Biochemical and Biophysical Research Communications 414 (2011) 20–24

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Molecules from apoptotic pathways modulate HIV-1 replication in Jurkat cells

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ARTICLE INFO

Article history:
Received 26 August 2011
Available online 14 September 2011

Keywords:
Apoptosis
Bax
Fas
HIV-1
Replication

ABSTRACT

The replication of viruses involves control of some aspects of host cell homeostasis by modification of target cell metabolism and regulation of the apoptotic machinery. It is not well known whether molecules involved in apoptotic pathways affect human immunodeficiency virus type 1 (HIV-1) replication and regulate viral yields. Using the susceptible Jurkat cell line, we studied the relationship of apoptosis-associated molecules with HIV-1 virus production using a sensitive real-time RT-PCR assay. Here, we found that expression of proapoptotic proteins, including Fas ligand (FasL), FADD, or p53 significantly increased HIV-1 virus production. In contrast, the expression of antiapoptotic molecules, such as FLIP, Bcl-X_L, and XIAP, decreased HIV-1 virus production. Knockdown of Bax with siRNA and FADD with expression of its antisense mRNA also inhibited viral replication and the caspase-3 inhibitor, Z-DEVD, and decreased virus production. These data indicate that HIV-1 infection regulates the apoptosis process to facilitate viral replication and inhibition of apoptosis may inhibit HIV-1 replication and cytopathogenesis. We also discuss the effects of MAPK signaling pathways and apoptosis on HIV-1 replication.

1. Introduction

HIV-1 infection has been characterized by progressive depletion of the number of $CD4^+$ T lymphocytes, which involves apoptosis-mediated cell death. HIV directly kills CD4-expressing infected cells, such as $CD4^+$ T helper, T lymphocytes and dendritic cells [1]. In addition, HIV kills bystander cells through cell-exposed proteins (such as the Envelope glycoprotein, Env) or secreted proteins, including Vpr, Tat, Nef, Vpu, Vif and the viral protease [1]. Killing of non-infected bystander cells is also driven by the proinflammatory state that is associated with ongoing viral replication and responsible for the activation of apoptotic pathways [1].

Cells infected with HIV-1 can initiate apoptosis by two known major pathways: an extrinsic (death receptor-mediated, such as Fas/Fas ligand) and an intrinsic (Bax/mitochondrial mediated) pathway [2,3]. FasL (or Fas agonistic antibodies) interaction with Fas initiates the Fas-mediated apoptosis pathway, forming a death-inducing signal complex (DISC) with the rapid recruitment of FADD (Fas-associated death domain protein) and caspase-8 to the cytoplasmic death domain of Fas. Activated caspase-8 subsequently activates caspase-3 in Jurkat cells [4]. In response to HIV-1 infection, the pro-apoptotic Bcl-2 family protein, Bax, initiates the intrinsic apoptotic pathway by forming channels on the mitochondria, which increases the outer mitochondrial membrane permeability, and thereby facilitates the release of cytochrome c and other pro-apoptotic factors from the mitochondrial intermembrane space [4]. After release into the cytosol, cytochrome c forms an apoptosome complex with Apaf-1, which activates caspase-9, and in turn, its downstream caspase-3, resulting in the morphological features of apoptosis [4].

A recent report from our group has shown that the expression of a caspase-8 agonist partner, known as Fas-associated death domain (FADD) interleukin-1β-converting enzyme (FLICE)-like inhibitory protein (FLIP), and an anti-apoptotic Bcl-2 family protein, Bcl-X_L, are able to decrease HIV replication significantly [2], suggesting that the change of protein expression involved in apoptotic pathways may affect HIV-1 replication in host cells. Here, we studied the effects of proteins of both Fas and Bax-mediated apoptotic pathways on HIV-1 replication in susceptible Jurkat cells.

