Various plus unique: Viral protein U as a plurifunctional protein for HIV-1 replication

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Impact statement

Viral protein U (Vpu) is a unique protein encoded by human immunodeficiency virus type 1 (HIV-1) and related lentiviruses, playing multiple roles in viral replication and pathogenesis. In this review, we briefly summarize the most up-to-date knowledge of HIV-1 Vpu.

Abstract

Human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome, encodes four accessory genes, one of which is viral protein U (Vpu). Recently, the study of Vpu has been of great interest. For instance, various cellular proteins are degraded (e.g. CD4) and down-modulated (e.g. tetherin) by Vpu. Vpu also antagonizes the function of tetherin and inhibits NF-κB. Moreover, Vpu is a viroporin forming ion channels and may represent a promising target for anti-HIV-1 drugs. In this review, we summarize the domains/residues that are responsible for Vpu's functions, describe the current understanding of the role of Vpu in HIV-1-infected cells, and review the effect of Vpu on HIV-1 in replication and pathogenesis. Future investigations that simultaneously assess a combination of Vpu functions are required to clearly delineate the most important functions for viral replication.

Keywords: Viral protein U, human immunodeficiency virus type 1, acquired immunodeficiency syndrome, simian immunodeficiency virus, virus, accessory protein

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Introduction

In 1988, five years after the discovery of human immunodeficiency virus type 1 (HIV-1) as the causative agent of acquired immunodeficiency syndrome,[1,2] Strebel *et al.*[3] and Cohen *et al.*[4] identified a novel gene within the HIV-1 genome encoding a 16 kDa, 77–86 amino acid protein which they named Viral protein U (Vpu). Importantly, Vpu is unique to the HIV-1 lineage including HIV-1, simian immunodeficiency viruses (SIVs) in chimpanzee (SIVcpz) and gorilla (SIVgor), and four SIVs in four species of Old World monkeys (OWMs): greater spot-nosed monkey (SIVgsn), mona monkey (SIVmon), mustached monkey (SIVmus), and Dent's mona monkey (SIVden).[5–8] In contrast, Vpu is not encoded by human immunodeficiency virus type 2 (HIV-2) and the majority of SIVs.[9]

Vpu is translated from a bicistronic *vpu/env* mRNA in the late stage of the HIV-1 life cycle at levels similar to those of *gag* and *env*.[9,10] The *vpu* gene is located immediately downstream of the 3′ end of the first coding exon of *tat/rev* and runs 28 amino acids into the start of the first open reading frame of the HIV-1 envelope protein (Env) (Figure 1(a)).[9] The HIV-1 Rev protein regulates the expression of Env and Vpu from the same mRNA by binding to the Rev response element located in *env* and promoting their export to the cytoplasm.[10] Vpu is expressed late in the viral life cycle, due in part to its dependence on Rev.[10]

HIV-1 encodes at least 109 different spliced RNAs, of which at least 12 encode bicistronic *vpu/env* mRNAs.[10,14,15] HIV-1 mRNAs use the modified scanning model of translation[16,17] for selecting AUG initiation sites.[10] Relative to the initiation site, if a purine and guanine are simultaneously found at positions −3 and +4, respectively, there is a much greater likelihood of successful translation.[17] Alternatively, ribosomal shunting can also occur, resulting in bypassing of the *vpu* ORF and efficient translation at the downstream *env* AUG.[18]

Vpu codes for an integral membrane phosphoprotein that contains a 31-amino acid hydrophobic N-terminal transmembrane anchor. As shown by nuclear magnetic resonance, Vpu is predicted to have one transmembrane

