underscores the idea that Env is, at least *in vitro*, the principal apoptosis-inducing protein encoded by the HIV-1 genome.^{12–15}

The Env glycoprotein (gp) precursor protein (gp160) undergoes proteolytic maturation to generate gp41 (membrane inserted) and gp120 (membrane inserted or shed from the cell surface). Soluble gp120 can stimulate proapoptotic signal via an action on chemokine receptors (CXCR4 for lymphotropic Env variants, CCR5 for monocyctotropic Env variants)^{12,16,17} (Figure 1). Although soluble gp120 has been detected in body fluids, it has been doubted whether it would reach concentrations high enough to induce cell death *in vivo*.¹⁸ The membrane-anchored gp120/gp41 complex expressed on the surface of HIV-1-infected cells can induce apoptosis through an interaction with uninfected cells expressing the receptor (CD4) and the chemokine coreceptor CXCR4 or CCR5. This type of bystander killing is obtained by at least three distinct mechanisms (Figure 1). First, the two interacting cells (one which expresses Env and the other that expresses CD4 plus the coreceptor) may not fuse entirely and simply exchange plasma membrane lipids, after a sort of hemifusion process, followed by rapid death.¹⁹ Second, the interaction between the two cells can induce cellular fusion (cytogamy)^{14,15,20} followed by nuclear fusion (karyogamy) within the syncytium.²¹ Syncytia are condemned to die from apoptosis after a latency phase, presumably when conflicts in cell cycle between the daughter nuclei are detected or when

the polyploidy checkpoint is activated.²² Third, it is possible that virus-infected cells, on the point of undergoing apoptosis, fuse with CD4-expressing cells, in which case apoptosis is rapidly transmitted from one cell to the other and thus occurs in a ‘contagious’ fashion.²³

In this paper, we will review the lethal signal transduction pathways elicited by Env *in vitro* and discuss their possible contribution to HIV-1 pathogenesis *in vivo*.

Cell Killing by Soluble gp120

Soluble gp120 can induce apoptosis in lymphocytes,¹² but also in neurons,^{24–27} cardiomyocytes,^{17,28} kidney epithelial cells^{29,30} and hepatocytes.³¹ These potent cytotoxic effects have been implicated in the HIV-1-induced lymphodepletion, HIV-associated dementia (also called HIV encephalitis or neuro-AIDS), AIDS-cardiomyopathy, as well as AIDS associated nephropathy and hepatopathy.

In lymphocytes, the effect of gp120 mainly involve interactions with CD4 and CXCR4 (Figure 2). Antibody-mediated crosslinking of CD4 (or CXCR4) in the absence of CD3 stimulation can sensitize T lymphocytes to apoptosis induction,³² and similar findings have been reported for the gp120-mediated activation of T cells.³³ Stimuli converging on CD4 may activate the CD95/CD95L-dependent death receptor pathway or, alternatively, activate the Bax-dependent mitochondrial pathway to apoptosis.^{34,35} In Jurkat T cells, CD4 engagement by the Leu3a mAb results in a rapid and strong increase of Lck kinase activity, its association with the T-cell