2. Materials and methods

2.1. Chemicals and reagents

Rabbit polyclonal antibodies against β-actin, Erk, Jnk, p38, and human Bax siRNA were from Santa Cruz Biotechnology (Santa Cruz, CA). The full-length *Bcl-X_L*, anti-*FADD, FADD, FasL, FLIP*, *p53*, and *LacZ* inserted in an adenovirus expression system were obtained from the Molecular Medicine Institute Programs of Excellence in Gene Therapy Vector Core Facility, University of Pittsburgh. Caspase-3 fmk Inhibitor Z-DEVD was obtained from R and D systems (Minneapolis, MN). All other chemicals were from Sigma (St. Louis, MO).

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doi:10.1016/j.bbrc.2011.09.007

2.2. Cell culture and treatments

The human Jurkat T cell line (clone JE6.1) was obtained from American Type Culture Collection (Manassas, VA), and cultured at 37 °C in 5% CO_2 in RPMI 1640 medium containing 10% fetal calf serum, 2 mm glutamine, 50 μg/ml penicillin, and 50 μg/ml streptomycin. For adenoviral infections, cells were grown in the medium with 10% fetal calf serum containing 10^6 CPU/ml of an adenoviral vector inserted with a gene. Infected cells were incubated for an additional 2 days. The expression levels of target gene products in adenoviral vector with Western blot analysis could be seen in our previous reports [3,4] (data not shown here).

2.3. siRNA transfection

A small interfering RNA (siRNA) transfection kit corresponding to Bax (Human) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Jurkat cells were transfected with Bax siRNA or the control siRNA for 48 h according to the manufacturer's protocol, and collected the supernatants.

2.4. HIV-1 infection

Jurkat cells were seeded at 2×10^5 cells/ml for 24 h, and infected with known amounts of HIV-1 (MN, 10^9 copies per 10^6 cells) for 2 h, washed twice with PBS, and cultured for periods of time indicated.

2.5. Real-time PCR

Quantitative real-time reverse-transcriptase (RT) PCR was used for detection of virus. Viral RNA was isolated from 140 μl of culture supernatant by using the QIAamp Viral RNA Mini Kit (Valencia, CA 91355) according to the manufacturer's protocol. The primers and TaqMan probe were designed in the *gag* p24 region, which is the variable region among most of the HIV-1 subtype B isolate sequences according to GenBank database. The forward primer was 5′-GACATCAAGCAGCCATGCAA-3′, corresponding to nucleotides 1367–1386, and the reverse primer was 5′-CTATCCCATTCTGCA GCTTCCT-3′, corresponding to nucleotides 1430–1409. The TaqMan probes was oligonucleotides 5′-ATTGATGGTCTCTTTTAAC A-3′, corresponding to nucleotides 1488–1507, coupled with a reporter dye [6-carboxy fluorescein] (FAM) at the 5′ end and a non-fluorescent quencher and a minor groove binder (MGB), which is a Tm enhancer, at the 3′ end. The nucleic acids were amplified and detected in an automated TaqMan 7500 Analyzer by using QuantiTect™ Probe RT-PCR kit (Qiagen Inc., Valencia, CA). The 25-μl PCR mixture consisted of 100 nM primers and 100 nM probe. Following three thermal steps at 55 °C for 5 min, at 50 °C for 30 min and at 95 °C for 10 min, 45 cycles of two-step PCR at 95 °C for 15 s and at 60 °C for 1 min were performed.

2.6. Statistical analysis

The unpaired Student's *t* test was used for data analyses as indicated, and a value of $p < 0.05$ was considered significant.

3. Results

Fas (CD95) and its ligand (FasL) are key molecules for antigen receptor-induced apoptosis in activated mature T cells [5]. Activation of Fas signaling pathway with human Fas agonist antibody, CH11, which effectively activates Fas signaling pathway in Jurkat