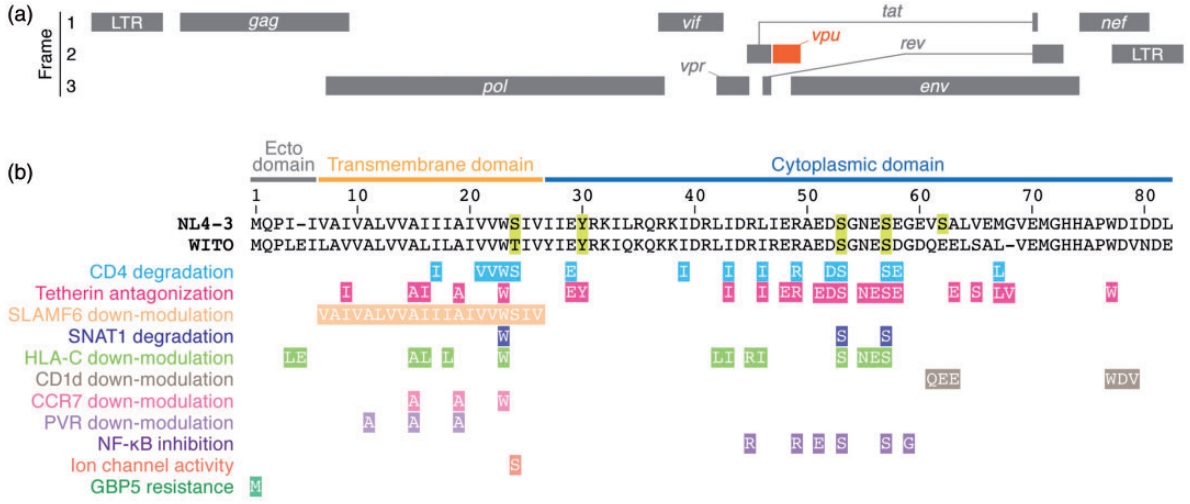

Figure 1 Vpu functions. (a) A schematic of the HIV-1 genome. The *vpu* gene is indicated in orange. (b) Summary of Vpu functions. The amino acid numbers of HIV-1 Vpu proteins of strain NL4-3 (a laboratory-adapted virus; accession number M19921) and strain WITO (a primary transmitted/founder virus)[11,12] are indicated. The putative phosphorylation residues are indicated with yellow boxes. The amino acid residues responsible for each function are summarized (note that residue 62 of strain WITO is glutamic acid, not serine). GBP5; although GBP5 does not directly interact with Vpu, the loss of the *vpu* gene boosts Env expression and overcomes the GBP5-mediated antiviral effect.[13]

α-helix and two cytoplasmic α-helixes between amino acids 6–29, 32–51 and 52–72, respectively (Figure 1(b); as the length of Vpu differs among viral strains, the residue numbers are based on the consensus sequence of HIV-1 clones NL4-3 and WITO). Vpu also has putative phosphorylation sites at serines at positions 24, 53, 57, and 62, as well as tyrosine at position 30 (Figure 1(b)).[9] Moreover, it has been reported that Vpu associates with multiple cellular proteins and elicits various responses that potently affect HIV-1 replication, infection, and pathogenesis. In the following sections, we describe the roles of Vpu in both *in vitro* cell culture systems and *in vivo*.

CD4 degradation

CD4, a 55 kDa membrane glycoprotein, is the main receptor involved in HIV infection[19,20] and is expressed on the surface of $CD4^+$ T lymphocytes and some myeloid cells such as macrophages and dendritic cells (DCs).[21] It is well known that CD4 molecules expressed on the surface of HIV-1-infected cells are severely down-regulated, and that this decrease is mediated by viral Env, Nef, and Vpu proteins.[22] Specifically, Vpu degrades CD4 molecules through a ubiquitin/proteasome-dependent pathway.[22,23]

