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HIV-1 Vpu mediated downregulation of CD155 requires alanine residues 10, 14 and 18 of the transmembrane domain

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ABSTRACT

HIV-1 NL4-3 Vpu induces downregulation of cell surface CD155, a ligand for the DNAM-1 activating receptor of NK and $CD8^+$ T cells, to evade NK cell mediated immune response. Here we show that the conserved alanine residues at positions 10, 14 and 18 in the TM domain of Vpu are required for the efficient downregulation of cell surface CD155. In contrast, the CK-2 phosphorylation sites and the second α-helix in the cytoplasmic Vpu domain have no influence on the surface expression of CD155. Thus, compared to Vpu's effect on CD4, NTB-A and tetherin, the Vpu mediated downregulation of CD155 is an independent Vpu function. We finally show that in contrast to other lentiviral strains, only Vpu and Nef from HIV-1 M NL4-3 potently interfere with CD155 surface expression. Thus, Vpu seems to subvert NK cell responses against HIV-1 infected T cells by modulation of receptors necessary for NK cell activation.

Introduction

The human immunodeficiency virus type 1 (HIV-1) accessory protein Vpu is a 15–20 kDa oligomeric type 1 integral membrane phosphoprotein (Cohen et al., 1988; Maldarelli et al., 1993; Strebel et al., 1988), which is encoded exclusively by HIV-1 and related simian immunodeficiency viruses (SIVs), but not by the majority of SIVs and HIV-2. Vpu has been shown to induce the degradation of newly synthesized CD4 by retaining it in the endoplasmic reticulum (ER) (Magadan et al., 2010) and thereby targeting CD4 to the ER-associated protein degradation (ERAD)-pathway. This specific function of Vpu depends on the CK-2 phosphorylation sites in its cytoplasmic domain (Binette et al., 2007; Magadan et al., 2010; Schubert et al., 1998; Willey et al., 1992).

Furthermore, it has been shown that Vpu supports HIV-1 virion release by counteracting the cellular restriction factor tetherin (also known as CD317, BST-2 or HM1.24) (Neil et al., 2008; Van Damme et al., 2008). Vpu induces an accumulation of tetherin in the trans-Golgi network (TGN), which results in the downregulation of cell surface tetherin (Dube et al., 2010; Schmidt et al., 2011). Vpu and tetherin interact via their TM domains, and this interaction is required for the efficient downregulation of cell surface tetherin (Banning et al., 2010; Dube et al., 2010; Iwabu et al., 2009; Rong et al., 2009). Recently, it has been demonstrated that several highly conserved residues in the TM domain of Vpu, particularly Ala-10, Ala-14 and Ala-18, are required for efficient downregulation of tetherin from the cell surface (Skasko et al., 2012; Vigan and Neil, 2010).

In addition to its role in tetherin antagonism and CD4 degradation, HIV-1 Vpu induces the downregulation of the co-activating NK-cell receptor NK-cell, T-cell, B-cell antigen (NTB-A) (also termed CD352 or SLAMF6) as well as the activating NK cell receptor CD155 (also termed polio virus receptor (PVR) or NECL-5) from the cell surface to evade NK-cell mediated lysis of HIV-1 infected cells (Matusali et al., 2012; Shah et al., 2010). Moreover, Vpu affects the anterograde transport of newly synthesized NTB-A molecules by retention of NTB-A within the Golgi-compartment (Bolduan et al., 2013). With the exception of HIV-1 group N, the Vpu mediated downregulation of cell surface NTB-A is conserved among diverse HIV-1 and SIV strains (Bolduan et al., 2013). In contrast, it is currently not known how Vpu downregulates CD155 or if this function is conserved among different lentiviral Vpu variants.