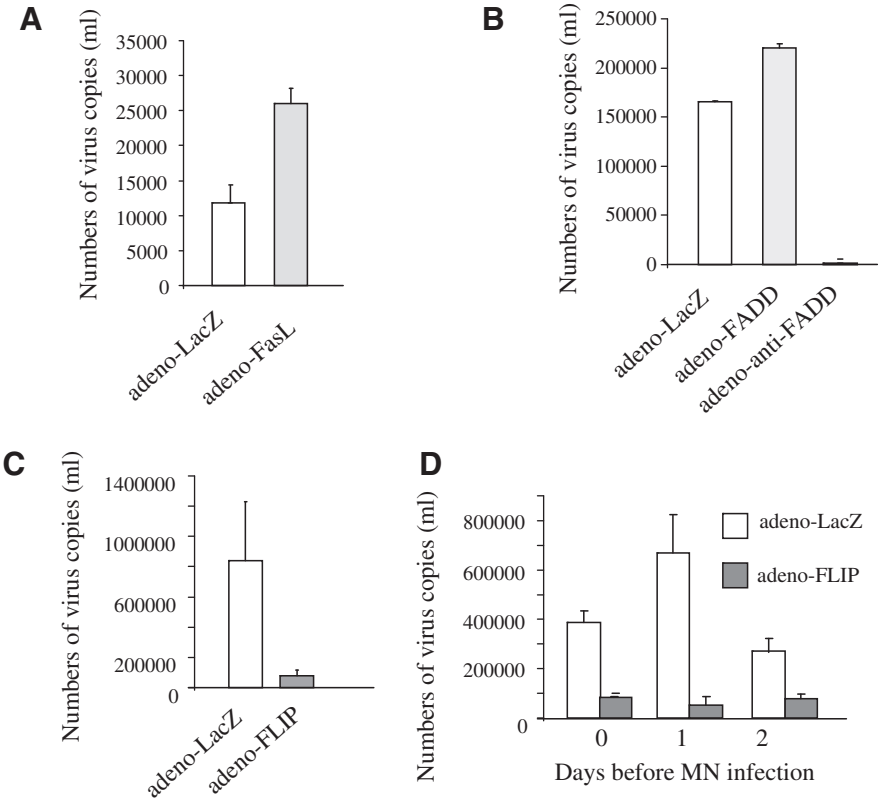

Fig. 1. The effects of molecules from Fas-mediated apoptotic pathway on HIV-1 replication. Jurkat cells, infected with HIV-1 (MN) for 7 days, were subjected to infection with adenovirus inserted a gene, LacZ, FasL (A), FADD and anti-sense FADD (B), or FLIP (C) for another 2 days. (D) Jurkat cells, infected with aden-FILP for 0, 1, or 2 days indicated, were infected with HIV-1 (MN) for another 7 days. 140 μl of culture supernatants containing HIV-1 particles were used to isolate viral RNA. 10 μl in 50 μl of the RNA was used as template to perform real-time PCR. Known concentrations of HIV-1 (MN) viral RNA (serially diluted: 10^8 to 100 copies) were used as templates and quantitative RT-PCR performed to generate a standard curve. Each value represents the average concentration of six reactions in triple isolated repeats based on the standard curve.

cells caused significant changes of HIV-1 productions statistically in the manner of dose dependence (Sup. Fig. S1A).

3.1. The effects of FasL and components of DISC on HIV-1 production

FasL, a member of the TNF superfamily, plays a crucial role in controlling excessive lymphoproliferation by inducing apoptosis in T cells [5]. It has been well-documented that HIV-1 infection and some HIV-1 proteins induce Fas-signaling apoptosis, and that infection increases FasL expression in host cells [6]. To examine the effects of FasL expression, we infected Jurkat cells with an adenovirus construct containing FasL or LacZ gene for 2 days, after the cells were infected with HIV-1 for 7 days. FasL expression caused significantly increased viral RNA yields, relative to LacZ infected control (Fig. 1A).

Given that FasL expression increases HIV-1 replication, we hypothesized that the components of the death-inducing signal complex (DISC) formation, which are required to initiate Fas-mediated apoptosis, such as FADD, may also regulate HIV-1 RNA production. Jurkat cells were infected with HIV-1 (MN) for 7 days, and were subjected to infection with FADD, or LacZ for another 2 days. Real-time PCR analysis showed that Jurkat cells infected with FADD displayed an upregulation of viral yield, relative to LacZ infected control (Fig. 1B). In contrast, the expression of the antisense mRNA of FADD caused a significantly decreased HIV yield relative to LacZ control and FADD (Fig. 1B). These data indicate that increased expression of proapoptotic molecules produce more HIV-1 virus, while the expression of decreased proapoptotic proteins reduced HIV-1 virus production.

