HIV-1 Vpu Accessory Protein Induces Caspase-mediated Cleavage of IRF3 Transcription Factor

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Background: The transcription factor IRF3 is not properly activated during HIV-1 infection.

Results: Infection with VSV-G-pseudotyped HIV-1 induces caspase-mediated cleavage of IRF3, and Vpu contributes to this event.

Conclusion: The product of IRF3 cleavage by HIV-1 interferes with IRF3-regulated gene expression.

Significance: Our findings contribute to the understanding of how HIV-1 attenuates the innate anti-viral response.

Vpu is an accessory protein encoded by HIV-1 that interferes with multiple host-cell functions. Herein we report that expression of Vpu by transfection into 293T cells causes partial proteolytic cleavage of interferon regulatory factor 3 (IRF3), a key transcription factor in the innate anti-viral response. Vpu-induced IRF3 cleavage is mediated by caspases and occurs mainly at Asp-121. Cleavage produces a C-terminal fragment of ~37 kDa that comprises the IRF dimerization and transactivation domains but lacks the DNA-binding domain. A similar cleavage is observed upon infection of the Jurkat T-cell line with vesicular stomatitis virus G glycoprotein (VSV-G)-pseudotyped HIV-1. Two other HIV-1 accessory proteins, Vif and Vpr, also contribute to the induction of IRF3 cleavage in both the transfection and the infection systems. The C-terminal IRF3 fragment interferes with the transcriptional activity of full-length IRF3. Cleavage of IRF3 under all of these conditions correlates with cleavage of poly(ADP-ribose) polymerase, an indicator of apoptosis. We conclude that Vpu contributes to the attenuation of the anti-viral response by partial inactivation of IRF3 while host cells undergo apoptosis.

Vpu is a 16-kDa transmembrane protein encoded by HIV-1 and some simian immunodeficiency virus (SIV) strains that interferes with multiple host-cell functions to enhance viral propagation and pathogenesis (1). One of the best characterized effects of Vpu is the down-regulation of CD4, a component of the host immune system that doubles as the primary HIV-1 receptor. CD4 down-regulation impairs the immune response, prevents superinfection, and promotes the release of infectious virions. This function of Vpu involves an interaction with newly synthesized CD4 in the endoplasmic reticulum, leading to CD4 ubiquitination mediated by the SCFβ-TrCP ubiquitin ligase and targeting to the endoplasmic reticulum-associated degradation pathway (2–4). Another well established function of Vpu is the counteraction of tetherin, a plasma membrane protein that prevents the release of viral progeny (5, 6) and acts as a virus sensor to induce NF-κB-dependent pro-inflammatory gene expression (7). Tetherin down-regulation by Vpu occurs by a different mechanism, involving internalization, intracellular retention, and/or lysosomal degradation (8, 9). Mounting evidence indicates that Vpu has additional functions that contribute to HIV-1 escape from the host immune system. For example, Vpu down-regulates the natural killer cell receptor NK-T-B-antigen and ligand poliovirus receptor from the surface of HIV-1-infected cells (10, 11). Furthermore, in dendritic cells, Vpu inhibits the surface expression of the antigen-presenting protein CD1d by interrupting its recycling from intracellular compartments (12).

Interferon regulatory factor 3 (IRF3) is a key transcription factor in the innate immune response against viral infection. This response is triggered by pattern recognition receptors, including Toll-like receptors and RIG-1-like receptors, that recognize viral pathogen-associated molecular patterns. Through pattern recognition receptor signaling pathways, IRF3 induces the expression of type I IFNα/β genes as well as other interferon-stimulated genes (13). In HIV-1 infection, anti-viral responses are not properly deployed and only show a limited profile of gene expression (14, 15). Because of the blunted anti-viral response to HIV-1, it has been suggested that this virus might have a mechanism to antagonize IRF3 function. Indeed, two other HIV-1 accessory proteins, Vif and Vpr, impair the innate immune response by promoting degradation of IRF3 (16). Recently, Vpu was shown to induce lysosomal degradation of IRF3 (17), although this finding was challenged by another study (18).
We were intrigued by the possibility that a cytosolic/nuclear protein such as IRF3 could be targeted for degradation in the lysosomal lumen. To investigate this mechanism, we started by re-examining the effect of Vpu on IRF3. However, our experiments did not show any evidence of lysosomal degradation of IRF3 induced by Vpu. Instead, we found that Vpu causes partial cleavage of IRF3 in a caspase-dependent manner. A similar cleavage was elicited by transfection of 293T cells with a Vpu-expressing plasmid or by infection of Jurkat T-cells with vescicular stomatitis virus G glycoprotein (VSV-G)-pseudotyped HIV-1. In addition to Vpu, two other HIV-1 accessory proteins, Vpr and Vif, also contributed to the induction of IRF3 cleavage. Finally, we found that the C-terminal fragment of IRF3 produced by the cleavage can act as a negative regulator of IRF3-dependent gene activation.