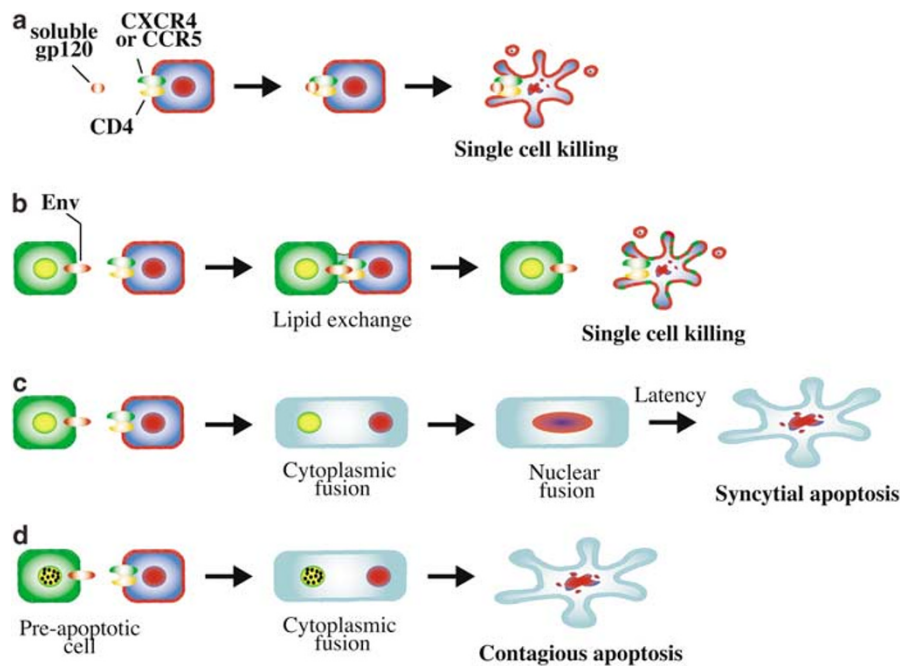


Figure 1 Four distinct mechanisms of Env-mediated killing. Soluble gp120 interacting with CD4 and chemokine receptors (CXCR4 or CCR5) can cause a single cell killing (a). Membrane-bound Env (gp120/gp41) can induce apoptosis by interactions with its receptors through at least three distinct mechanisms. First, the interaction between Env- and CD4/CXCR4 (or CCR5)-overexpressing cells leads to single cell killing through transient interactions with hemifusion-mediated exchange of lipids on the membranes of the interacting cells (b). Second, Env-expressing cells can fuse with Env-negative cells expressing suitable combinations of receptors and coreceptors resulting in syncytium formation and death after a period of latency (c). Finally, preapoptotic Env-positive cells can kill CD4-positive cells upon fusion, in a rapid ‘contagious’ process (d)

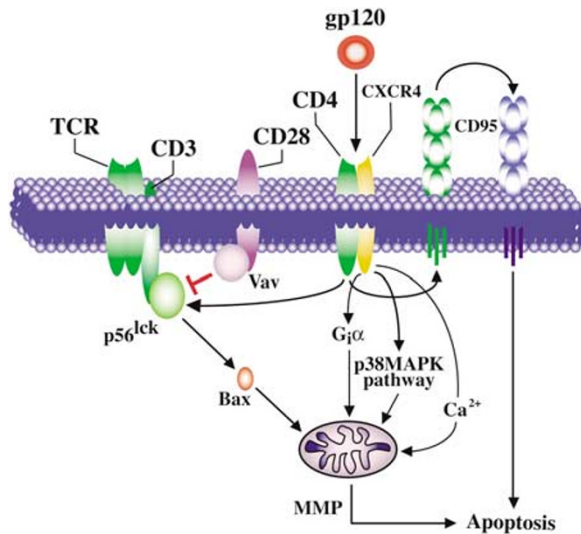


Figure 2 Mechanisms of gp120-induced killing. Soluble gp120 triggers cell death through interactions with CD4 and/or CXCR4. Binding of gp120 to CD4 may stimulate the CD95/CD95L-dependent cell death pathway or trigger a Bax-dependent mitochondrial apoptosis, which requires p56lck activity. In addition, interactions between gp120 and CXCR4 can cause mitochondrial membrane permeabilization (MMP) through pertussis toxin-sensitive G proteins (Giα), p38 MAPK pathway and/or Ca²⁺-dependent mechanisms

receptor, upregulation of Bax, subsequent alterations of the mitochondrial transmembrane potential ($\Delta\Psi_m$), and apoptosis.³⁶ This effect can be counteracted by Vav, a signaling molecule that cooperates with CD28 to boost TCR signals.³⁶ This may explain why CD28 can inhibit gp120-induced cell death.³⁷ A monoclonal antibody against CXCR4 also triggers rapid T lymphocyte death preceded by $\Delta\Psi_m$ dissipation. In this particular model, cell death occurs in a caspase-independent fashion.³² Cell lines transfected with a truncated form of CD4 that binds gp120 but lacks the ability to transduce signals (due to absence of the cytoplasmic domain of CD4), still manifest $\Delta\Psi_m$ dissipation in response to gp120 acting on CXCR4.¹⁶ The signal triggered by gp120 involves chemokine receptors—in neurons mainly CXCR4,^{16,17} and downstream of such receptors, pertussis toxin-sensitive G proteins,¹⁷ the p38 mitogen-activated protein kinase pathway,³⁸ and/or a rapid cytosolic Ca²⁺ increase³⁹ (Figure 2).