FLIP has been characterized as an inhibitor of apoptosis induced by death receptors such as Fas or the tumor necrosis factor-related apoptosis-inducing ligand receptors [4]. cFLIP (cellular FLICE inhibitory protein) is structurally related to procaspase-8 and -10 but lacks enzymatic activity [3,4]. Previously, we found that FLIP expression blocked caspase-8 recruitments into DISC formation and inhibited apoptotic cell death induced by HIV infection [3], and displayed a downregulation of HIV-1 p24 expression and HIV-2 p27 expression [2]. We infected Jurkat cells with an adenovirus construct expressing FLIP or LacZ gene for 2 days, after cells were infected previously with HIV-1 for 7 days, and found that FLIP expression decreased HIV-1 virus yield significantly relative to LacZ infected control in Jurkat cells (Fig. 1C). We also examined the effects of FLIP pre-treatment on HIV-1 production. Jurkat cells were infected with the FLIP or LacZ containing adenoviruses for 0, 1, or 2 days and subsequently subjected to infection with HIV-1 (MN) and incubation for 7 days, and then isolated HIV-1 virus from culture supernatants and detected the RNA quantities with real-time PCR assay. Cells expressing FLIP displayed significantly decreased HIV-1 yields (Fig. 1D). These data indicate that FLIP inhibits both HIV-1 replication (Fig. 1D) and expression of viral proteins [2].

3.2. The effects of molecules from Bax-mediated pathway on HIV-1 RNA yields

It has been known that HIV infection and some HIV-1 gene products, such as envelope, nef, and transactivator (tat), protease, and vpr can activate the intrinsic (Bax/mitochondrial-mediated) apoptotic pathway with a downregulation of the expression of Bcl-X_L, activation of Bax, and an increased p53 expression [7]. We tested the hypothesis that p53 expression may diminish HIV-1 RNA transcription. Jurkat cells infected with HIV-1 (MN) for 7 days were subjected to infection with p53 or LacZ expressing

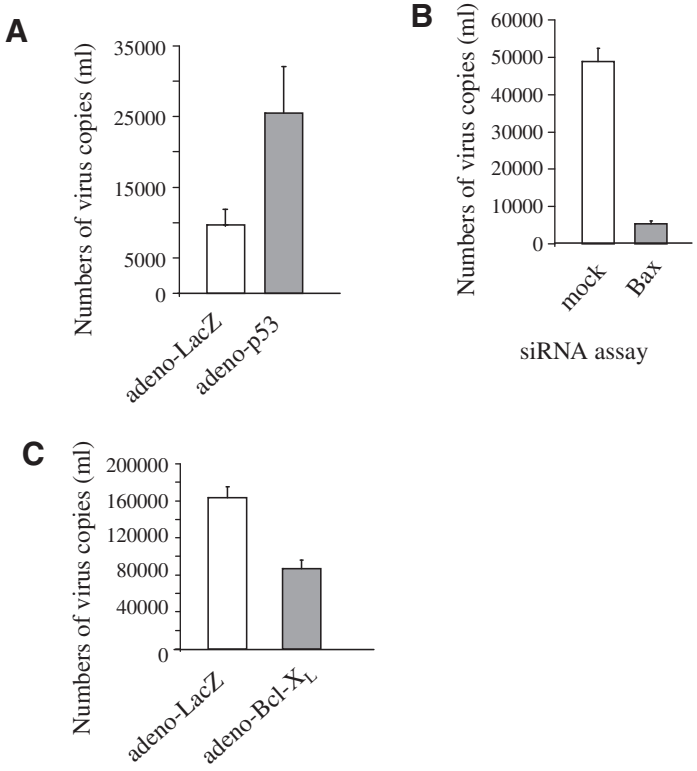

Fig. 2. The effects of molecules from Bax/mitochondrial-mediated apoptotic pathway on HIV-1 replication. Jurkat cells, infected with HIV-1 (MN) for 7 days, were subjected to infection with adenovirus inserted a gene, LacZ, p53 (A), or Bcl-X_L (C), for another 2 days. (B) Jurkat cells, were infected with siRNA Bax or siRNA control for 2 days, were infected with HIV-1 (MN) for another 7 days. 140 μl of culture supernatants containing HIV-1 particles were used to isolate viral RNA. 10 μl in 50 μl of the RNA was used as template to perform real-time PCR. Known concentrations of HIV-1 (MN) viral RNA (serially diluted: 10^8 to 100 copies) were used as templates and quantitative RT-PCR performed to generate a standard curve. Each value represents the average concentration of six reactions in triple isolated repeats based on the standard curve.