Vpu-mediated CD4 degradation depends on the interaction of the cytosolic domains of these two proteins.[24] Vpu contains two phosphorylated serine residues in a canonical DpSGxxpS sequence within its cytoplasmic domain (residues 53 and 57; Figure 1(b)) that binds cellular beta-transducin repeat-containing E3 ubiquitin protein ligases (BTRCs, also known as β-TrCPs).[25] This recruitment leads to the addition of ubiquitin to lysine residues of the cytoplasmic tail of CD4, which marks the protein for proteasomal degradation.[26,27] Vpu is not ubiquitinated or degraded itself, as it has an equivalent function to ubiquitin ligase adaptors.[28] The formation of the Vpu–BTRC complex and the interaction between Vpu and CD4 through their transmembrane domains also increases the amount of time CD4 proteins are retained in the endoplasmic reticulum (ER).[29]

The molecular mechanism of Vpu-mediated degradation of CD4 molecules follows an ER-associated protein degradation (ERAD)-dependent pathway. Proteins in the secretory pathway that fail to acquire a native structure are usually degraded via the canonical ERAD pathway in a proteasome-dependent manner. Experiments using specific inhibitors of the proteasome pathway reported partial or complete blocking of CD4 degradation in a polyubiquitination-dependent manner. This system is, however, independent of the activity of the ER chaperone calnexin.[27] The polyubiquitination pathway requires the joining of several ubiquitin moieties to a protein. The CD4 cytoplasmic tail is considered to be the main target for the activity of ubiquitin-activating enzyme E1. The CD4 cytoplasmic tail contains four lysine residues that are potential targets for ubiquitin, and substitution of these amino acids by arginine (which cannot be joined to ubiquitin) leads to a complete blockade of CD4 degradation.[27]

However, the function of Vpu in ERAD appears to be non-canonical.[30] In 2010, Magadan *et al.*[29] reported the convergence of Vpu-induced CD4 degradation with the valosin-containing protein (VCP)–ubiquitin fusion degradation protein 1 homolog (UFD1L)–nuclear protein localization protein 4 (NPL4) complex, a key component of the ERAD pathway. The main function of this complex is to remove ubiquitinated substrates from the ER, making them available for proteasome degradation.[31–34] This activity was reported to be dependent not only on lysine residues within the cytoplasmic tail of CD4 but also on serine/threonine residues. This was the first time a ubiquitin ligase activity was shown to rely on serine/threonine activity for the degradation of an ERAD substrate. The three proteins in the VCP–UFD1L–NLP4 complex have unique functions.

VCP is a member of the AAA-ATPase superfamily and has an N-terminal domain that binds UFD1L and NPL4 and two AAA-ATPase domains. Both domains are required to degrade CD4.[29] The VCP–UFD1L–NPL4 complex is composed of a heterodimer of UFD1L–NPL4 joined with a homohexamer of VCP. Although both UFD1L and NPL4 have sequences to join ubiquitin chains that have been K48-linked or K63-linked to a target, Magadan *et al.*[29] confirmed that VCP provides energy to the complex, UFD1L binds K48 CD4 ubiquitin chains, and that NPL4 stabilizes UFD1L.

Tetherin antagonization

Previous studies indicate that Vpu potently enhances HIV-1 replication and production in certain cell lines (e.g. HeLa cells). A heterokaryon experiment conducted by Varthakavi *et al.*[35] identified the presence of a restriction factor that is expressed in HeLa cells whose action is antagonized by Vpu. Then, Neil *et al.*[36] have reported that the expression of this restriction factor is induced by type I interferon (IFN). In 2008, two groups identified this restriction factor as tetherin (also known as bone marrow stromal antigen 2).[37,38] Tetherin strictly impairs HIV-1 release from the surface of infected cells (i.e. this protein ''tethers'' budding virions). Conversely, some Vpu proteins antagonize tetherin-mediated antiviral action by down-modulating tetherin surface expression on HIV-1-producing cells (Van Damme[38]; also well summarized in the literature[7,29,39]).