**EXPERIMENTAL PROCEDURES**

**Cells, Transfection, and Reagents—**293T cells and HeLa cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 100 units/ml penicillin-streptomycin at 37 °C in a 5% CO₂ atmosphere. 293T cells were transiently transfected using Lipofectamine 2000 (Invitrogen). The Lipofectamine 2000 DNA mixture was made according to the manufacturer’s protocol, and the mixture was directly added to the cells with growth medium. HeLa cells were transfected using Lipofectamine 2000 according to the manufacturer’s protocol. Chloroquine (Sigma) was used at 20 μM, MG132 (Sigma), Z-VAD-FMK (Sigma), Z-IE TD-FMK (Sigma), and Z-DEVD-FMK (Sigma) were used at 10 μM, and bafilomycin A1 (Sigma) was used at 50 nM. The following antibodies were used in this study: rabbit anti-IRF3 (Cell Signaling), rabbit anti-Vpu (Acris Antibodies), mouse anti-cleaved caspase-8 (Cell Signaling), rabbit anti-poly(APD-ribose) polymerase (PARP) (Cell Signaling), rabbit anti-cleaved PARP (Cell Signaling), HRP-conjugated mouse anti-HA (Cell Signaling), mouse anti-HA (Sigma), human anti-HIV-1 (obtained from the National Institutes of Health AIDS Research and Reference Reagent Program), mouse anti-Vif (Abcam), rabbit anti-Vpr (Proteintech), mouse anti-clathrin heavy chain (BD Biosciences), HRP-conjugated donkey anti-mouse IgG (Santa Cruz Biotechnology), HRP-conjugated donkey anti-rabbit IgG (Santa Cruz Biotechnology), and HRP-conjugated goat anti-human IgG (Santa Cruz Biotechnology).

**Plasmids—**The IRF3 open reading frame was PCR-amplified from pCMV-SPORT6-IRF3 (Open Biosystems) and subcloned into pcDNA5/FRT/TO using KpnI and XhoI. A tobacco etch virus protease cleavage site and an HA tag were inserted at the 3’ end of the IRF3 open reading frame using Xhol and Apal (pcDNA5 IRF3-HA). Constructs expressing mutant IRF3 were generated from pcDNA5 IRF3-HA by site-directed mutagenesis (QuickChange II kit; Stratagene). pcDNA5 3’×FLAG-IRF3 was generated by insertion of a 3×FLAG sequence at the 5’ end of the IRF3 open reading frame of pcDNA5 IRF3-HA construct using HindIII and KpnI. A sequence encoding the C-terminal IRF3 cleavage product (amino acids 126–427) was amplified by PCR and subcloned into pcDNA5/FRT/TO with the addition of an ATG codon at the 5’ end of the cleaved IRF3 open reading frame (pcDNA5 c-IRF3). Sequences encoding WT and mutant IRF3 were verified by DNA sequencing. pcDNA3.1-codon-optimized HIV-1 NL4-3 Vpu was previously described (3). Codon-optimized open reading frames of Vpu from HXB2, JR-CSF, ANT70, MVP5180, YBF30, and SIVVcpz strains of HIV-1 and the EK505 and GAB1 strains of SIVcpz were synthesized by chemical gene synthesis (GenScript) and cloned into pcDNA3.1 with sequences encoding a C-terminal HA tag. p55C1B-Luc was a kind gift from Dr. Takashi Fujita (Tokyo Metropolitan Institute of Medical Science, Japan).