CD155 is a nectin-like protein that serves as a ligand for the DNAX accessory molecule-1 (DNAM-1) activating receptor of NK and $CD8^+$ T cells (Takai et al., 2008; Vassena et al., 2013), thereby

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triggering T and NK cell-mediated cytotoxicity (Matusali et al., 2012; Shibuya et al., 1996). Interestingly, while HIV-1 Nef and Vpu are required for optimal downregulation of cell surface CD155 to avoid DNAM-1 mediated immune response against HIV-1 infected cells (Matusali et al., 2012), HIV-1 Vpr induces an upregulation of cell surface and steady state CD155 levels in HIV-1 infected $CD4^+$ T cells. As a consequence, the effect of Vpr seems to decrease the anti-CD155 activity of HIV-1 Nef (Vassena et al., 2013).

In order to elucidate the importance of the TM domain of Vpu in downregulating cell surface CD155, several well-established Vpu TM mutants, particularly A10N, A14N and A18N, were analyzed for their capability to affect the surface expression of CD155. We show that the mutation of Ala-10, Ala-14 and Ala-18 to asparagine impairs the ability of Vpu to downregulate cell surface CD155. In contrast, the CK-2 phosphorylation sites and the second α-helix in the cytoplasmic domain of Vpu have no influence on the surface expression of CD155. Interestingly, the Vpu Δ23 mutant, which lacks the second cytoplasmic α-helix, exhibits an attenuated ability to downregulate cell surface tetherin and NTB-A (Bolduan et al., 2013; Dube et al., 2009), but was fully active on CD155. Thus, the downregulation of CD155 is an independent function of Vpu and can be separated from its effects on CD4, NTB-A or tetherin. Furthermore, we show that Vpu causes intracellular accumulation of CD155 in perinuclear compartments and a Vpu mutant, which is retained in the ER/cis Golgi, still interferes with CD155 cell surface expression. Altogether, these data indicate that Vpu might inhibit trafficking of CD155 to the cell surface. Finally, our data reveal that Vpu and Nef proteins derived from HIV-1 M NL4-3 are most potent agonists of CD155 cell surface expression, whereas those from other strains, except SIVgor CP2139, were strongly attenuated. Thus, these results are in line with a model suggesting that a variety of Vpu and Nef functions mediate HIV-1 immune evasion and add to the full pathogenic potential of HIV-1.

Results

Ala-10, Ala-14 and Ala-18 in the Vpu TM domain are critical for the downregulation of cell surface CD155

Recently, it was shown that HIV-1 NL4-3 Vpu downregulates cell surface CD155 to evade NK cell mediated immune response against HIV-1 infected cells (Matusali et al., 2012). Moreover, the randomization of Vpu's TM domain impairs its ability to affect the surface expression of CD155 (Matusali et al., 2012). In order to analyze the effect of Vpu's TM and cytoplasmic domain on the surface expression of CD155, we analyzed specific Vpu TM and cytoplasmic deletion mutants, which are summarized in Fig. 1. HeLa cells, which constitutively express endogenous CD155, were transfected with NL4-3 *wt* Vpu or mutants translated along with GFP via an internal ribosomal entry site (Vpu-IRES-GFP) to monitor transfected cells. 24 h post-transfection cells were harvested, stained for cell surface (Fig. 2A) and intracellular (Fig. 2B) CD155 using a CD155 specific antibody, and analyzed by flow cytometry. The surface expression of CD155 in Vpu expressing cells was calculated as the mean fluorescence intensity (MFI) of CD155 expression in GFP positive cells relative to GFP negative cells. In addition, cells were lysed and the soluble protein fraction was analyzed by Western blot (Fig. 2C). Fig. 2A shows that NL4-3 *wt* Vpu as well as the m2/6 mutant efficiently downregulate cell surface CD155, indicating that the CK-2 phophorylation sites have no influence on the surface expression of CD155. The C-terminal deletion mutant Vpu Δ23, which exhibits a decreased localization in the TGN (Dube et al., 2009; Pacyniak et al., 2005), and the Vpu KKDQ mutant, which is retained in the ER and the cis-Golgi compartment (Shikano and Li, 2003; Skasko et al., 2011; Vigan and Neil, 2011), both downregulate CD155 to the same degree as *wt* Vpu (Fig. 2A).