To determine the Vpu residues responsible for tetherin antagonization, Vigan and Neil[40] conducted alanine scanning mutagenesis of HIV-1 Vpu (strain NL4-3) and determined that A15, W23, and, to a lesser extent, A19 are required for tetherin antagonism (Figure 1(b)).[40] Additionally, the S53/S57 mutant partially lost its antitetherin activity, suggesting that the BTRC-mediated pathway is also important.[41–43] Moreover, Kueck *et al.*[44] revealed that Vpu degrades tetherin via the endosomal degradation pathway dependent on endosomal sorting complexes required for transport machinery.[44] In addition to the interaction with tetherin via its transmembrane domain, two motifs in Vpu, the DSGNES phosphoserine motif (residues 52–57 in Figure 1) and the adjacent ExxxLV acidic dileucine motif (residues 60–65 in Figure 1), are required for clathrin-dependent sorting, which leads to tetherin antagonism.[44]

Although the potency of HIV-1 Vpu to degrade CD4 is highly conserved in all HIV-1 groups and related SIVs, the activity of Vpu to antagonize tetherin is only conserved in HIV-1 group M, a global pandemic strain.[45] In contrast to HIV-1 group M, the Vpu proteins of HIV-1 groups N, O, and P, which are endemic in Africa, either do not or only weakly antagonize tetherin.[45–49]

The Vpu proteins of SIVcpz and SIVgor, precursors of HIV-1, do not possess antitetherin activity,[45] implying that Vpu only obtained this function, once it infected humans as HIV-1. This change quite possibly contributed to its pandemic status. Indeed, a multilayered experimental–mathematical investigation has addressed this scenario and suggests that tetherin plays the role of a host restriction factor, providing ''intrinsic herd immunity,'' while Vpu has evolved in HIV-1 group M as a tetherin antagonist.[50]

Although SIVcpz Vpu does not antagonize tetherin, the Vpu proteins of SIVgsn/mon/mus, precursors of SIVcpz in OWMs, possess antitetherin activity.[45] This fact suggests that SIVcpz Vpu has lost its antitetherin activity during its evolution. It appears that SIVcpz received its *nef* gene from the SIVrcm lineage, whereas its *vpu* gene originated from the SIVgsn/mon/mus lineage.[51] The viral protein Nef of SIVcpz, SIVgor, and SIVrcm also possesses the ability to antagonize the tetherin of its host.[45,52–54] This antitetherin activity by Nef extends to HIV-1 group O.[55] It appears that the switching of viral tetherin antagonists between Vpu and Nef has frequently occurred during the evolution of primate lentiviruses including SIVs and HIV-1.

Down-modulation of cellular transmembrane proteins

In addition to CD4 and tetherin, Vpu potently degrades and/or down-modulates a variety of cellular transmembrane proteins. In this section, we summarize how Vpu degrades and/or down-modulates these cellular transmembrane proteins and the relevance of each down-regulation to HIV-1 infection.

SLAMF6/NTB-A

Signaling lymphocytic activation molecule family member 6 (SLAMF6, also known as NTB-A) is expressed on the surface of human leukocytes such as T-cells, B-cells, and natural killer (NK) cells[56] and induces the cytotoxicity mediated by NK cells.[56,57] It is also known that SLAMF6 facilitates NK cell-mediated killing of HIV-1-infected cells[58] and that HIV-1 down-modulates SLAMF6 from the surface of infected cells.[58,59] Previous studies revealed that Vpu (strain NL4-3) down-modulates SLAMF6 from the surface of infected $CD4^+$ T-cells.[60,61] A Vpu mutant with serine residues 53 and 57 substituted for asparagines was shown to be unable to bind BTRC but could still down-modulate SLAMF6 independent of BTRC. On the other hand, a Vpu derivative with a mutated transmembrane domain failed to bind to SLAMF6 (Figure 1(b)). These observations suggest that Vpu interacts with SLAMF6 via its transmembrane domain, although it was not specified which amino acid residues in the transmembrane domain are responsible for this activity.[60] Neither a lysosome inhibitor (bafilomycin) nor proteasome inhibitors (epoxomicin and MG132) were able to affect the surface expression level of SLAMF6, implying that SLAMF6 is not degraded but only down-modulated by Vpu from the cell surface.[60,61] As Vpu-mediated down-modulation of CD4 and tetherin is impaired by these reagents,[25,43,62] these observations suggest that SLAMF6 is targeted in a manner different from those of CD4 and tetherin. Additionally, a cytolytic assay suggested that Vpu-mediated SLAMF6 down-modulation leads to the evasion of HIV-1-infected cells from NK cell-mediated cytolysis.[60] Vpu's ability to down-modulate SLAMF6 has been observed to be conserved across various HIV-1 and SIV strains.[49,61]

