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HIV Accessory Proteins versus Host Restriction Factors

Klaus Strebel

Laboratory of Molecular Microbiology, Viral Biochemistry Section, National Institute of Allergy and Infectious Diseases, NIH, Bethesda, Maryland, 20892-0460;

Abstract

Primate immunodeficiency viruses, including HIV-1, are characterized by the presence of accessory genes such as *vif*, *vpr*, *vpx*, *vpu*, and *nef*. Current knowledge indicates that none of the primate lentiviral accessory proteins has enzymatic activity. Instead, these proteins interact with cellular ligands to either act as adapter molecules to redirect the normal function of host factors for virus-specific purposes or to inhibit a normal host function by mediating degradation or causing intracellular mislocalization/sequestration of the factors involved. This review aims at providing an update of our current understanding of how Vif, Vpu, and Vpx control the cellular restriction factors APOBEC3G, BST-2, and SAMHD1, respectively.

Overview

Since its identification as the causative agent of AIDS, HIV-1 has been made responsible for the deaths of millions of people worldwide. And there is no light at the end of the tunnel just yet. One might think that viruses should not have an inherent interest in killing their hosts; after all, why bite the hand that feeds you? In fact, many viruses have adapted to their hosts in a manner that allows a relatively peaceful coexistence. In addition, many viral infections are effectively controlled by preventive vaccination. So why is it that HIV-1 or other viruses such as Influenza still cause worldwide pandemics? The answer to that question is quite simple: (i) preventive vaccines are unavailable or ineffective and (ii) our immune system has not (yet) adapted to these viral challenges. In the case of HIV, the virus infects and kills CD4⁺ T cells thereby profoundly damaging the host's ability to mount an effective adaptive T- or B-cell response [1,2]. On top of that, HIV can integrate into memory cells and long-lived macrophages, thereby establishing a latent or chronic infection. That is not to say that humans are not equipped with tools to fight HIV infections. Indeed, there is a growing list of host restriction factors that can, at least in principle, target human immunodeficiency viruses, including Apolipoprotein B mRNA-editing enzyme 3G (APOBEC3G), bone marrow stromal cell antigen 2 (BST-2), cyclophilin A, and tripartite motif protein 5alpha (Trim5α) (for review see [3]). The most recent addition to this growing list of antiviral restriction factors is SAMHD1, which was identified in a proteomics screen as a Vpx-interacting factor. SAMHD1 is now known to be responsible for the Vpx-sensitive restriction of HIV and SIV in myeloid cell types such as macrophages and dendritic cells [4,5]. APOBEC3G, SAMHD1, BST-2, Trim5α, or cyclophilin A are all part of the innate or intrinsic immune system and represent a rapid response system to fight infections.

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Correspondent address: Klaus Strebel, Viral Biochemistry Section, Laboratory of Molecular Microbiology, NIAID, NIH; Bldg. 4, Room 310; 4 Center Drive MSC 0460; Bethesda, MD 20892-0460, Tel: 301 496-3132; Fax: 301 402-0226; kstrebel@nih.gov.

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Natural infection of African green monkeys by SIV typically does not induce AIDS-like symptoms despite persistent virus infection [6]. So why is it that SIV-infected monkeys live happily ever after while infection of humans by the closely related HIV-1 causes severe and generally fatal immune deficiency? Again, the answer - at least in part - is lack of adaptation. HIV-1 is believed to have evolved from chimpanzee immunodeficiency virus (SIVcpz) and was only recently introduced into humans [7]. While there is evidence for positive selection of host restriction factors [8], population-wide adaptation of the innate and intrinsic immune system to new viral challenges is slow and probably needs to be measured in centuries rather than weeks. This is probably the reason why in the case of HIV-1, viral countermeasures do currently have the upper hand.

When HIV-1 was first cloned and sequenced, researchers were surprised to find that in addition to the prototypical retroviral *gag*, *pol*, and *env* genes, this virus contained several additional open reading frames. Collectively, they were termed accessory genes due to a lack of understanding of their biological functions. HIV-1 has four such accessory genes, *vif*, *vpr*, *vpu*, and *nef*. HIV-2 and SIV also encode *vif*, *vpr*, and *nef* but they lack for the most part *vpu*. Instead, HIV-2 and most SIVs encode a *vpx* gene. However, it is important to point out that Vpu and Vpx are not functional homologues and they target different host factors as described in more detail below. While each accessory protein targets different cellular factors, the strategies employed are strikingly similar: none of the HIV accessory proteins has enzymatic activity; instead, they all seem to act as molecular adapters to manipulate the host cell, resulting in most instances in the proteolytic degradation of the cellular target (Fig. 1).

