The Role of HIV-1 Vpu in down regulate cell surface expression of MHC class I proteins, mediated CD4 degradation and phosphorylation as potential target for vaccine development (Part 15)

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Abstract

BST2/tetherin, an antiviral restriction factor inhibits the release of enveloped viruses from the cell surface. Human immunodeficiency virus-1 (HIV-1) antagonizes BST2 through viral protein u (Vpu), which down regulates BST2 from the cell surface.

Vpu mimics a canonical acidic dileucine-sorting motif to bind AP1 in the cytosol, while simultaneously interacting with BST2 in the membrane. These interactions enable Vpu to build on an intrinsic interaction between BST2 and AP1, presumably causing the observed retention of BST2 in juxtanuclear endosomes and stimulating its degradation in lysosomes.

In previous study of the function of the HIV-1 Vpu protein, only HIV-1 has evolved to encode a gene, vpu, that has two distinct functions, i.e., the rapid ER degradation of CD4 receptor and the enhancement of virus release from infected cells which, at the same time, reduces HIV-induced cytopathic effects.

In this article, I discuss the Biology of Vpu, HIV-1 Vpu binds to multiple subunits of both AP1 and AP2, but not μ3 of AP3, Fusion of BST2CD and VpuCD enhances binding to AP1, A novel open conformation is observed for the BST2/Vpu-activated AP1, Vpu and tetherin, and Action of HIV-1 Vpu

Key Words: HIV-1 Vpu, MHC class I, CD4, and Vaccine

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1. Introduction

Vpu is an 81-residue integral membrane protein that is post-translationally modified by phosphorylation and has the ability to form oligomeric structures in membranes (1),(2),(3),(4),(5). In the absence of Vpu, viral proteins accumulate in infected cells as a result of intracellular budding, a phenomenon that is accompanied by increased cytopathicity of *vpu*-deficient isolates (5). Vpu has also been shown to induce specifically the degradation of CD4 trapped in the endoplasmic reticulum (ER) of cells by the envelope glycoprotein precursor gp160 (6),(7),(8),(9). Even though Vpu and Env are co-expressed from a bicistronic mRNA (10), the gp160 envelope product does not appear to be directly involved in either biological activities of Vpu (11),(12), (8),(12). Although the two biological functions of Vpu appear to converge and result in the increased production of infectious virions, they are distinct and mechanistically separable.The degradative activity of Vpu appears to be specific for CD4 (13), to involve physical interactions with target molecules (5), and to require Vpu phosphorylation at two conserved serine residues (14),(15). On the other hand, Vpu enhancement of particle release operates through a more generalized mechanism. Indeed, Vpu has been shown to augment release of chimeric viruses bearing the *gag-pol* regions of retroviruses that naturally lack a *vpu* open reading frame (ORF), such as HIV-2, visna virus, and Moloney murine leukemia virus (16). Vpu associates with BST2 through an anti-parallel interaction between the transmembrane domains of each protein (17),(18),(19). This interaction is species-specific and essential for the antagonism of BST2 by Vpu (20),(18). A component of the ESCRT-0 machinery, HRS, has also been suggested to recognize ubiquitinated BST2 and target it for lysosomal degradation (21). However, these degradation pathways are only partially responsible for the antagonism of BST2 by Vpu (22),(23),(17). Efficient BST2 down regulation from the cell surface can occur in the absence of BST2 degradation(24),(25),(26). Moreover, Vpu induces the mistrafficking of BST2 (26),(27),(28). causing the accumulation of BST2 at the *trans*-Golgi network (TGN). Both recycled and newly synthesized BST2 are retained at the TGN, blocking the resupply of BST2 to the plasma membrane and eventually leading to its depletion at the cell surface (29),(28), Moreover, mutations in the juxta-membrane hinge region of Vpu that interfere with the localization of Vpu to the TGN impair the antagonism of BST2 (30),(19). Clathrin-dependent trafficking pathways have been suggested to be involved in the Vpu mediated mistrafficking of BST2(31),(29),(32). Such pathways regulate the trafficking of cellular membrane proteins by selectively packaging these membrane cargos into clathrin-coated vesicles (CCV) (33),(34),(35). The clathrin adaptor

protein (AP) complexes mediate this cargo selection. In the absence of Vpu, the natural trafficking of endogenous BST2 depends on the clathrin-associated pathways, and the involvement of both AP1 and AP2 has been suggested ((36),(37). In Vpu, a putative clathrin sorting motif, ExxxLV (ELV), located in the membrane-distal half of the protein's cytoplasmic domain (VpuCD) was shown to be important for BST2 antagonism (31),(38). Furthermore, Vpu-induced virion release and removal of BST2 from the cell surface are inhibited by a dominant negative mutant of AP180, a protein required for the assembly of the CCV at the lipid membrane (31),(29).