**Virus Preparation—**Virus stocks were prepared by transfecting 293T cells with the indicated plasmids using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s recommendations. The plasmids used to prepare virus stocks were: full-length molecular clone pNL4-3 (21) and Vpu-defective (pNL4-3delVpu) (22), Vpr-defective (pNL4-3delVpr) (23), and Vif-defective (pNL4-3delVIf) (24) clones. The VSV-G expression vector pCMV-ΔG (25) was generously provided by Dr. Jane Burns (University of California, San Diego, La Jolla, CA). VSV-G pseudotyped virus stocks were prepared by co-transfecting 293T cells with pCMV-ΔG and the indicated molecular clones. One day after transfection, virus in the supernatant was collected, and reverse transcriptase activity was measured.

**Infection of Jurkat Cells—**Jurkat cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum. One half-million Jurkat cells were infected with reverse transcriptase-normalized VSV-G pseudotyped virus stocks (at 3–5 cpm of reverse transcriptase activity/cell) and spinoculated for 2 h at room temperature. Virus in the supernatant was removed, and cells were resuspended in 1 ml of RPMI 1640. Under these conditions, a majority of Jurkat cells were infected. After culturing at 37 °C, cells were pelleted and lysed in buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, and protease inhibitor mixture (Roche Applied Science). Proteins were denatured by boiling in SDS-PAGE sample buffer and subjected to immunoblot analysis with the indicated antibodies.

**Luciferase Gene Reporter Assay—**For Dual-Luciferase gene reporter assay, 293T cells were transiently transfected with p55C1B-Luc (200 ng), pRL-TK (50 ng), pcDNA5 IRF3 (100 ng), and pcDNA5 c-IRF3 (various amounts from 0 to 200 ng). At 48 h after transfection, firefly and Renilla luciferase activity was measured.
IRF3 Cleavage by HIV-1 Vpu

measured using the Dual-Luciferase reporter assay system (Promega).

RESULTS

Vpu Induces IRF3 Cleavage—To test whether Vpu induces lysosomal degradation of IRF3, we performed a cycloheximide (CHX) chase assay using transfected 293T cells. The experimental protocol consisted of transfecting cells with a plasmid encoding IRF3 tagged at its C terminus with the HA epitope (IRF3-HA), plus or minus another plasmid encoding WT Vpu (unless otherwise indicated, Vpu was from the HIV-1 NL4-3 strain). At 48 h after transfection, cells were incubated for 2 h at 37 °C in the absence or presence of the lysosomal acidification inhibitor chloroquine (20 μM) or the proteasomal inhibitor MG132 (10 μM). This was followed by the addition of 100 μg/ml CHX and further incubation for different times at 37 °C. IRF3 levels were then detected by immunoblotting with antibodies to the C-terminal region of the protein or to the HA epitope. Using this protocol, we observed that Vpu expression caused a slight decrease in the half-life of IRF3 (from greater than 8 h to ~6.7 h) (Fig. 1A). However, chloroquine or MG132 had no effect on the half-life of IRF3 in the presence of Vpu (Fig. 1A). Furthermore, we did not observe co-localization of Vpu with IRF3 in either the presence or the absence of chloroquine (Fig. 1B). Under all conditions tested, Vpu was mostly accumulated in the juxtanuclear area, whereas IRF3 was diffusely distributed throughout the cytoplasm (Fig. 1B). Thus, we were unable to confirm that Vpu targets IRF3 for degradation in lysosomes.

Interestingly, prolonged exposure of the blots in Fig. 1A revealed a faster migrating (~37-kDa) IRF3 species in cells expressing Vpu but not in control cells. This species could be detected with antibodies to both IRF3 and the HA epitope (Fig. 1C), indicating that it corresponded to a C-terminal fragment of IRF3. The appearance of this species was not prevented by chloroquine or MG132 (Fig. 1A), and was therefore not dependent on lysosomal or proteasomal degradation. This fragment was quite stable over the 8-h CHX chase period under all incubation conditions (Fig. 1A). Mutation of the Vpu phosphoacceptor sites Ser-52 and Ser-56 involved in β-TrCP recruitment (SN mutant) (26) greatly decreased, although did not completely abrogate, the appearance of this fragment (Fig. 1C), implying that IRF3 cleavage was largely dependent on the ability of Vpu to interact with β-TrCP.