adenovirus constructs for another 2 days. We found that p53 expression yielded more virus relative to LacZ infected control (Fig. 2A).

To examine the effect of preapoptotic Bcl2 family, Bax, a molecule at the downstream of p53 signaling in Bax-mediated apoptotic pathway, on HIV-1 replication, Jurkat cells were infected with HIV-1 (MN) for 7 days, after the cells were subjected to transformation with Bax siRNA, or control siRNA for 2 days. We found that knockdown of Bax with siRNA assay significantly blocked HIV-1 virus production, relative to control siRNA treatment (Fig. 2B).

Bcl-X_L, an antiapoptotic Bcl2 family member, has been reported to inhibit the production of p24 protein of HIV-1 and p27 protein of HIV-2 [2]. We infected Jurkat cells with Bcl-X_L, or LacZ expressing adenovirus constructs for 2 days after the cells were infected with HIV-1 for 7 days, and found that Bcl-X_L expression also inhibited HIV-1 RNA transcription relative to LacZ infected control (Fig. 2C).

3.3. The effects of caspase-3 on HIV-1 virus production

The important biomarker of apoptosis is the activation of caspase-3. To examine effect of activation of caspase-3 on HIV-1 virus production in cell culture supernatants, Jurkat cells were infected with HIV-1 (MN) for 7 days, and caspase-3 inhibitor, Z-DEVD, for an additional 2 days. As shown in Fig. 3A, caspase-3 inhibitor treatment caused significantly decreased viral RNA yield relative to the DMSO treated control.

3.4. The effect of XIAP expression on HIV-1 replication

X-linked inhibitor of apoptosis protein (XIAP), which belongs to a family of inhibitors of apoptosis proteins (IAPs), also known as baculovirus IAP repeat-containing proteins, are evolutionarily conserved proteins defined by structural similarity [8]. The capacity of XIAP to inhibit the catalytic activity of caspases-3, 7, and 9 is well determined [8]. XIAP was reported to be downregulated by HIV-1 infection in Jurkat cells [8]. We tested the hypothesis that XIAP may inhibit HIV-1 replication due to its inhibition of effecter caspases. Jurkat cells infected with HIV-1 (MN) for 7 days, and then subjected to infection with XIAP or LacZ expressing adenovirus constructs. The cells infected with XIAP displayed a significantly decreased viral particle quantity, relative to LacZ infected control (Fig. 3B).

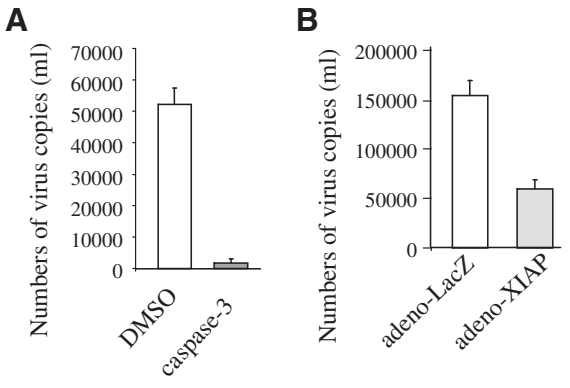

Fig. 3. The effects of caspase-3 or XIAP on HIV-1 replication. Jurkat cells, infected with HIV-1 (MN) for 7 days, were subjected to treatment with caspase-3 fmk Inhibitor Z-DEVD (A) for 2 days, or infection with adenovirus inserted a gene, LacZ, XIAP (B), for another 2 days. 140 μl of culture supernatants containing HIV-1 particles were used to isolate viral RNA. 10 μl in 50 μl of the RNA was used as template to perform real-time PCR. Known concentrations of HIV-1 (MN) viral RNA (serially diluted: 10^8 to 100 copies) were used as templates and quantitative RT-PCR performed to generate a standard curve. Each value represents the average concentration of six reactions in triple isolated repeats based on the standard curve.