Gp120 induces apoptosis of cultured neurons and sensitizes them to oxidative stress and excitotoxins. Gp120 also causes neuronal dysfunction and death in rodents *in vivo*.⁴⁰ Specific inhibitors of both the Fas/tumor necrosis factor- α /death receptor pathway and the mitochondrial caspase pathway can reduce the gp120-induced neuronal apoptosis.⁴⁰ When added to mixed cultures of murine neurons and glial cells, gp120 only causes apoptosis when such cells are derived from p53-expressing mice. Reconstitution experiments in which p53^{+/+} and p53^{-/-} neurons and glial cells were mixed demonstrated that both cell types (neuronal + glial) were required for the lethal response to gp120 and that both of them had to express p53 so that neurons would die upon gp120 addition.²⁵ Thus, p53 may participate in the proapoptotic tuning of cellular networks. An accumulation of p53 has been observed in the brain of patients with HIV-

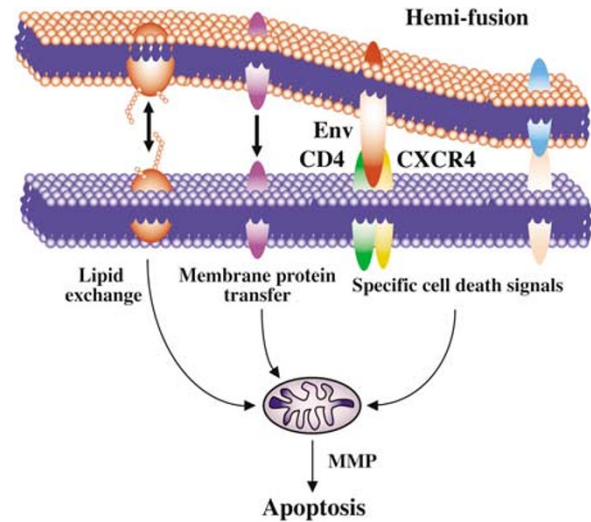


Figure 3 Single cell killing triggered by cell membrane-bound Env. This process implies transient membrane contacts and hemifusion events with exchange of lipids between the interacting plasma membranes. The putative implication of membrane protein transfer and/or specific receptor-mediated death signals is also depicted

associated dementia.⁴¹ Similarly, p53 accumulates in the brain tissue from monkeys with simian immunodeficiency virus (SIV) encephalitis.⁴² These observations suggest that, at least in neurons, p53 may be a critical proapoptotic protein elicited by HIV-1 products (and in particular gp120) *in vivo*.

Single Cell Killing by Membrane-anchored Env: The Kiss of Death

Bystander cells expressing CD4 and CXCR4 or CCR5 die after coculture with cells engineered to express surface-bound Env (gp120/gp41). Cell killing may be secondary to fusion of the interacting cells, but it may also affect single cells^{16,19,43–46} (Figure 3). Apparently, the ‘decision’ whether cell fusion occurs or not is governed at the level of the CD4/coreceptor-expressing cell, through yet-to-be-elucidated mechanisms, since different cell lines expressing such coreceptors (e.g. Jurkat cells and U937 cells) behave in a differential fashion when exposed to the same Env-expressing cell type (e.g. Env-transfected HeLa cells) (Ferri *et al.*, 2000a).⁴⁵ Moreover, the caspase inhibitor Z-VAD.fmk, which can reduce single cell killing, reportedly increases the amount of syncytia formed in such cocultures.⁴³ Agents that inhibit the interaction between gp120 and CD4 or CXCR4 suppress both syncytium formation and single cell killing by surface-exposed Env.^{19,43,45} In addition, fusion inhibitors blocking the function of gp41 (such as C34 and T-20) also block the death of single target cells.^{19,43}