SNAT1/SLC38A1

Using the stable isotope labeling by amino acids in cell culture technique, Matheson *et al.*[63] have conducted proteomic analyses of the kinetics of transmembrane proteins expressed on plasma membranes of HIV-1-infected cells and identified sodium-coupled neutral amino acid transporter 1 (SNAT1, also known as solute carrier family 38 member 1 [SLC38A1]).[63] SNAT1 is a transporter of certain amino acids such as glutamine and alanine and plays an important role in the uptake of glutamine by neurons.[64–66] Western blotting showed that Vpu decreases total SNAT1 expression. As the S53A/S57A mutation abolished SNAT1 degradation (Figure 1(b)), Vpu degrades SNAT1 in a BTRC-dependent manner.[63] Interestingly, the W23A mutation abolished down-regulation of all 3 Vpu substrates (CD4, tetherin, and SNAT1) (Figure 1(b)), while CD4 and SNAT1 targeting were retained in the A15L mutant. Therefore, the pathway for SNAT1 degradation by Vpu utilizes the cellular machinery used for antagonism of tetherin but occurs independently of tetherin down-regulation and may be dissociated from it by the A15L mutation.[63] Moreover, SNAT1 degradation is rescued by treatment with vacuolar ATPase (lysosome) inhibitors (concanamycin A or bafilomycin A), but not with proteasome inhibitors (MG132 and lactacystin), suggesting that (as for tetherin) SNAT1 is degraded in a lysosome-dependent manner.[63] Furthermore, the SNAT1 down-modulation suppresses $CD4^+$ T-cell proliferation. As SNAT1 is an alanine transporter, its down-modulation from the cell surface leads to the decrease of alanine uptake and subsequent mitogenesis.[63]

Based on these findings, the authors have suggested that HIV-1 interferes with immunometabolism by degrading SNAT1. In fact, Vpu-mediated SNAT1 down-modulation is observed in various strains of HIV-1 group M (e.g. strains NL4-3, YU2, JRCSF, and ZM246F), while it is absent in HIV-1 group O, SIVgor, SIVcpz*Pts*, and SIVgsn/mon/mus/den.[63] Therefore, it is assumed that Vpu's ability to degrade SNAT1 has been recently acquired (i.e. after the emergence of HIV-1 from SIVcpz).[63] Furthermore, since this ability is common in HIV-1 group M, which is largely responsible for the current pandemic (http://www.unaids.org/globalreport/), it may be reasonable to assume that Vpu-mediated SNAT1 degradation is critical for viral replication *in vivo* and/or viral pathogenicity. However, the relevance of SNAT1 degradation to viral replication has not been fully explained at this time.