These data indicates that HIV-1 virus production is associated with the inhibition of effect or caspase activities downstream of the apoptotic pathways.

4. Discussion

HIV-1 establishes a persistent infection characterized by progressive depletion of $CD4^+$ lymphocytes and immunosuppression [1]. HIV kills CD4-expressing primarily infected cells directly, while bystander cell killing is achieved through cells exposed to HIV proteins [1,7]. Killing of non-infected bystander cells is also driven by the proinflammatory state that is associated with ongoing viral replication and is responsible for the activation of both extrinsic and intrinsic pathways of apoptosis [1,7]. HIV infection induces Fas apoptotic signaling system, with an increased expression of FasL, Bid and caspase-3 in lymph nodes of patients [9]. Proteins from HIV-1, Env, Nef, and Tat increase FasL expression and Fas signaling activation in bystander cells. HIV-1 proteins, such as Env, Nef, Tat, protease, or Vpr in bystander cells, can also activate Bax-mediated pathways [7] with upregulation of Bax and downregulation of Bcl-2, Bcl-X_L [10] (Fig. 4).

HIV manipulates host cells to ensure its own survival and propagation by controlling some crucial aspects of host cell homeostasis, modifying target cell metabolism, influencing the cell cycle and regulating the apoptotic machinery. In particular, HIV is likely to induce cell death pathways at different levels [9]. Obviously, rapid death of infected cells will limit the production of the virus. Therefore, to ensure its spread, the virus needs to establish a balance between the apoptosis-prone activation state and the replication-unfavorable environment of host cells (comparison of Sup. Fig. S1A with S1B). In the initial phase of the infection, HIV must prevent the rapid elimination of host cells. The increased expression of FasL, FADD, and p53 are helpful for viral replication (Figs. 1, 2 and 4); while increased expressions of FLIP and Bcl-2 [2] decrease HIV-1 replication (Figs. 1, 2 and 4). HIV induces DNA damage responses as a consequence of retroviral DNA integration, and cell death induced at this stage would block viral infection. At late stages of the viral life cycle, these responses kill the infected target cell to ensure their propagation in the adjacent tissues. So far the mechanism by which proapoptotic molecules increase HIV-1 replication and antiapoptotic proteins decrease viral RNA yields is not known.

Cells from lymph nodes of patients infected with HIV-1 display increased caspase-3 expression [9]. In addition to self cleavage [4], caspase-3 was reported to truncate Bid [4], cleave its downstream proteins [4], and activate molecules in the autophagy signaling pathway which increases HIV-1 yields [11]. The inhibition of caspase-3 activity significantly blocks HIV-1 RNA production (Fig. 3A), it may be because the inhibition can affect many activities of caspase-3 in the signaling pathways of apoptosis and/or autophagy during HIV infection.

HIV-1 infection increases expression of MAPK proteins, Erk, p38, and JNK, which may regulate cellular apoptotic machinery [12–14] (Fig. 4). Fas-agonistic antibody CH11, which effectively activates Fas-mediated pathway, regulates expression of Erk and p38 in a trend that is a positive relation with HIV-1 replication at the dose-dependent manner (S. 2A). The p38 kinase family is one of three major known Mitogen-Activated Protein Kinase (MAPK) pathways in humans and contains four P38 isoforms (α, β, γ, and σ) [15]. Cells expressing different p38 isoforms after HIV-1 infection displayed different effects on HIV-1 virus production (Sup. 2B). These data indicate that HIV-1 infection activates MAPK pathways, and regulates the apoptosis process to facilitate viral replication.