Importantly, Env-induced single cell death is associated with the gp41-mediated transfer of lipids from the membrane of Env-expressing cells to the target cell, a phenomenon which occurs without detectable cytoplasmic mixing, and thus involves a hemifusion-like event.¹⁹ However, basic information is lacking on this ‘kiss of death’ (Figure 3). It is not clear if surface proteins are transferred during the cell–cell contact,

and whether a simple membrane perturbation or more specific signals lead to cell death. The exact cellular signals involved in target cell killing remain largely unexplored apart from the fact that they do not involve CD95¹⁶ and that they act in a rapid, pertussis toxin-resistant fashion,⁴³ leading to $\Delta\Psi_m$ loss and subsequent plasma membrane permeabilization.^{19,43,45} Overexpression of Bcl-2 can inhibit single cell killing⁴⁵ and, as mentioned above, inhibition of caspases diverts single cell killing to syncytium formation,⁴³ suggesting that the process does follow canonical apoptotic signaling pathways (Figure 3).

Syncytial Apoptosis Induced by Env

Syncytium formation resulting from the Env/CD4 interaction leads to apoptosis, and this mode of cell death induction may participate in the AIDS-associated depletion of CD4+ T lymphocytes.^{15,47–50} A positive correlation between CD4+ T cell decline and infection by syncytium-inducing HIV-1 or SIV-1 variants has been established *in vitro*^{15,20,48,51} and, more importantly, *in vivo*, in humans with AIDS,^{47,49} humanized SCID mice,⁵² and monkeys.⁵³

We have studied several *in vitro* models of syncytial apoptosis, in particular a coculture system involving HeLa cells transfected with the HIV-1^{LAI} Env gene and HeLa cells transfected with CD4.⁵⁴ In such an *in vitro* model, apoptosis occurs only in syncytia, as a result of a stepwise process (Figure 4), affecting 50% of the syncytia around 36–48 h after their formation. After an initial stage during which the cells contain well separated nuclei with intact envelopes, nuclear fusion (karyogamy) occurs.²¹ This nuclear fusion is the expression of an abortive entry into the mitotic prophase stimulated by the transient activation of the cyclin B-dependent kinase-1 (Cdk1),⁵⁵ accompanied by the permeabilization of the nuclear envelope, presumably due to the Cdk1-mediated phosphorylation of lamin (which favors lamin depolymerization and hence disassembly of the nuclear envelope). It is only after karyogamy has occurred that syncytia die from apoptosis.^{21,56–60}

Molecular ordering of the process has been achieved using a systemic approach involving microarrays, macroarray, transcription factor profiling, and proteomics. This systemic approach has been complemented by hypothesis-driven research involving a battery of chemical inhibitors of kinases, microinjection of specific inhibitors (of Bax, VDAC, AIF, cytochrome *c* (Cyt *c*)), transfection with dominant-negative constructs (of Cdk1, p38 MAPK, MKK3/6, and p53), as well as positive manipulations (microinjection of the cyclin B-dependent kinase-1, transfection with mammalian target of rapamycin (mTOR), microinjection of recombinant Bax or purified Cyt *c*). After a transient activation of the mitotic progression factor (MPF, composed of cyclin B and cyclin-dependent kinase 1, Cdk1) with activation and phosphorylation of the checkpoint kinase-2 (Chk2)⁵⁹ as well as activation of NF- κ B,⁶¹ cells undergo an abortive entry into the prophase of mitosis, where they arrest and manifest nuclear fusion (karyogamy) within the heterokaryon.²¹ It is at that point (but not during the interphase) that syncytia manifest p53 phosphorylation both on serine 15^{55,62} and serine 46.⁶¹ Moreover, a series of p53 target genes including two proapoptotic Bcl-2 family numbers,