To examine whether Vpu-induced IRF3 cleavage involved a direct interaction between these proteins, extracts from cells co-expressing IRF3-HA and either WT or SN mutant Vpu were subjected to immunoprecipitation with antibody to the HA epitope followed by immunoblotting with antibody to Vpu (Fig. 1D). We did not observe co-precipitation of either Vpu species with IRF3-HA (Fig. 1D). Under the same conditions, SN mutant Vpu exhibited robust co-precipitation with HA-tagged CD4 (Fig. 1E), as reported previously (27). The degradation of CD4-HA caused by expression of WT Vpu precluded detection of the co-precipitation of these proteins (Fig. 1E). From these experiments, we concluded that Vpu induces partial cleavage of IRF3, independently of lysosomal and proteasomal degradation and of a physical interaction between these proteins in 293T cells.

Caspase Activity Is Required for Vpu-induced IRF3 Cleavage—It was previously reported that infection with Sendai virus or adenovirus caused cleavage of IRF3 by caspase-8 (28). To examine whether Vpu-induced IRF3 cleavage is dependent on caspase activity, 293T cells co-expressing IRF3 and Vpu were incubated for 8 h with the pan-caspase inhibitor Z-VAD and of the proteasomal inhibitor bafilomycin A1 (BFM), MG132, or vehicle (DMSO) as controls. As shown in Fig. 2A, Z-VAD blocked IRF3 cleavage, in contrast to BFM and MG132, which had no effect. Caspase activation plays a critical role in apoptosis. Indeed, previous studies showed that Vpu induces activation of caspase-8 by suppressing NF-κB-dependent activation of anti-apoptotic genes (29), inhibits degradation of the apopto-
Asp-121 and Asp-125 of IRF3 Are the Sites of Caspase-dependent Cleavage—Caspases are cysteine-dependent, aspartate-directed proteases that recognize canonical four-amino acid sequences and hydrolyze the peptide bond C-terminal to the aspartate residue. From the molecular weight of the IRF3 fragment generated in the presence of Vpu, we inferred the location of the caspase cleavage site to amino acid residues 111–140 of IRF3. To identify the exact site, we mutated each aspartate in this region to alanine (Fig. 4A). The IRF3 mutants were expressed along with WT Vpu or empty vector in 293T cells and analyzed by immunoblotting. We found that none of the mutations completely abrogated Vpu-induced IRF3 cleavage (Fig. 4B). The D121A mutant, however, showed decreased cleavage to a slightly smaller fragment (Fig. 4B). This observation suggested the existence of an alternative cleavage site that is used when Asp-121 is mutated. Indeed, a D121A/D125A double mutant showed no cleavage, whereas a D116A/D121A double mutant exhibited reduced cleavage similar to that of the D121A single mutant (Fig. 4C). We concluded that Asp-121 is the primary site for Vpu-induced, caspase-mediated IRF3 cleavage, and that alternative cleavage can occur at Asp-125 when Asp-121 is mutated (Fig. 4C).

The IRF3 N-terminal Fragment Is Rapidly Degraded by the Proteasome—To investigate the fate of the N-terminal fragment of IRF3 generated by caspase-mediated cleavage, we expressed an IRF3 construct tagged with an N-terminal triple FLAG epitope in the absence or presence of Vpu. This fragment was barely detectable, but became evident upon incubation of the Vpu-expressing cells with MG132 (Fig. 5, arrow). Therefore, unlike the IRF3 C-terminal fragment, the N-terminal fragment product of caspase cleavage is rapidly degraded by the proteasome.

HIV-1 Infection Induces Caspase-mediated IRF3 Cleavage—We next sought to determine whether IRF3 cleavage could be observed in a T-cell infection setting. To this end, Jurkat T-cells were infected with VSV-G-pseudotyped NL4-3 HIV-1 and analyzed by immunoblotting at different times after infection. We observed expression of HIV-1 proteins beginning at 24 h and greatly increasing at 48 h after infection (Fig. 6A). Interestingly,
cleavage of endogenous IRF3 and PARP was detected at 48 h (Fig. 6A), consistent with a requirement for expression of HIV-1 late genes. We then tested whether IRF3 and PARP cleavage in this system also required caspase activity. This was done by infecting Jurkat cells with pseudotyped HIV-1, and at 40 h after infection incubating the cells in the presence of Z-VAD, BFM, or MG132 for an additional 8 h. Similar to the results obtained in the transfection system (Fig. 2A), cleavage of endogenous IRF3 and PARP caused by infection with pseudotyped HIV-1 was inhibited by Z-VAD but not BFM or MG132 (Fig. 6B). MG132 actually increased the levels of cleaved IRF3 and PARP (Fig. 6B), suggesting that these fragments were subject to proteasomal degradation in infected cells. The percentage of cleaved IRF3 relative to full-length was greater in the Jurkat HIV-1 infection system than in the 293T Vpu transfection system (Fig. 1), reaching 33% in untreated cells (Fig. 6A) and >50% in MG132-treated cells (Fig. 6B). Thus, infection with pseudotyped HIV-1 also causes caspase-mediated cleavage of IRF3 and PARP.