Vif

Vif (Viral infectivity factor) is critical for the production of infectious virus *in vivo*. While *vif*-deficient viruses can replicate unimpaired in many tissue culture systems, there are no known replication competent *vif* defective primary HIV or SIV isolates [9]. Vif targets APOBEC3G, a cellular cytidine deaminase that in the absence of Vif is packaged into virions and causes severe damage to the viral genome by deaminating cytidine residues during reverse transcription of the viral genome [10]. Deamination of cytidine produces deoxyuridine that is misread by the reverse transcriptase as thymidine during second strand cDNA synthesis and results in the insertion of alanine instead of guanine (reviewed in [11]). The presence of deoxyuridine in single-stranded viral cDNA can also lead to activation of the cellular DNA repair machinery and result in lethal fragmentation of the viral cDNA. In the presence of Vif, APOBEC3G is excluded from virions, thus allowing the virus to replicate unharmed in APOBEC3G-expressing cells. This makes Vif an interesting target for the development of novel antivirals. Indeed, several small molecule inhibitors targeting Vif/APOBEC3G have been identified [12,13]. However, none of them has proven very potent when tested in tissue culture. Dominant-negative mutants of Vif that interfere with the activity of virus-encoded Vif may offer an alternative approach but their potential for development into clinically useful antivirals remains to be explored [14].

One of the important points to consider when designing Vif-based antivirals is that incomplete inhibition of Vif could be counterproductive and provide the virus with a selective advantage. Indeed, naturally occurring HIV-1 variants with partially defective *vif* genes rapidly developed drug resistance when put under selection pressure [15]. The reason is that sublethal levels of APOBEC3G will not completely block virus replication but will promote deamination-induced mutagenesis of the viral genome, which in turn accelerates viral evolution in response to environmental challenges such as antiviral drug therapy. So how does Vif neutralize APOBEC3G? The commonly accepted and most widely studied mechanism is proteasomal degradation. Vif interacts with APOBEC3G and at the same time

assembles a Cul5-based E3 ubiquitin ligase complex [16]. This molecular adapter function of Vif results in ubiquitination of APOBEC3G and subsequent degradation by the cellular proteasomal machinery (Fig. 1). It was also reported that Vif can inhibit the packaging of APOBEC3G through degradation-independent pathway(s) [17]. Although not well understood at the molecular level, a degradation-independent mechanism may be especially critical early in infection when a virus enters a cell that is loaded with pre-existing APOBEC3G. Controlling pre-existing APOBEC3G is not a trivial job. Experimental evidence indicates that Vif preferentially degrades newly synthesized APOBEC3G while pre-existing APOBEC3G, which is presumably engaged in high-molecular mass complexes with other host proteins and RNA, is relatively insensitive to Vif-induced degradation [18]. Yet, such pre-existing APOBEC3G is efficiently packaged into HIV virions and potentially blocks viral infectivity unless Vif is present to prevent APOBEC3G encapsidation. Long-term exposure of cells to Vif will eventually result in depletion of APOBEC3G [19]. However, early on when Vif levels are still low, APOBEC3G clearly outnumbers Vif. Thus, if APOBEC3G degradation were the only mechanism available to Vif, one would expect viruses produced early during infection, when there are still significant amounts of APOBEC3G in a cell, to be less infectious than later on when Vif has reached steady-state levels and cells are depleted of APOBEC3G. Such a phenomenon was, however, never observed experimentally. In fact, the relative infectivity of viruses produced from HIV-infected macrophages decreased rather than increased with time even though levels of APOBEC3G in the cultures gradually decreased [19].

More recently, Vif-induced degradation of APOBEC3G was found to involve CBF β [20,21] a cellular transcription factor known to form hetero-dimeric complexes with RUNX proteins to enhance their DNA binding activity [22]. The interaction of CBF β with Vif was identified in a proteomics screen [20,21]. How a cellular transcription factor enhances Vif-induced degradation of APOBEC3G is not entirely clear. However, CBF β does not appear to directly interact with APOBEC3G or components of the E3 ubiquitin ligase complex (i.e., elongin B/C, or Cul5) but instead seems to ensure proper folding of Vif. CBF β also seems to increase the stability of Vif [21,23]. Again, the molecular mechanism is unclear. It is worth noting though that even in the presence of CBF β , Vif is unstable and subject to proteasomal degradation. The impact of CBF β on the production of infectious virus has yet to be determined. Given that production of infectious viruses is not absolutely dependent on APOBEC3G degradation, the reported impact of CBF β on APOBEC3G degradation may not be directly reflected in its effect on viral infectivity.