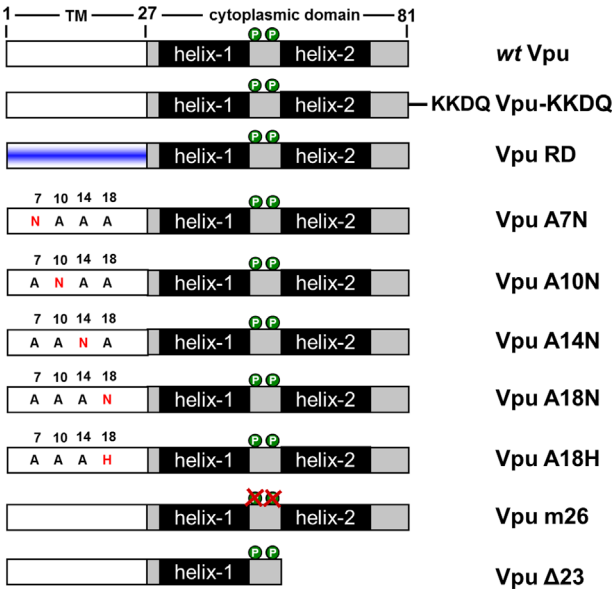

Fig. 1. Schematic representation of *wt* Vpu (A) or Vpu mutants KKDQ (B), RD (C), A7N (D), A10N (E), A14N (F), A18N (G), A18H (H), m26 (I) or Δ23 (J).

In contrast to the Vpu A7N mutant, the Vpu TM mutants A10N, A14N and A18N were strongly attenuated in their ability to decrease the surface expression of CD155, implying that this stretch of conserved residues is critical for the Vpu induced downmodulation of cell surface CD155. For control, the expression levels of all Vpu proteins were comparable (Fig. 2C). In Fig. 2B, we analyzed the ability of Vpu to affect the intracellular expression of CD155. Here we show that neither *wt* Vpu nor any of the mutants does alter steady state protein levels of CD155 (Fig. 2B).

Next, we analyzed the impact of Vpu's TM domain, particularly A18, to affect the surface expression of CD155 in $CD4^+$ SupT1 T cells (Fig. 3). Thus, $CD4^+$ SupT1 cells were infected with either VSV-G pseudotyped Vpu deletion mutant HIV-1 NL4-3 Vpu Del-1, HIV-1 NL4-3 *wt,* HIV-1 NL4-3 Vpu RD or HIV-1 NL4-3 Vpu A18H, and surface expression of CD155 was analyzed by flow cytometry. As expected, HIV-1 NL4-3 *wt* was capable of inducing downregulation of CD155 from the cell surface (Fig. 3). However, the Vpu deletion mutant HIV-1 NL4-3 Vpu Del-1 and the Vpu mutants HIV-1 NL4-3 Vpu RD and HIV-1 NL4-3 Vpu A18H lost their ability to induce efficient downregulation of cell surface CD155 (Fig. 3). These results suggest that alanine 18 in the TM domain of Vpu is required to induce downregulation of cell surface CD155 also in the context of infected $CD4^+$ T cells.

In summary, these results suggest that the TM domain of Vpu, particularly Ala-10, Ala-14 and Ala-18, is crucial for cell surface downregulation of CD155, while the CK-2 phophorylation sites and the second α-helix in the cytoplasmic domain of Vpu are dispensable for this phenomenon.

NL4-3 Vpu alters the subcellular localization of CD155 by entrapment in perinuclear compartments

Since NL4-3 *wt* Vpu induces downregulation of cell surface CD155, we investigated by immunofluorescence whether Vpu has an influence on the subcellular localization of CD155. HeLa cells were transfected with, NL4-3 Nef–YFP or NL4-3 Vpu- fusion proteins (Banning et al., 2010) containing the indicated mutations A10N, A14N, A18N or RD. 24 h post-transfection, cells were fixed, permeabilized and stained for CD155. Confirming the results shown in Fig. 2A,