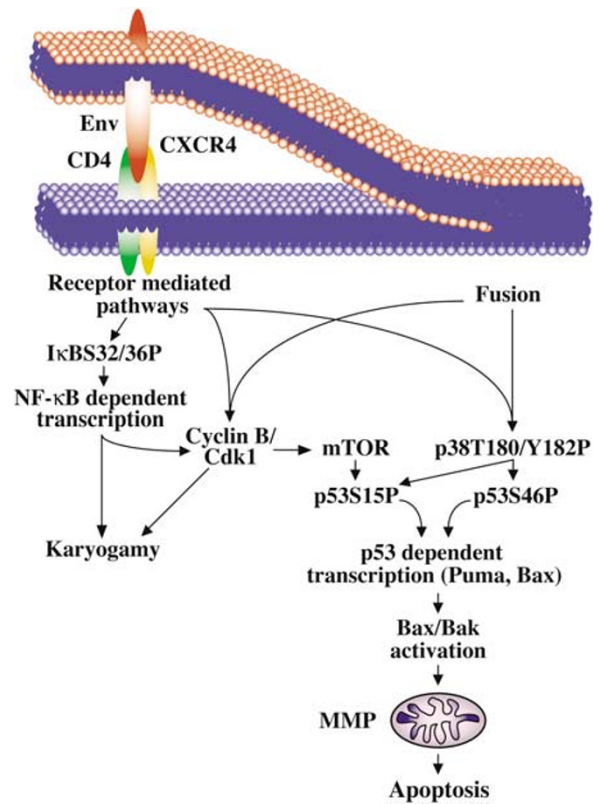


Figure 4 Mechanisms of syncytial apoptosis. Syncytia arising from the fusion of cells expressing Env with cells expressing CD4 and CXCR4 die after a latency through a mitochondrion-dependent apoptosis pathway. Specific signals mediated by plasma membrane receptor and/or a perturbation of cellular metabolism induced by the fusion event can trigger a series of proapoptotic kinases. Phosphorylation of the inhibitor of NF- κ B ($I\kappa$ B) on serine 32 and on serine 36 induces its degradation and initiates NF- κ B-dependent transcription. Cyclin B-dependent kinase-1 (Cdk1) is induced and causes an abortive entry into the mitotic prophase, resulting in the fusion of several nuclei within the same cytoplasm (karyogamy). mTOR and p38MAPK, two kinases activated by cell fusion, phosphorylate p53 on serines 15 and 46, thereby facilitating p53-dependent transcription. Puma and Bax, two p53 target genes, are induced and trigger mitochondrial membrane permeabilization (MMP) and consequent caspase activation

namely Bax and Puma, are transcribed,^{55,61,62} thus activating the mitochondrial pathway of apoptosis with Cyt *c*-dependent caspase activation.^{21,45,63,64} Thus, p53 emerges as a critical mediator of syncytial apoptosis (Figure 4).

One of the p53-phosphorylating enzymes has been identified as mTOR, which is enriched in karyogamic nuclei and coimmunoprecipitates with p53.^{55,62} Blockade of the aberrant advancement in cell cycle, by inhibition of Cdk1, Chk2, or NF- κ B strongly reduces the phosphorylation of p53 on both S15 and S46.^{58,59,61,62,65} However, inhibition of mTOR with rapamycin only partially reduced the phosphorylation of p53 on S15 (p53S15P), by about 50–60%.^{55,61,62}

Another p53 kinase that has been identified more recently, cytoplasmic p38 mitogen-activated protein kinase (MAPK), was found to undergo an activating phosphorylation (on T180/Y182P) before karyogamy and to translocate into karyogamic nuclei, where p38T180/Y182P colocalized and coimmuno-

precipitated with p53 alone.⁶⁶ Activated recombinant p38 protein phosphorylated recombinant p53 on serines 15 and 46 *in vitro*. Inhibition of p38 MAPK by pharmacological inhibitors, dominant-negative p38, or small interfering RNA (siRNA), suppressed p53S46P (but not p53S15P), the expression of p53-inducible genes, the conformational activation of proapoptotic Bax and Bak, the release of Cyt *c* from mitochondria, and consequent syncytial apoptosis. Importantly, we found that the combined inhibition of mTOR and p38 led to a much more pronounced reduction of p53S15P than the inhibition of mTOR alone.⁶⁶ Thus, the principal p53 kinase acting on S46 is p38 MAPK, while there are at least two kinases acting on S15, namely mTOR and p38 MAPK (Figure 4).