Vpu, Vpr, and Vif All Contribute to IRF3 Cleavage in HIV-1-infected Cells—We next examined whether Vpu is responsible for the IRF3 cleavage caused by HIV-1 infection. Additionally, we investigated a possible contribution of Vpr and Vif because these proteins can induce degradation of IRF3 (16). Jurkat cells were infected with either WT or Vpu-, Vpr-, or Vif-null mutant pseudotyped HIV-1 for 48 h. As expected, infection with Vpu-null HIV-1 elicited less IRF3 and PARP cleavage than its WT counterpart (Fig. 7). Interestingly, both Vpr-null and Vif-null viruses also induced less IRF3 and PARP cleavage relative to the WT control (Fig. 7). Thus, Vpu, Vif, and Vpr all contribute to IRF3 and PARP cleavage in infected T-cells. We could not rule out, however, that decreased cleavage by the mutant viruses was due to a less robust infection (particularly noticeable in the immunoblot for HIV-1 proteins in cells infected with Vpr-defective virus in Fig. 7). Thus, we turned again to the 293T transfection system to determine whether Vpr or Vif expression could also induce IRF3 cleavage. In these experiments, cells were co-transfected with plasmids encoding IRF3-HA plus plasmids encoding Vpu, Vpr, or Vif. Interestingly, at 24 h after transfection, we could only observe IRF3 cleavage in cells expressing Vpu, and not Vpr or Vif (Fig. 8A). At 48 h, however, we observed cleavage in the Vpr- or Vif-expressing cells (Fig. 8A). The IRF3 D121A/D125A mutant failed to undergo cleavage by expression of any of the HIV-1 proteins (Fig. 8A). We next examined whether Vpu, Vpr, and Vif have additive affects. Indeed, we found that several combinations of these proteins caused enhanced cleavage at both 24 h and 48 h after transfection (Fig. 8B). The highest level of cleavage was observed upon co-expression of the three proteins (Fig. 8B). This cleavage was inhibited by Z-VAD (Fig. 8C), indicating that it was also mediated by caspases. Taken together, these experiments indicated that Vpr and Vif cooperate with Vpu to induce IRF3 cleavage, although their individual effects are weaker than those of Vpu.
Cleaved IRF3 Interferes with IRF3-regulated Gene Expression—The C-terminal fragment of IRF3 comprises the IRF association domain that is important for IRF3 dimerization and the auto-inhibitory domain that is responsible for transcriptional activation via phosphorylation by TBK1, but lacks the DNA-binding domain (Fig. 4A). Thus, we hypothesized that the C-terminal fragment of IRF3 might act as an inhibitor of IRF3 function by competing for TBK1 signaling or diminishing DNA binding through formation of hybrid dimers with full-length IRF3. To test this hypothesis, we co-expressed by transfection in 293T cells increasing amounts of the C-terminal fragment of IRF3 (c-IRF3, amino acids 126–427) with full-length IRF3 and a p55C1Bluc reporter construct having multiple IRF-binding elements upstream of the luciferase gene (32). Expression of full-length IRF3 increased luciferase activity 15-fold (Fig. 9A). Co-expression of approximately equal amounts of c-IRF3, however, decreased luciferase activity by 50% relative to expression of full-length IRF3 alone (Fig. 9A). The IRF3 C-terminal fragment thus behaves as a competitive inhibitor of full-length IRF3.