2. The Biology of Vpu

Vpu promotes degradation of CD4 in these complexes, thus allowing Env transport to the cell surface for assembly into viral particles. Vpu is an 81-residue oligomeric integral membrane protein with an N-terminal 24-residue hydrophobic membrane-spanning domain and a C-terminal cytoplasmic tail (39),(40). Amino acids important for receptor binding and degradation have been mapped to the C-terminal region of Vpu and to putative ®-helices in the cytoplasmic tail of CD4 (41). Coimmunoprecipitation experiments have shown that Vpu associates with wild-typeCD4or with recombinant proteins containing the CD4 cytoplasmic tail, but it is not yet known if the interaction is direct or indirect (63). These complexes are probably relevant to CD4 degradation because there is a direct correlation between the extent of Vpu association and their relative levels of degradation (11). The effect of Vpu on CD4 degradation appears to be regulated by posttranslational modification. Vpu is phosphorylated on Ser52 and Ser56 by a casein kinase-2-related protein, and mutation of these positions decreases the levels of CD4 degradation (39). The mechanism of degradation is not clear but may involve the cytoplasmic proteasome, because Vpu-mediated degradation can be blocked by proteasome inhibitors such as lactacystin (42). Vpu can also down regulate cell surface expression of MHC class I proteins, which may protect infected cells from recognition and killing by cytotoxic T lymphocytes (43). In addition to its role in CD4 degradation, Vpu can also stimulate virion release, and it has been proposed to be an ion channel (40). In Vpu mutant viruses, significantly increased numbers of particles either remain associated with the cell surface or are localized to intracellular membranes (39),(40). In contrast to Vpu-mediated CD4 degradation, its effect on particle release requires the hydrophobic N-terminal domain and is not influenced by serine phosphorylation (39),(40). The mechanism appears to be relatively nonspecific in that Vpu can also promote the release of heterologous retroviral particles (39),(40). Also, absence of Vpu resulted in virions containing multiple cores and in viral budding into vacuolar compartments as opposed to the plasma Membrane (5),(44). Hence, expression of Vpu is associated with the proper maturation and targeting of the virions and with their efficient release. Another well-documented effect of Vpu expression is the reduction of syncytium-mediated cytopathicity, presumably due to the efficient release of virions and the lack of accumulation of viral proteins at the cell surface (45),(44). However, some evidence suggests that the reduction of cytopathic effect by syncytium formation may at least in part stem from a separate effect, as some mutants of Vpu, in spite of efficient viral release, showed no reduction of syncytial killing (46). More recently, Vpu has been demonstrated to degrade CD4 in the endoplasmic reticulum (ER). This effect is functionally relevant to the virus, as CD4 traps viral envelope precursors in the ER, thus affecting their transport to the cell surface (47),(8). The degradative effect of Vpu was CD4 specific, as Vpu did not mediate degradation of CD8 similarly retained in the ER(6). Vpu-specific response sequences appear to be present in the cytoplasmic domain of CD4, and the

degradative effect is dependent on Vpu being associated with the same membrane compartment as CD4 (6),(15),(7). The facilitation of virion release and the effect on CD4 are not related events, as virion release was not dependent on the envelope or CD4 expression (48). While Vpu-mediated degradation of CD4 occurs in the ER, Vpu-mediated facilitation of virion release takes place at the plasma membrane. Also, facilitation of virion release is efficiently inhibited when Vpu is retained in the ER, suggesting that these two functions occur in two separate cellular compartments(15). Phosphorylation of two seryl residues (amino acids 52 and 56) by casein kinase II has been reported to affect Vpu functional phenotypes(46),(3). While phosphorylation status only partially affected the release function, it was necessary for efficient degradation of CD4, suggesting that Vpu phosphorylation status is likely to constitute an important control mechanism(15). From a mechanistic standpoint, it is interesting that Vpu expression facilitates the release of divergent retroviruses, suggesting that its function is not specific to HIV(49). Vpu has structural and biochemical similarities with the influenza virus M2 protein, which has been suggested to exist as a multimeric ion channel capable of affecting luminal conditions such as pH in the Golgi compartment (5),(50). Similarly, Vpu has been demonstrated to be a type 1 integral membrane protein capable of homo-oligomerization (15).