Little is known on the upstream signals leading to the activation of mTOR or p38 MAPK. In both cases, karyogamy occurs independent from these kinases and is required for their translocation to the nucleus. Syncytium formation of HeLa CD4 cells, induced for instance by polyethyleneglycol, is sufficient to activate p38 MAPK, to phosphorylate p53 on serine 46 and to cause apoptosis, suggesting that CD4 and chemokine receptor-mediated signaling is not required for initiation of this particular proapoptotic cascade. Dominant-negative MKK3 or MKK6 inhibited syncytial activation of p38, p53S46P, and apoptosis in the HeLa CD4/HeLa Env coculture system.⁶⁶ However, the exact cascade of molecular events linking syncytium formation to the activation mTOR and p38 MAPK remains to be elucidated.

Can the results obtained with cell lines engineered to overexpress the gp120/gp41 complex (that is in HIV-1-free systems) be extrapolated to the pathophysiology of HIV-1 infection? Infection of primary human lymphoblasts *in vitro*, with lymphotropic HIV-1 isolates, causes cell death mainly by syncytium formation, associated with a series of alterations that resemble those induced by Env *in vitro*, namely $\Delta\Psi_m$ loss,^{46,67} mitochondrial release of Cyt *c* and AIF,^{67,68} increased ROS production,⁶⁹ phosphorylation of p53 on serine 15,^{55,67} phosphorylation of p53 on serine 46,⁶¹ activation of p38 MAPK,⁶⁶ and the p53-dependent induction of Bax⁶⁷ and Puma.⁶¹ The amount of Bax is found to be increased in the mitochondrion-enriched heavy membrane fraction of HIV-infected CD4+ T cells as compared to uninfected controls.^{45,67} *In vitro*, pharmacological inhibition of Cdk1 (with roscovitine),^{55,62} mTOR (with rapamycin),^{55,62} p38 MAPK (with SB203580 or other inhibitors),^{66,70} or p53 (with cyclic pifithrin- α)^{61,62} suppresses the apoptosis induced by HIV-1 infection, while caspase inhibition (with Z-VAD.fmk) has no or little cytoprotective effects.^{45,68} Altogether, these data suggest that the cascade of events delineated above (cell fusion \rightarrow activation of Cdk1/cyclin B \rightarrow p53 phosphorylation on serine 15 and serine 46 by mTOR and p38 MAPK \rightarrow transcriptional activation of p53 target genes such as Bax and Puma \rightarrow Bax translocation to mitochondria with consequent MMP \rightarrow caspase independent cell death) is induced by HIV-1 infection *in vitro*.

A few reports suggest that the pathway delineated above is also activated in HIV-1-infected individuals, *in vivo*, at least in some cell types. Thus, cyclin B has been found to be overexpressed in circulating T lymphocytes from HIV-1 carriers, and this upregulation disappears upon successful

antiretroviral therapy.^{71,72} Similarly, mTOR and the phospho- neopeptide indicating phosphorylation of p53 on serine 15 were found to be overexpressed among a fraction of peripheral blood mononuclear cells, correlating with viral load,^{55,62} as well as with the frequency of cells expressing tissue transglutaminase-2, a marker of preapoptosis.⁷³ Highly active antiretroviral therapy (HAART) corrected the abnormally high mTOR and p53S15P levels of the patients.⁶² Syncytia containing p38 with phosphorylated threonine 180 and tyrosine 182 (p38T180/Y182P) were detected in lymph node biopsies from HIV-1 carriers, in the brain of patients with HIV-1-associated dementia, and in cocultures of HeLa expressing the HIV-1 envelope (Env) with HeLa cells expressing CD4.⁶¹ Activated, phosphorylated p53 with phosphorylated serine 46 (p53S46P) was also detected in HIV-1-induced syncytia, *in vivo*, in patients' lymph nodes and brains from patients with HIV-1-associated dementia, yet lacked in uninfected control subjected or in brains of HIV-1-infected patients without neurodegeneration.⁶⁶ Thus, it appears possible that the syncytial pathway leading to apoptosis (or a closely related pathway) is activated *in vivo*, in HIV-1 carriers.