In conclusion, We provide direct evidence that proapoptotic molecules from both death receptor-mediated and Bax/mitochondrea-mediated apoptotic pathways increase HIV-1 replication,

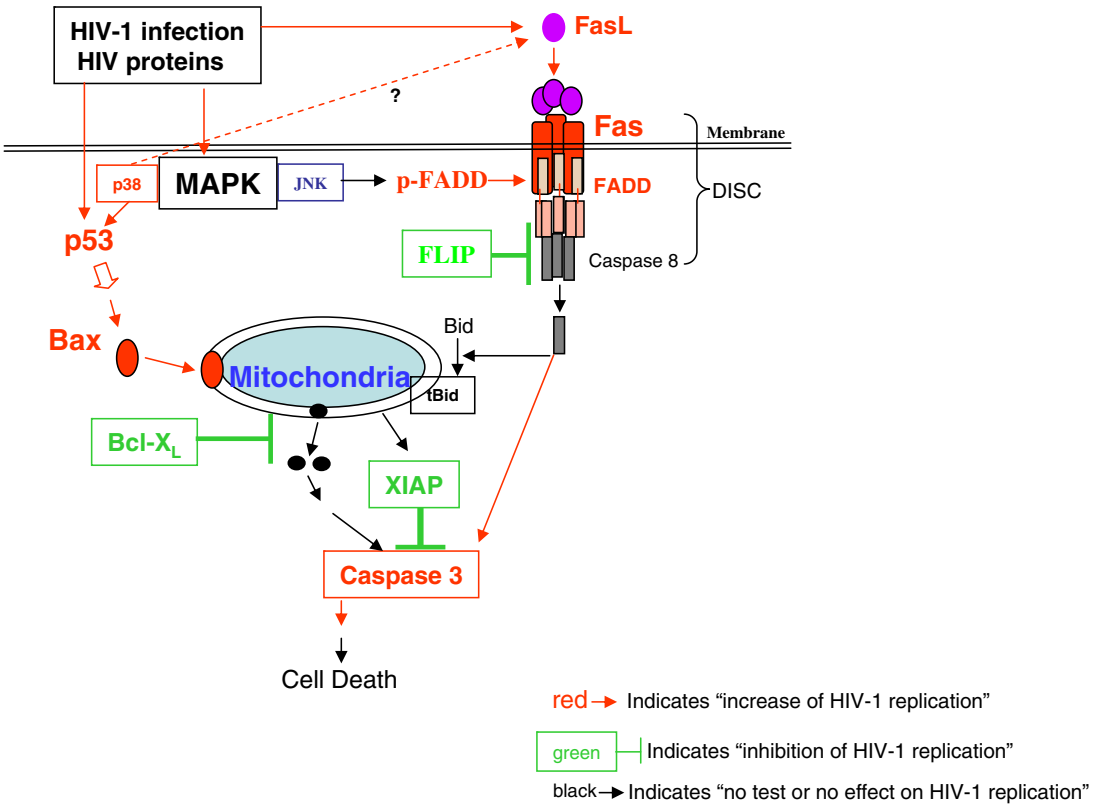

Fig. 4. Association of HIV-1-induced cell death pathways with HIV-1 replication. The diagram depicts the pathways by which HIV-1 infection triggers cell death. HIV-1 triggers both Bax/mitochondrial (intrinsic) and death receptor (Fas)-dependent (extrinsic) apoptotic pathways via inducing MAPK signaling pathways, by increasing the expressions of FasL, p53, and Bax; promoting the activation of Bax, FADD, and caspases; and decreasing the expressions of Bcl-X_L and FLIP. The increased expression of preapoptotic molecules, FasL, FADD, p53, and Bax, promotes HIV-1 replication; and inhibition of caspase-3 activation and increased expression of FLIP, Bcl-X_L, and XIAP inhibits HIV-1 replication. (For interpretation of the references to color in this figure legend the reader is referred to the web version of this article.)

while antiapoptotic proteins inhibit viral RNA yield (Fig. 4), suggesting that apoptotic inhibition is able to reduce HIV-1 replication and its cytopathogenesis, and molecules that disrupt the HIV-mediated signal transduction chain influence viral replication and pathogenesis. More detailed analysis of these cellular and molecular events associated with HIV-1 replication will provide new insights and targets for controlling HIV associated disease.