3. HIV-1 Vpu binds to multiple subunits of both AP1 and AP2, but not μ3 of AP3

Indeed, MBP152 VpuCD co-migrated with the μ1-truncated AP1 as a complex on a size exclusion column. Importantly, the binding was abolished by the alanine-mutation of the ELV motif, signifying the crucial role of the ELV motif in the interaction between Vpu and AP1. These interactions demonstrate that Vpu has evolved the ability to associate with multiple subunits of AP1, potentially allowing it to modulate the cellular trafficking machinery to target host proteins such as BST2. Altogether, these results suggest specific interactions between Vpu and AP1 and AP2, which may allow the viral protein to hijack the associated trafficking pathways. However, because BST2CD specifically binds only to μ1, but not to μ2 or μ3, AP1 may play a more significant role than AP2 in the Vpu-mediated antagonism of BST2. This notion is supported by multiple observations, with one exception (51), that Vpu does not increase the rate of BST2 internalization from the cell surface (52),(26),(32),(28).

4. Fusion of BST2CD and VpuCD enhances binding to AP1

The ability of Vpu to interact simultaneously with BST2 and AP1 suggests that Vpu may enhance a native but weak affinity between BST2 and AP1 to increase their binding and consequently retain BST2 in endosomes including the TGN and/or target it to lysosomes. BST2 and Vpu each have a transmembrane (TM) helix through which the two proteins associate. The C-terminus of BST2CD and the N-terminus of VpuCD are placed close to each other by the interacting TM helices (18).This facilitates the convenient design of a 10-amino acid fusion linker that mimics the restraints exerted by the TM helices and links the cytoplasmic domains in an appropriate spatial arrangement. The fusion protein exhibited strong binding to the GST tagged AP1 in the pull down assay.

5. Crystal structure of BST2 and Vpu binding to the open AP1 core

The AP1 core adopts an activated, open conformation, with both of its cargo binding sites exposed for interaction with BST2CD-VpuCD. The AP1 in the current structure adopts an open

conformation distinct from that observed previously for AP2 and AP1(53),(54) BST2CD binds to the tyrosine motif-binding site on AP1 through critical interactions involving the YxY motif, while VpuCD occupies the acidic dileucine motif-binding site of AP1 through the ELV motif. Only a short region of VpuCD flanking the ELV motif is well ordered and successfully built in the structure. There is no direct interaction between BST2CD and VpuCD. By combining the viral protein's affinity for AP1 *via* the ELV motif and the tight trans-membrane interaction with the host protein, Vpu appears to act as an adaptor to increase the affinity of AP1 for BST2.

6. A novel open conformation is observed for the BST2/Vpu-activated AP1

While the previously observed Arf1-bound AP1 exhibits the same level of opening as the activated AP2 (53),(54).(the AP1 in the BST2/Vpu-bound structure adopts a conformation that is much more open than the previously observed structures. When the BST2/Vpu-activated AP1 structure was overlaid with the Arf1-283 activated AP1 structure using the β1 subunits, a twisting of the γ and σ1 subunits was observed, which further exposes the dileucine-binding pocket at the γ/σ1interface in AP1. The conformational change involves a ~20° rotation of γ/σ1 around an axis at the base of γ where it contacts β1, with the largest Cα movement of ~35 Å at the tip of γ. As a result of the γ/σ1 movement, new interactions occur between the μ1-CTD and the N-terminal portion of γ. This new γ-μ1interface has a buried surface area of Å2, and consists of extensive, hydrogen bonding and salt bridge interactions between the two subunits. We further created a double mutation, γ Q28R and μ1 D319R, to disrupt this new interface and tested its role in the binding of AP1 to BST2/Vpu in the GST pulldown assay. The binding was decreased, indicating that this newly observed γ-μ1 interface is important for the Vpu mediated manipulation of AP1(34).

7. Vpu and tetherin

Accessory protein Vpu is required for optimal replication of HIV-1 in certain cell types that express tetherin. Tetherin specifically inhibits the virion release from cells and is countered by Vpu ((55),(56).; Vpu degrades cellular tetherin and CD4 effectively. It is generally accepted that Vpu enhances virion release from the cell surface by down-regulation of tetherin, and thereby promote viral replication. However, Vpu proteins of HIV-1 and some SIVs cannot efficiently antagonize simian tetherin molecules relative to those of SIVs with a high ability ((57).In fact, HIV-1 NL4-3 scarcely suppressed the anti-viral activity of the rhesus tetherin. Study demonstrated that, it can be concluded that tetherin is associated with the species-tropism of HIV-1. The positive effect of Vpu on viral replication is much smaller than those of Vif and Gag-CA. Moreover, another functional activity of Vpu to degrade cellular CD4 is considered to be irrelevant to the HIV-1 species tropism (56),(58).