Contagious Apoptosis Facilitated by the HIV-1 Envelope

HIV-1 can kill the cells that it infects (and which express Env on the surface) in a cell-autonomous fashion. We recently discovered that, when the apoptotic pathway is initiated in Env+ cells ('donor cells'), coculture with a healthy CD4+ fusion partner ('acceptor cells') results in rapid apoptosis of the syncytium and thus is 'contagious'²³ (Figure 5). As an initiating signal, we could use a short pulse of staurosporine treatment (3 μ M, 3 h) or, alternatively, actinomycin D or Vpr, meaning that different modes of apoptosis induction in 'donor' cells can trigger contagious apoptosis. The cell-to-cell transmission of the lethal signal was only observed when the nuclei from donor cells exhibited preapoptotic chromatin condensation (PACC), which precedes caspase activation, as well $\Delta\Psi_m$ dissipation.⁷⁴ However, no fusion between HeLa Env and HeLa CD4 cells and no contagious apoptosis was observed when the donor cells were already apoptotic.²³ PACC marks a preapoptotic event, upstream of the mitochondrial checkpoint. Cells with PACC exhibited partial chromatin condensation with irregular nuclear contours, no Tunel staining, no DNA fragmentation detectable by conventional or pulse field gel electrophoresis, yet manifested comet assay-detectable DNA strand breaks, as well as the phosphorylation of histone H2AX (on serine 139) and that of Chk2 (on threonine 68), which accumulated in discrete nuclear speckles indicative of DNA damage.^{23,74}

Transmission of the lethal signal from PACC+ cells to healthy cells resulted in mitochondrial alterations ($\Delta\Psi_m$ loss and Cyt *c* release), and caspase-dependent nuclear pyknosis with chromatinolysis affecting both the donor and the acceptor nuclei.²³ Thus nuclear apoptosis affected all nuclei, irrespective of their origin, within the same syncytium simultaneously. In the presence of caspase inhibitors, all nuclei of the syncytium formed by fusion of the preapoptotic Env-expressing and the healthy expressing cell manifested PACC,