HLA-C

It is well known that human leukocyte antigen (HLA) classes A and B, which are crucial membrane proteins for antigen presentation to cytotoxic T lymphocytes (CTLs),[67,68] are down-regulated by HIV-1 Nef.[67,69] Recently, Apps *et al.*[70] have reported that HIV-1 Vpu, but not Nef, specifically down-modulates HLA class C (HLA-C). The Vpu proteins of primary HIV-1 isolates (e.g. WITO, 2_87 and CH269) have the ability to down-modulate surface HLA-C, while the Vpu of HIV-1 strain NL4-3, a laboratory-adapted strain, did not efficiently perform this function.[70] Additionally, mutagenesis experiments revealed that multiple amino acid residues (summarized in Figure 1(b)) are associated with Vpu's ability to down-modulate HLA-C.[70] It should be noted, however, that the magnitude of each mutation's effect is different among each residue because the HLA-C targeting ability of Vpu varies among HIV-1 strains.[70] Furthermore, a viral replication assay (strains NL4-3 and AD8) using primary $CD4^+$ T-cells co-cultured with an Env-specific HLA-C*03-restricted CTL clone demonstrated that HIV-1 evades the CTL-driven antiviral effect through Vpu-mediated HLA-C down-modulation.[70]

CD1d

Moll *et al.*[71] have revealed that Vpu down-modulates CD1d molecules expressed on the surface of DCs infected with HIV-1. CD1d, a cellular membrane protein expressed on the surface of DCs, presents lipid antigens to NK T-cells.[72,73] HIV-1 Vpu (strain BaL) binds to CD1d and inhibits its recycling, but it does not affect the efficiency of CD1d endocytosis.[71] Interestingly, Vpu and Nef cooperatively down-modulate CD1d and inhibit CD1d-mediated lipid antigen presentation in HIV-1-infected DCs.[71]

CD1d down-regulation is a conserved function of Vpu proteins from HIV-1 groups M, O, and P as well as their direct precursors SIVcpz*Ptt* and SIVgor.[74] Among the strains of HIV-1 group M, subtype C Vpu proteins were significantly weaker CD1d antagonists than subtype B Vpu proteins.[74] Mutagenesis experiments revealed that residues in the C-terminal cytoplasmic domain are important for CD1d down-regulation (Figure 1(b)).[74]

Other cellular transmembrane proteins

In addition to the cellular proteins introduced above, Haller *et al.*[75] found that Vpu down-modulated 32 out of 105 cellular transmembrane receptors they analyzed, and interestingly, Nef also targeted all of these receptors. Particularly, surface expression of tetraspanin protein family members, such as CD37, CD53, CD63, CD81, and CD231, was reduced by both Vpu and Nef.[75] Lambele *et al.*[76] also demonstrated that two tetraspanin molecules, CD81 and CD82, are down-modulated by HIV-1 Vpu (strain NL4-3), and specifically, that CD81 is reduced independently of the serine residues at positions 53 and 57 (Figure 1(b)). Interestingly, these tetraspanin proteins including CD37, CD53, CD63, CD81, and CD231 have the ability to modulate HIV-1 infectivity by incorporating into nascent released viral particles[77] and impair cell-to-cell HIV-1 infection.[78,79] Therefore, Vpu might potently enhance HIV-1 replication by down-modulating tetraspanin proteins from the surface of infected cells.

Similar to CD81, CCR7, a chemokine receptor,[80] and Selectin L (SELL, also known as CD62L and L-selectin)[81] are down-modulated independently of the two serines at positions 53 and 57 (Figure 1(b)). Vpu inhibits the transport of newly synthesized SELL molecules to the cell surface, although the amino acids responsible for this action are not determined.[81] In the case of CCR7 down-modulation, the three residues in the transmembrane domains at positions 15, 19, and 23 are crucial.[80] Moreover, poliovirus receptor (also known as CD155) is also down-modulated

by HIV-1 Vpu (strain NL4-3).[82] This ability of Vpu is highly conserved in a variety of Vpu proteins encoded by HIV-1 group N (strain CK1.62), HIV-1 group P (strain RBF168), SIVcpz (strain EK505), SIVgor (strain CP2139), and SIVgsn (strain 166) and is determined by at least three residues in the transmembrane domain at positions 11, 15, and 19.[82]

Inhibition of nuclear factor kappa light chain enhancer of activated B-cells (NF-κB) signaling