8. Action of HIV-1 Vpu

Vpu, which is encoded in the genomes of HIV-1 and a few SIV strains, is an 81-amino acid type I transmembrane protein. It comprises an amino-terminal single TM α-helix domain that also acts as an uncleaved signal peptide, and a carboxy-terminal CT domain in which two cytosolic α-helices are separated by a short flexible connector loop. Vpu mediates proteasomal degradation of CD4 by interacting with newly synthesized CD4 molecules in the endoplasmic reticulum, together with the β-transducin repeat-containing protein (βTrCP) 1 and 2 subunits through its phosphoserine residues in the CT domain(59),(60). This βTrCP dependency of Vpu is only partially common to the anti-tetherin activity since βTrCP-binding-defective mutant viruses still retain half of the wild-type activity (23). The models of intracellular sites of Vpu's action in tetherin down-regulation have been controversial. First, it was proposed that Vpu interferes with the membrane transport of newly synthesized tetherin by sequestering the restriction factor in the trans-Golgi network (TGN)(61),(62),(63). Second, Vpu might be able to block the recycling of tetherin by sequestering the latter protein in the recycling endosomes after its internalization from the cell surface(61),(62),(63),(64). Third, it was suggested that Vpu might directly internalize tetherin from the cell surface leading to lysosomes(23),(65),(66). possibly in a cell-type-dependent manner(67). In terms of the intracellular fate of tetherin, Vpuinduced down-regulation of the restriction factor might be mediated in part through proteasomal degradation(68),(69). This possibility is based on experiments in which the treatment by proteasomal inhibitors resulted in increased levels of tetherin and loss of Vpu-mediated viral release enhancement(70). Indeed, the latter degradation pathway has been suggested by evidence that the treatment with inhibitors of the lysosomal pathway blocks the Vpu-mediated tetherin degradation (23),(64),(66). resulting in a clear colocalization of these two proteins to lysosomal compartments(23),(68),(67). It has been reported that tetherin is constitutively degraded in lysosomes by HRS, a key component of the ESCRT-0 complex that sorts ubiquitinated membrane proteins to lysosomes, and this is accelerated by interaction with Vpu(66). The ability of Vpu to bind tetherin through TM-TM interaction is crucial for viral antagonism of this restriction factor (23),(68),(71),(72). This interaction is highly specific at the amino acid level requiring residues I34, L37, and L41 of tetherin(73), and A14, A18 and W22 of Vpu(74), on the hydrophobic faces of the helices that contribute an interactive surfaces. Recent NMR spectroscopy analysis showed that V30 of tetherin and A10 of Vpu together with the aforementioned residues contribute to form an anti-parallel, lipid-embedded helix-helix interface(75). Importantly, species specificity of tetherin antagonism by primate Vpu proteins is determined by their TM-TM interaction. Indeed, non-human primate tetherin proteins are mostly insensitive to Vpu antagonism(69),(76),(77),(78), due to the difference of the amino acid positions 30–45 of the TM sequence(68), (76),(79), that correspond to the interaction surface as described above.

9. Concluding remarks

In the case of antiviral proteins that are not counteracted by accessory proteins, the virus may have found an alternative means of escape. HIV-1 escapes TRIM5α by altering its capsid protein, for example. For antiviral proteins such as MxB, an interferon-induced protein that targets the viral capsid, the virus may simply live with a decreased ability to replicate. With regard to Vpu-mediated enhancement of virus secretion, no reasonable explanation for the lack of a Vpu-likfactor in HIV-2 could be offered. The absence of a Vpu counterpart in HIV-2 seemed especially paradoxal in light of the fact that Vpu was shown to be able to enhance HIV-2 particle release similar to that of HIV-1(16). Recent study confirmed that, the effect of Vpu observed on

the release of HIV-2 particles was influenced by the experimental design and was demonstrable only in the context of chimeric viruses. The fact that Vpu can fully replace the gp140 envelope to enhance particle release by the NLgp2/Udel-1 virus demonstrates that the Vpu-like activity of the gp140 envelope protein does not simply reflect the requirement for HIV-2 Gag-Pol to interact with envelope proteins for efficient particle release. Although there is evidence in the literature that specific interactions between HIV-1 Gag and envelope proteins exist (80),(81),(82),(83),(84), it has also been shown that HIV-1 as well as HIV-2 particles can be formed in the absence of Env (85),(86),(87),(88),(89),(90). The experiment demonstrated that Vpu, trans membrane (TM) domain of Vpu plays a crucial function in this process (2). Recent evidence that this domain of Vpu has the propensity to form ion channels in planar lipidbilayers(91). In addition, the ability of the Vpu TM domain to form ion-conductive membrane pores is directly correlated with its ability to enhance virus release from the cell surface(91). (43). gp140 and Vpu are also similarly regulated, since in HIV-1, Vpu and Env are translated from the same bicistronic mRNA(10). In addition, both proteins are sensitive to BFA(15), (47) indicating that they may act in similar subcellular compartments.

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