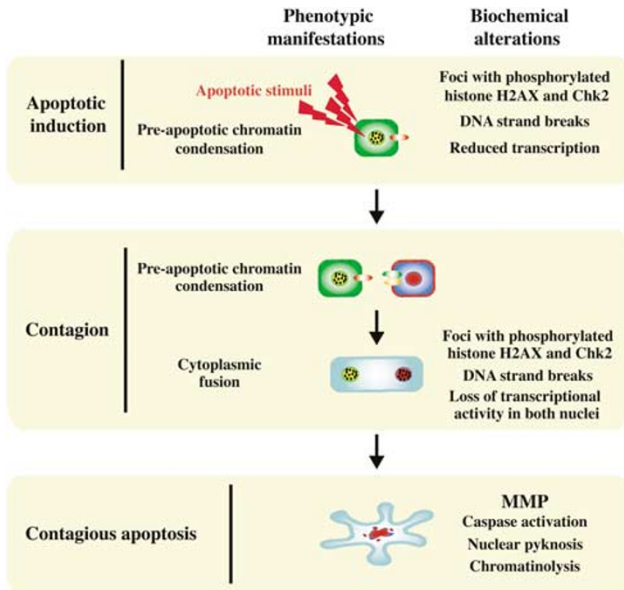


Figure 5 Mechanisms of contagious apoptosis. When apoptosis is initiated in Env⁺ cells, preapoptotic Env⁺ cells can fuse with healthy CD4⁺ cells, thus forming syncytia that die rapidly through caspase-dependent apoptosis. This 'contagious' apoptosis is exclusively observed, when treated Env⁺ cells show a preapoptotic chromatin condensation (PACC), a phenomenon that precedes caspase activation and mitochondrial membrane permeabilization (MMP). PACC⁺ cells manifest other biochemical alterations such as DNA strand breaks and DNA foci with phosphorylated histone H2AX on serine 135 (HZAXS139P) and Chk2 on threonine 68 (Chk2T68P). The transmission of lethal signals requires cytoplasmic (but not nuclear) fusion and leads first to PACC of the nuclei originating from the CD4⁺ cells and then to full-blown apoptosis with MMP, caspase activation, nuclear pyknosis, and chromatolysis

exhibited DNA lesions, and lost transcriptional activity. Transmission of the lethal signal did not require donor cells to contain a nucleus or mitochondrial DNA (and thus was independent from DNA lesions), yet was inhibited at all levels when two mitochondrion-stabilizing proteins, Bcl-2 or vMIA, were overexpressed (Figure 5). Importantly, contagious apoptosis was mechanistically different from spontaneous syncytial apoptosis because it occurred more rapidly (killing around 50% of the cells within 4–6 h) and because it was not inhibited by pharmacological inhibitors of Cdk1 (e.g. roscovitine), mTOR (e.g. rapamycin), or p53 (e.g. cyclic pifithrin α).²³ Thus, contagious apoptosis relies on mitochondrial permeabilization, upstream of caspase activation, linked to a yet-to-be characterized cytoplasmic event, which is different from the known actors involved in slow, spontaneous syncytial death.

We specifically investigated whether fusion was required for contagious apoptosis or whether hemifusion-like events would be sufficient for the transmission of the lethal signal, based on the knowledge that two T-lymphoid cell lines (Jurkat and CEM) fuse with HeLa Env cells, while the myelomonocytic U937 cells do not fuse with HeLa Env and rather undergo transient interactions that involve transfer of plasma membrane lipids through a hemifusion-like process, the 'kiss of death'.¹⁹ STS-pulsed HeLa Env cells with PACC did induce killing of Jurkat or CEM cells, yet had no major apoptosis-inducing effect on U937 cells, in accord with the notion that

fusion (rather than hemifusion) is required for cell death induction. Contagious apoptosis could be induced in primary human C4⁺ T lymphocytes, as well as *in vivo*, in the peritoneal cavity of mice, in which human T cells were exposed to dying Env-expressing cells.²³

Based on these data, we speculate that contagious apoptosis could potentially affect CD4⁺ T cells in HIV-1 infection. In conditions in which viral replication overwhelms the cellular defense response and triggers (pre-)apoptosis of the 'donor' Env⁺ cells, fusion with 'recipient' CD4⁺ cells might contribute to the depletion of interacting CD4⁺ T lymphocytes by contagious apoptosis.

Concluding Remarks

The HIV-1 envelope can cause the demise of uninfected cells through a plethora of different mechanisms, as summarized in this review. At least *in vitro*, cells expressing the CD4 receptor and/or suitable coreceptors can die upon exposure to the soluble Env derivative gp120 or to cells expressing Env (gp120/gp41) on their surface, through a process that may or may not involve hemifusion (the 'kiss of death'), cytoplasmic and nuclear fusion ('syncytial apoptosis'), and/or rapid cell-to-cell transmission of apoptotic signals ('contagious apoptosis'). Beyond Env and its coreceptors, the lethal signal transduction may involve a number of kinases (e.g. IKK, p38 MAPK, mTOR), some transcription factors (in particular, NF- κ B and p53), as well as typical ingredients of the mitochondrial apoptotic cascade (Puma, Bax, Bak, Cyto *c*, etc.).

In view of the scarcity and the cost of animal models of HIV-1 infection, it will be particularly important to investigate the relative contribution of these processes and molecules to AIDS pathogenesis by careful examination of circulating cells or biopsies from HIV-1 carriers. Future will tell whether the modulation of apoptosis induced by HIV-1 (rather than the infection by HIV-1 itself) will provide some clinical benefit for the control of one of the major menaces to human longevity and civilized life.

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