NF-κB is a transcription factor that is ubiquitously expressed and essential for effective transcription of lentiviral genomes.[83] Galao *et al.*[84] have reported that tetherin acts as a virus sensor to induce NF-κB-dependent proinflammatory gene expression, while HIV-1 Vpu (strain NL4-3) potently suppresses the signal transduction triggered by tetherin. Recently, Sauter *et al.*[85] have reported that Vpu contributes to the strong suppression of NF-κB activity through inhibiting tetherin-triggered signaling and inhibition of nuclear translocation of p65. This activity of Vpu to suppress NF-κB is conserved among the Vpu proteins of most primate lentiviruses.[85] Vpu's ability to inhibit tetherin-induced NF-κB activation was not associated with its ability to reduce tetherin-mediated antiviral action.[85] Mutagenesis experiments revealed that Vpu-mediated recruitment of BTRC, which is mediated by serine residues at positions 53 and 57, was not sufficient for inhibition of NF-κB signaling. Further mutagenesis determined that adjacent residues, particularly R45, R49, E51, and G59 were required for efficient Vpu-mediated inhibition of NF-κB signaling (Figure 1(b)). Thus, this region of Vpu appears to affect NF-κB activity by both BTRC-dependent and -independent mechanisms. Based on these findings, the authors have emphasized that a tight interplay occurs between early and late viral products to control NF-κB activity[85]: early HIV-1 gene products such Nef and Tat promote NF-κB activation, whereas late products such as Vpu suppress it. However, it should be noted that the relevance of Vpu-mediated NF-κB inhibition in viral replication remains unclear. This issue should be addressed in a future study.

Ion channel activity and Vpu inhibitors

Before the identification of its role in targeting cellular proteins (summarized above), Vpu was characterized primarily as a cation-selective ion channel.[86] Vpu forms homo-oligomers[87] and is designated a ''viroporin.''[88] As an ion channel, Vpu depolarizes the plasma membrane by inhibiting the background potassium current and the membrane depolarization contributes to viral release.[89–91] Also, the Vpu channel can be blocked by amiloride analogs, and importantly, an amiloride analog, called BIT225, blocks the ion channel activity of Vpu in cell cultures.[92,93] Thus, this Vpu inhibitor that entered into clinical trials targets its viroporin function.[94] This compound was obtained after the finding that amiloride derivatives that inhibit ion channels could depress the release of HIV-1 virions in macrophages.[92,93] Furthermore, acylguanidine-containing compounds have been recognized as inhibitors against a broad range of viruses such as hepatitis C virus and coxsackievirus B3,[95] and a acylguanidine-containing compound, called SM111, potently impairs the late stage of HIV-1 replication.[96] More importantly, SM111-mediated inhibition of HIV-1 replication was partially overcome by a Vpu I17R substitution.[96] These observations suggest that SM111 targets Vpu and that acylguanidine-containing compounds including SM111 provide promising opportunities to develop new treatments targeting Vpu.

The ion channel activity of HIV-1 Vpu is independent of tetherin antagonism,[97] and the serine residue at position 24 is crucial for channel activity (Figure 1(b)).[98] Vpu's ion channel activity is conserved among HIV-1 groups M, N, and P and also among SIVs from chimpanzees and from greater spot-nosed monkeys, despite their sequence variability.[99] Although this suggests an involvement of Vpu's ion channel activity for viral replication, survival, and pathogenicity, its relevance *in vivo* has remained unclear. Interestingly, it has been reported that the viroporins encoded by some viruses such as influenza virus M2 protein[100] and encephalomyocarditis virus 2B protein[101] harbor the ability to trigger inflammasome activation. Therefore, it is conceivable that HIV-1 Vpu has similar activity.