Vpu

Vpu is unique to HIV-1 and its predecessor SIV strains. The initial functional property attributed to Vpu was to facilitate virus release from infected cells [24]. It became apparent early on that this function of Vpu was host cell-dependent, suggesting the involvement of host cell factor(s). However, not until 20 years after the initial discovery of Vpu and its effect on virus release was the involvement of BST-2 unraveled [25,26]. Prior to the identification of BST-2, different functional models of how Vpu regulates virus release had been proposed. Among them were regulation of virus release by a Vpu ion channel activity or inactivation of a cellular background channel by Vpu [27–29]. However, the identification of BST-2 as a target for Vpu induced a major shift in research focus and investigating the role of Vpu in the antagonism of BST-2 is unquestionably the most active aspect of current Vpu-related research. Nevertheless, one should not ignore the fact that Vpu controls additional functions. These include degradation of the HIV receptor CD4 or the inhibition of NF κ B activation. These functions of Vpu are relatively well understood on a mechanistic level but their significance for HIV replication is still not fully clear [24,30].

BST-2 inhibit virus release by tethering Vpu-defective virions to the plasma membrane [25,26]. BST-2 is a transmembrane protein with two membrane anchors. Based on its homology to rat BST-2 [31], human BST-2 is generally thought to contain a C-terminal glycosylphosphatidylinositol (GPI) anchor even though experimental evidence points to a second transmembrane domain [32]. The two membrane anchors are connected by an ectodomain containing three cysteine residues that are involved in the formation of functionally critical cysteine-linked dimers [33,34]. Interestingly, there is significant flexibility regarding size and structure of the BST-2 ectodomain [35]; in fact, an artificial BST-2 construct assembled from various heterologous proteins had tethering activity but was insensitive to Vpu [34]. BST-2 can be neutralized by three different lentiviral proteins: HIV-1 employs Vpu, HIV-2 uses its Env glycoprotein, and SIV uses Nef. All three proteins are thought to interfere with BST-2 function via direct physical interaction ultimately leading to depletion of BST-2 from the cell surface. However, the observation that Vpu can enhance virus release in acutely infected T cells in the absence of appreciable surface downmodulation [36], suggests that surface depletion of BST-2 is a downstream consequence of Vpu activity, caused at least in part by interference of Vpu with the resupply of surface depleted BST-2 from the pool of newly synthesized protein [24,37]. The fact that lentiviruses have evolved three proteins to independently target BST-2 highlights the importance of controlling BST-2 function. It remains a matter of debate, however, exactly why control of BST-2 is of such importance to HIV. One argument is that the expression of BST-2 on human target cells may limit transmission of HIV-1 from its predecessor SIV [38]. This is because Vpu from SIVcpz, while capable of degrading CD4, is unable to target chimpanzee or human BST-2. In these viruses, Nef has assumed the role of Vpu to enhance virus release. However, Nef cannot target human BST-2 because its target sequence (G/D)DIWK located in the cytoplasmic domain of BST-2 has been lost in human BST-2. On the other hand, Vpu from certain HIV-1 isolates is capable of targeting macaque BST-2 [39] suggesting that species-specificity of Vpu is not as absolute as initially thought. It is not quite clear how or even why Vpu acquired anti-BST-2 activity. For instance HIV-2, which lacks a *vpu* gene, has learned to use its Env glycoprotein to antagonize human BST-2 [40,41]. Of note, even some HIV-1 isolates, such as the R5-tropic AD8 isolate, can make use of their Env protein to regulate virus release from human cells [42] raising the interesting question of whether following the initial zoonosis, the Env protein temporarily assumed Vpu-like function in HIV-1. At any rate, the ability to target BST-2 is not a feature of Vpu that arose only as a result of the transmission of SIV into humans. Indeed, Vpus encoded by SIVgsn, SIVmon, SIVmus, and SIVden are active against the BST-2 of their native hosts but not against human BST-2 [43,44]. Nevertheless,