Conflict of interest

None declared.

Acknowledgments

The authors wish to acknowledge of Dr. Mingjie Zhang, Dr. Krishnakumar Devadas for critical review of this manuscript. The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.09.007.

References

[1] .P. Matarrese, W. Malorni, Human immunodeficiency virus (HIV)-1 proteins and cytoskeleton: partners in viral life and host cell death, Cell Death Differ. Suppl. 1 (2005) 932–941.
[2] X. Wang, R. Viswanath, J. Zhao, S. Tang, I. Hewlett, Changes in the level of apoptosis-related proteins in Jurkat cells infected with HIV-1 versus HIV-2, Mol. Cell Biochem. 337 (2010) 175–183.
[3] X. Wang, J. Zhao, S. Tang, S. Lee, R.I. Glazer, I. Hewlett, C-FLIPL regulates PKC via AP-2 to inhibit Bax-mediated apoptosis induced by HIV-1 gp120 in Jurkat cells, Mol. Cell Biochem. 330 (2009) 23–29.
[4] X. Wang, Y. Wang, J. Zhang, H.P. Kim, S.W. Ryter, A.M. Choi, FLIP protects against hypoxia/reoxygenation-induced endothelial cell apoptosis by inhibiting Bax activation, Mol. Cell Biol. 25 (2005) 4742–4751.
[5] S.T. Ju, D.J. Panka, H. Cui, R. Ettinger, M. el-Khatib, D.H. Sherr, B.Z. Stanger, A. Marshak-Rothstein, Fas(CD95)/FasL interactions required for programmed cell death after T-cell activation, Nature 373 (1995) 444–448.
[6] M.O. Westendorp, R. Frank, C. Ochsenbauer, K. Stricker, J. Dhein, H. Walczak, K.M. Debatin, P.H. Krammer, Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120, Nature 375 (1995) 497–500.
[7] D.J. Shedlock, D. Hwang, A.Y. Choo, C.W. Chung, K. Muthumani, D.B. Weiner, HIV-1 viral genes and mitochondrial apoptosis, Apoptosis 13 (2008) 1088–1099.
[8] L. Dubrez-Daloz, A. Dupoux, J. Cartier, IAPs: more than just inhibitors of apoptosis proteins, Cell Cycle 7 (2008) 1036–1046.
[9] Q. Li, A.J. Smith, T.W. Schacker, J.V. Carlis, L. Duan, C.S. Reilly, A.T. Haase, Microarray analysis of lymphatic tissue reveals stage-specific, gene expression signatures in HIV-1 infection, J. Immunol. 183 (2009) 1975–1982.
[10] Z.T. Wan, X.L. Chen, Mechanisms of HIV envelope-induced T lymphocyte apoptosis, Virol. Sin. 25 (2010) 307–315.
[11] T. Koike, H. Yamagishi, Y. Hatanaka, A. Fukushima, J.W. Chang, Y. Xia, M. Fields, P. Chandler, M. Iwashima, A novel ERK-dependent signaling process that regulates interleukin-2 expression in a late phase of T cell activation, J. Biol. Chem. 278 (2003) 15685–15692.
[12] P.S. Cohen, H. Schmidtmayerova, J. Dennis, L. Dubrovsky, B. Sherry, H. Wang, M. Bukrinsky, K.J. Tracey, The critical role of p38 MAP kinase in T cell HIV-1 replication, Mol. Med. 3 (1997) 339–346.
[13] R.L. Furler, C.H. Uittenbogaart, Signaling through the P38 and ERK pathways: a common link between HIV replication and the immune response, Immunol. Res. 48 (2010) 99–109.
[14] B. Hemonnot, C. Cartier, B. Gay, S. Rebuffat, M. Bardy, C. Devaux, V. Boyer, L. Briant, The host cell MAP kinase ERK-2 regulates viral assembly and release by phosphorylating the p6gag protein of HIV-1, J. Biol. Chem. 279 (2004) 32426–32434.
[15] P.P. Roux, J. Blenis, ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions, Microbiol. Mol. Biol. Rev. 68 (2004) 320–344.