GBP5 Antagonism: Trade-off of antitetherin activity and resistance to GBP5

Recently, guanylate binding protein 5 (GBP5), a novel restriction factor that potently suppresses HIV-1 replication, was identified by a genome screening analysis.[102] GBP5 belongs to an IFN-inducible subfamily of guanosine triphosphatases (GTPases) with host defense activity against intracellular bacteria and parasites.[103] Krapp *et al.*[13] revealed that GBP5 suppresses the infectivity of HIV-1 and other retroviruses by interfering with N-linked glycosylation of the viral Env, which is critical for correct processing and viral infectivity. GBP5 is endogenously expressed in human monocyte-derived macrophages (MDMs) and $CD4^+$ T-cells and its expression is induced by IFN-α and IFN-γ.[13] Additionally, the authors revealed that *GBP5* gene expression is up-regulated by HIV-1 replication in infected individuals and that its expression level is inversely correlated with the infectious HIV-1 yield in human MDMs.[13] Mutational analysis revealed that the cysteine residue at position 583 of GBP5 is critical for its antiviral activity.[13]

Importantly, the authors demonstrated that mutation of the *vpu* initiation codon or the insertion of premature stop codons in *vpu* gene increased Env expression and conferred partial resistance to GBP5.[13] Particularly, HIV-1 strains NL4-3, CH058, CH198, CH293, and CH167 with *vpu* deletion enhanced Env expression and were significantly more resistant to GBP5-mediated antiviral effects than *vpu*-proficient viruses.[13] Altogether, these observations suggest an unusual trade-off mechanism involving enhanced Env expression at the cost of an intact *vpu* gene. It is particularly noteworthy that Vpu potently antagonizes tetherin-mediated anti-HIV-1 effect, as described above. Therefore, HIV-1 must choose either activity: antitetherin activity (by maintaining *vpu* gene) or resistance to GBP5

(by augmenting Env expression through the loss of the *vpu* gene). Since most of the clinically isolated HIV-1s encode the *vpu* gene,[13,104] it is assumed that Vpu-mediated antitetherin activity is more crucial for HIV-1 to establish person-to-person transmission than acquiring resistance to GBP5. However, it should be noted that the initiation triplet AUG of *vpu* in certain HIV-1 strains such as strains HXB2,[105] BH8,[106] MAL,[107] and Zr6[108] has been primarily deleted. This fact further raises the possibility that the resistance to GBP5 is important for viruses to expand and disseminate in certain tissues or organs in infected individuals. Further investigations will shed light on the importance of the trade-off relationship between antitetherin activity and GBP5 resistance.

Impact of Vpu on HIV-1 replication and future direction

In addition to the multiple roles of Vpu described above, some virological experiments have suggested that the deletion of *vpu* enhances cell-to-cell HIV-1 infection.[109,110] Although a study has suggested the association of Vpu's antitetherin activity with the efficiency of cell-to-cell viral spread,[110] this issue remains controversial,[111] and how *vpu* deletion enhances cell-to-cell transfer remains unclear.

To address the role of Vpu in HIV-1 replication and pathogenesis *in vivo*, at least two small animal models have been utilized. First, Aldrovandi and Zack have used the SCID-hu mouse model, mice that have severe combined immunodeficiency (SCID) and are transplanted with human thymus and liver. The authors demonstrated that *vpu* is dispensable for viral replication in the SCID-hu mouse model.[112] Second, another group as well as ours have used a human hematopoietic stem cell-transplanted ''humanized'' mouse model to show that Vpu is slightly associated with viral cytopathicity.[113,114] Moreover, we have revealed that Vpu particularly boosts viral expansion during the acute phase of infection.[114] However, it should be noted that all of the three studies described above have used *vpu* defective viruses. As described in previous sections, since Vpu possesses multiple functions (Figure 1(b)), it remains unclear which activities of Vpu are most important for viral replication *in vivo*. This should be addressed to elucidate Vpu's precise role in HIV-1 replication and pathogenesis *in vivo* to hopefully provide useful knowledge for the development of novel anti-HIV-1 drugs.

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DECLARATION OF CONFLICTING INTERESTS

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