Inhibition of virus release should not be mistaken for inhibition of virus replication. Even though tetherin potently inhibited replication of Mo-MLV in interferon-stimulated NIH 3T3 cells, no difference in plasma viremia was observed under physiological conditions between untreated normal or tetherin knockout MoMLV-infected mice [45]. Furthermore, while tetherin seemed to delay the cell-to-cell transmission of *vpu*-defective HIV-1 in short-term single cell analyses [46], the phenotype of a *vpu* defect in HIV-1 in spreading infections is characterized by reduced levels of cell-free virus but not by reduced viral replication kinetics [47,48]. This indicates that *vpu*-defective virus can employ a cell-to-cell mode of transmission that, at least in tissue culture, is as effective as cell-free transmission by wild type virus. Thus, it may be more appropriate to regard BST-2 as modulator of the mode of virus transmission (i.e. cell-free versus cell-to-cell) rather than a restriction factor inhibiting virus replication. If virus transmission from person to person occurs via cell-free virus, viruses unable to target BST-2 will certainly have a selective disadvantage. On the other hand, the fact that a number of patient-derived primary HIV-1 isolates, including HXB2, MAL, and AD8, carried mutations in the *vpu* translation initiation codon [49,50], suggests that Vpu expression may provide selective advantages or disadvantages at certain times

during virus infection and may be turned on or off depending on the environmental pressures provided by the host. Such an “on-demand” regulation of virus release activity is also seen in HIV-2 where a single amino acid change in the Env TM subunit can switch its Vpu-like activity on or off [51].

Vpx

Vpx is an accessory protein unique to HIV-2 and SIV. It is believed to have originated by gene duplication from Vpr [52]. Indeed, Vpr and Vpx proteins share several common features: (i) they are both specifically packaged into viral particles through an interaction with the p6 domain of the viral Gag precursor; and (ii) they both assemble an E3 ubiquitin ligase complex consisting of DCAF1 (previously known as Vpr-binding protein, VprBP), DDB1 (damage-specific DNA binding protein1), Cul4 (cullin4), and Rbx1 (Ring box protein1) [53,54]. The association of Vpr and Vpx with components of an E3 ubiquitin ligase complex indicated that these proteins, like Vif and Vpu, target cellular protein(s) for proteasomal degradation (Fig. 1). The fact that both proteins are specifically packaged into virions at significant levels further suggests they may play a role early during infection. Indeed, in macrophages and dendritic cells, infection by Vpx-defective virus is blocked at a pre-reverse transcription step. Interestingly, the inhibition of infection by Vpx-deficient virus was prevented when cells were preloaded with Vpx prior to infection using Vpx-containing VLPs [55,56]. While the cellular target for Vpr remains elusive, the substrate of Vpx-dependent proteolysis was recently identified as Sterile alpha motif and HD domain protein 1 (SAMHD1) [4,5,57]. Silencing of SAMHD1 in monocyte-derived macrophages or dendritic cells rendered them susceptible to infection by Vpx-defective virus [4,5,57]. Interestingly, while HIV-1 does not possess a Vpx protein, Vpx also enhances HIV-1 infection of myeloid and dendritic cells, as well as resting CD4⁺ T-cells [58–63]. In susceptible cell types, SAMHD1 has been shown to restrict infection of lentiviruses at the reverse transcription step. Vpx counteracts this restriction by binding to and causing the proteasomal degradation of SAMHD1 via interaction with a Cul4/DDB1/DCAF1 ubiquitin ligase complex [4,5,64]. Consistent with this, knockdown of SAMHD1 enhanced HIV-1 infection and rendered it Vpx independent [4,5,57,62].

Several splice variants of SAMHD1 were identified in PMA-treated THP-1 cells although their functional significance remains unclear [65]. Furthermore, SAMHD1 has recently been shown to possess nucleic acid binding properties [66–68] and in one study was also reported to have exonuclease activity [68]. However, its main catalytic activity described to date is its dGTP-dependent dNTPase activity that allows it to degrade cellular dNTPs [69,70]. In this way, SAMHD1 is thought to restrict HIV-1 replication by decreasing the levels of cellular dNTP pools to below that required for reverse transcription [71,72]. Interestingly, while SAMHD1 has been shown to restrict HIV-1 replication in non-dividing cell types such as MDMs, dendritic cells, resting CD4 T cells as well as PMA-differentiated THP-1 and U937 cells (the latter requiring exogenous expression of SAMHD1) [4,5,57,62,73], SAMHD1 restriction does not strictly correlate with its expression. Indeed, fully HIV-1 permissive cells, such as activated CD4⁺ T cells or undifferentiated THP-1 cells also express high amounts of the SAMHD1 protein [5,62]. SAMHD1 restriction could be alleviated by silencing its expression in restrictive cell types. Intriguingly, however, transfer of SAMHD1 into permissive cell types failed to induce a restrictive phenotype even when the protein was over-expressed [73]. This distinguishes SAMHD1 from APOBEC3G and BST-2 whose restrictive phenotypes were readily transferred by expression in permissive target cells. The inability of SAMHD1 to restrict viral replication when over-expressed in HeLa or 293T cells suggested that SAMHD1 either exerts its effect in conjunction with other thus far unidentified host factor(s) or, alternatively, that the antiviral activity of SAMHD1 is regulated at a post-translational level. Such a regulation of SAMHD1 activity could explain