DISCUSSION

The results of our study demonstrate that HIV-1 infection causes partial caspase-mediated cleavage of IRF3 and that Vpu is a major contributor to this event. IRF3 plays important roles in the anti-viral immune response, promoting expression of type I IFNs and other anti-viral genes (33). In HIV-1 infected cells, IRF3 is not properly activated (15), suggesting that HIV-1 has mechanisms to interfere with IRF3 function. Previously, it was shown that Vpu depletes IRF3 by targeting it to lysosomal...
degradation (17). This finding, however, was disputed by another study showing that Vpu does not induce IRF3 degradation and that inhibition of NFκB activity by Vpu, Vpr, Vif, or empty vector in 293T cells. At 24 or 48 h after transfection, cells were analyzed by immunoblotting (WB) with antibodies to IRF3, Vpu, Vpr, Vif, or CHC (loading control). B, combinations of plasmids encoding Vpu, Vpr, Vif, or Vif, together with a plasmid encoding IRF3-HA, were transfected in 293T cells. After 24 or 48 h, cells were analyzed by immunoblotting with antibodies to IRF3, Vpu, Vpr, Vif, or CHC (loading control). C, IRF3-HA was expressed along with Vpu, Vpr, Vif, or empty vector by transfection in 293T cells. At 40 h after transfection, cells were treated with Z-VAD (10 μM), BFM (50 nM), or MG132 (10 μM) for an additional 8 h. Cells were then analyzed by immunoblotting with antibodies to IRF3, Vpu, Vpr, Vif, or CHC (loading control). The positions of molecular mass markers (in kDa) are indicated on the right.

IRF3 cleavage correlated with activation of apoptosis upon expression of Vpu or infection with HIV-1. HIV-1 has evolved various mechanisms to trigger apoptosis in infected cells (36). For instance, the expression of HIV-1 Tat down-regulates the anti-apoptotic protein Bcl-2 and induces apoptosis in hematopoietic cells (37). In addition, expression of HIV-1 Vpr induces apoptosis via rapid dissipation of the mitochondrial membrane potential and the release of cytochrome c (38). Vpu is also known to have pro-apoptotic activity dependent on phosphorylation of Ser-52 and Ser-56 (29, 30). In agreement with these studies, we found that Vpu expression induces activation of caspase-8 and cleavage of PARP in a Vpu phosphorylation-dependent manner. In our experiments, the block in IRF3 cleavage by a specific caspase-8 inhibitor in Vpu-expressing cells was quite effective but still partial (Fig. 2C), suggesting that other caspases might also be involved.

Our experiments also showed that Vpr and Vif contribute to caspase-mediated IRF3 cleavage. Vpr and Vif were already known to modulate the antiviral response through degradation of IRF3 (16). In addition, monocyte-derived dendritic cells infected with Vpr-null HIV-1 (although not Vif-null HIV-1) were shown to exhibit increased IFNβ mRNA expression (15). We found that expression of Vpr or Vif in 293T cells induces IRF3 cleavage, although not as effectively as Vpu expression.
Experiments involving transfection of 293T cells or infection of Jurkat T-cells with pseudotyped HIV-1 viruses carrying mutations of Vpu, Vpr, or Vif showed that all three proteins contribute to both IRF3 and PARP cleavage.

Caspase-mediated cleavage of IRF3 could be just an indirect consequence of the induction of apoptosis by Vpu. However, there is precedent for viruses exploiting caspase activation to evade host immune responses (39). We have already mentioned the induction of caspase-mediated cleavage of IRF3 by infection with Sendai virus and adenovirus (28). In addition, poxvirus infection promotes caspase-mediated cleavage of the cytoplasmic RNA helicase MDA-5, which senses the viral double-stranded RNA in the context of the innate anti-viral response (40). Like these viruses, HIV-1 may have been evolved to utilize caspase activation to interfere with IRF3 function.

Activation of caspases and the ensuing triggering of apoptosis could be considered a disadvantage for viral propagation. However, there is growing evidence that apoptosis of T-cells caused by HIV-1 infection is an important contributor to evasion of the host immune response (36). Loss of CD4-positive T-cells is one of the most striking events in HIV-1 infection (41). Importantly, HIV-1 preferentially infects and kills HIV-1-specific CD4-positive T-cells, resulting in rapid loss of HIV-specific CD4-positive T-cell responses (42). In addition, HIV-1 induces the apoptosis of anti-viral cytotoxic T lymphocytes through the CD95-mediated pathway (43). Thus, IRF3 cleavage and triggering of apoptosis likely represent two aspects of an immune evasion strategy based on Vpu-induced caspase activation.

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