why levels of SAMHD1 observed in undifferentiated non-restrictive THP-1 cells and in differentiated restrictive THP-1 cells are similar. Indeed, SAMHD1 was very recently found to be regulated by phosphorylation (Welbourn & Strebel [submitted] and [74,75]). Specifically, phosphorylation at a threonine residue near the C-terminus of SAMHD1 (T592) appears to inhibit SAMHD1's ability to restrict HIV infection. The sequence surrounding T592 (LIT*PQKK) conforms well to a cyclin-dependent kinase (CDK) consensus phosphorylation target site, S/T*PXR/K [76], suggesting T592 could be phosphorylated *in vivo* by a cyclin-dependent kinase. CDKs together with their partner cyclins promote cell proliferation by driving progression through the cell cycle [77]. The suggestion that regulation of SAMHD1 function is tied into the cell proliferation status is consistent with the observation that SAMHD1 restricts HIV-1 only in differentiated or non-dividing cells. During cell cycle progression, CDKs would be active to phosphorylate SAMHD1 at T592 and therefore render it inactive for HIV-1 restriction. On the other hand, SAMHD1 T592 would be in a largely unphosphorylated state in non-dividing/differentiated cells since CDKs would be inactive then. This model is supported by our finding that SAMHD1 phosphorylation is lower in differentiated THP-1 cells than in their cycling counterparts. It is tempting to speculate that phosphorylation of SAMHD1 at T592 impairs its catalytic ability to degrade dNTPs. Surprisingly, however, T592 phosphorylation did not significantly affect the catalytic dNTPase activity of SAMHD1 *in vitro* (Welbourn and Strebel, submitted). Further experimentation will be required to fully unravel the mechanism by which SAMHD1 blocks viral infection of non-dividing cells.

Conclusion

HIV accessory genes, which were once thought to play minor roles in the virus replication cycle have turned out to be key players in the control of the host's innate and intrinsic immune system. Among those, Vif is probably the most critical accessory protein since there are no primary HIV or SIV isolates that are defective in Vif. Vpu and Vpx, on the other hand, appear to have more specialized functions that control the mode of virus transmission or facilitate the infection of non-dividing cells, respectively. Non-dividing cells represent only a subset of natural target cells for HIV-1. However, their infection may be critical for the establishment of chronic infection. As such, treatment strategies targeting HIV accessory proteins may have great potential for inhibiting virus replication and preventing or delaying HIV-induced pathogenesis.

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that CBF β is important for Vif-induced degradation of APOBEC3G. Silencing of CBF β inhibits APOBEC3G degradation and reduces the infectivity of virions by raising the level of virus-associated APOBEC3G. CBF β does not directly bind to APOBEC3G or Cullin5 but increases the affinity of Vif to Cullin5, a component of the E3 ubiquitin ligase responsible for ubiquitination of APOBEC3G. [PubMed: 22190036]

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triphosphates to the constituent deoxynucleoside and inorganic triphosphate. The crystal structure of the catalytic core of SAMHD1 reveals that the protein is dimeric and indicates a molecular basis for dGTP stimulation of catalytic activity against dNTPs. The authors propose that SAMHD1 may restrict HIV-1 replication by hydrolysing the majority of cellular dNTPs thus inhibiting reverse transcription and viral cDNA synthesis. [PubMed: 22056990]

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- Host restriction factors are part of the innate immune system
- HIV encodes accessory proteins to control host restriction factors
- Vif degrades APOBEC3G and inhibits its encapsidation into virions
- Vpu degrades CD4 and downmodulates BST-2
- Vpx degrades SAMHD1

Figure 1.

HIV accessory proteins function as adaptor molecules. HIV accessory proteins have no enzymatic activity. Instead, they act as adaptor molecules to connect cellular substrates to other cellular pathways such as E3 ubiquitin ligases that then trigger ubiquitination and degradation of the substrates. In some cases, they connect their substrate to adapter protein (AP) complexes that trigger internalization and subsequent lysosomal degradation of the substrate. In most cases, the viral adapter molecule is recycled. Substrates are shown on the left and effectors on the right. For recent reviews on the function of Nef and Vpr see [78–81].