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Modulation of the severe CD4$^+$ T-cell loss caused by a pathogenic simian–human immunodeficiency virus by replacement of the subtype B *vpu* with the *vpu* from a subtype C HIV-1 clinical isolate

M. Sarah Hill [a], Autumn Ruiz [a], Erik Pacyniak [a], David M. Pinson [b], Nathan Culley [c], Bonnie Yen [d], Scott W. Wong [d], Edward B. Stephens [a,*]

[a] *Department of Anatomy and Cell Biology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA*
[b] *Department of Laboratory Medicine and Pathology, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA*
[c] *Laboratory Animal Resources, University of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160, USA*
[d] *Vaccine and Gene Therapy Institute, Oregon National Primate Research Center, Oregon Health Sciences Center, Beaverton, OR 97003, USA*

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Abstract

Previously, we showed that the Vpu protein from subtype C human immunodeficiency virus type 1 (HIV-1) was efficiently targeted to the cell surface, suggesting that this protein has biological properties that differ from the well-studied subtype B Vpu protein. In this study, we have further analyzed the biological properties of the subtype C Vpu protein. Flow cytometric analysis revealed that the subtype B Vpu (strain HXB2) was more efficient at down-regulating CD4 surface expression than the Vpu proteins from four subtype C clinical isolates. We constructed a simian-human immunodeficiency virus virus, designated as $SHIV_{SCVpu}$, in which the subtype B *vpu* gene from the pathogenic $SHIV_{KU\text{-}1bMC33}$ was substituted with the *vpu* from a clinical isolate of subtype C HIV-1 (strain C.96.BW16B01). Cell culture studies revealed that $SHIV_{SCVpu}$ replicated with slightly reduced kinetics when compared with the parental $SHIV_{KU\text{-}1bMC33}$ and that the viral Env and Gag precursor proteins were synthesized and processed similarly compared to the parental $SHIV_{KU\text{-}1bMC33}$. To determine if substitution of the subtype C Vpu protein affected the pathogenesis of the virus, three pig-tailed macaques were inoculated with $SHIV_{SCVpu}$ and circulating CD4$^+$ T-cell levels and viral loads were monitored for up to 44 weeks. Our results show that $SHIV_{SCVpu}$ caused a more gradual decline in the rate of CD4$^+$ T cells in pig-tailed macaques compared to those inoculated with parental subtype B $SHIV_{KU\text{-}1bMC33}$. These results show for the first time that different Vpu proteins of HIV-1 can influence the rate at which CD4$^+$ T-cell loss occurs in the SHIV/pig-tailed macaque model.

Keywords: Vpu; SHIV; Macaques; Pathogenesis; Subtype C HIV-1; CD4$^+$ T-cell depletion

Introduction

All the naturally occurring primate lentiviruses encode for Tat, Rev, Nef, Vif and Vpr accessory proteins. In addition to these accessory proteins, human immunodeficiency virus type 1 virus (HIV-1) and a select number of simian immunodeficiency virus (SIV) isolates (SIV_{cpz}, SIV_{den}, SIV_{gsn}, SIV_{mon}, SIV_{mus}) also encode for a Vpu protein (Barlow et al., 2003; Courgnaud et al., 2002, 2003; Dazza et al., 2005; Huet et al., 1990). The Vpu protein is a small transmembrane phosphoprotein synthesized off the same mRNA as the Env precursor and has two known functions within the cell (Schwartz et al., 1990; Maldarelli et al., 1993). Vpu is known to interact with the CD4 molecule within the rough endoplasmic reticulum (RER) and re-translocate the protein to the proteasome for degradation (Fujita et al., 1997; Schubert et al., 1998). By removing the CD4, it permits unbound gp160 to be transported to the cell surface and incorporated into the budding virion (Willey et al., 1992). Previous studies have shown that the interaction of the

* Corresponding author. Fax: +1 913 588 2710.
E-mail address: estephen@kumc.edu (E.B. Stephens).

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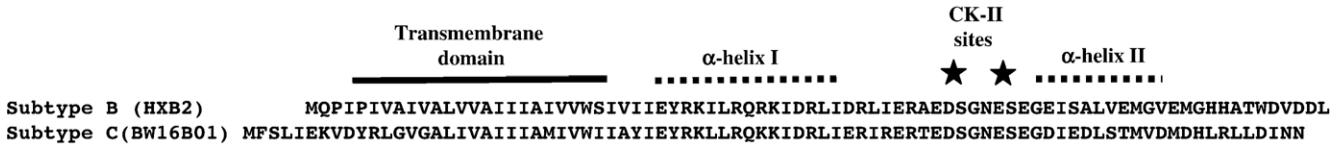

Fig. 1. Comparison of the Vpu sequences in $SHIV_{KU\text{-}1bMC33}$ and $SHIV_{SCVpu}$.

subtype B Vpu with the CD4 molecule is dependent on the presence of two casein kinase II (CK-II) phosphorylation sites (Paul and Jabbar, 1997). The Vpu protein also enhances virion release from infected cells (Klimkait et al., 1990). Although the mechanism of enhanced virion release is unknown, some investigators have linked this property of Vpu to the transmembrane domain and its ion channel properties (Cordes et al., 2001; Ewart et al., 1996; Grice et al., 1997; Park et al., 2003; Schubert et al., 1996a,b). Other investigators have shown that the certain ion channel-blocking compounds could inhibit the ion channel activity of Vpu (Ewart et al., 2002, 2004).

Because SIVmac strains commonly used in pathogenicity studies do not encode for the Vpu protein, we have used the pathogenic simian human immunodeficiency virus (SHIV), which has the *tat*, *rev*, *vpu* and *env* genes of HIV-1 in a genetic background of the $SIV_{mac}239$, to analyze the role of Vpu in pathogenicity. Infection with these pathogenic SHIVs results in high viral loads, a rapid loss of $CD4^+$ T cells within 1 month of infection, and severe depletion within lymphoid organs such as the thymus, lymph nodes and spleen. Using pathogenic molecular clones that express altered Vpu proteins, we showed that both the transmembrane domain and cytoplasmic domains of the Vpu protein contribute to the severe $CD4^+$ T-cell loss in these macaques (Singh et al., 2003; Hout et al., 2004, 2005).

To date, all studies assessing the role of Vpu in macaques have been performed using the well-studied subtype B Vpu protein from a laboratory-adapted HIV-1 isolate. Recently, we reported that the subtype C Vpu proteins were efficiently transported to the cell surface, indicating that the subtype C Vpu protein may have biological properties that differ from the better studied subtype B Vpu protein (Pacyniak et al., 2005). In this study, we report that the subtype C Vpu protein is less efficient at down-regulating CD4 from the cell surface than the subtype B Vpu. Using the pathogenic molecular clone, $SHIV_{KU\text{-}1bMC33}$, we report on the construction of a SHIV in which the subtype B *vpu* was exchanged with the *vpu* from a clinical isolate of subtype C HIV-1 ($SHIV_{SCVpu}$). Our results show that following inoculation into macaques, $SHIV_{SCVpu}$ had a decreased rate of $CD4^+$ T-cell loss compared with the parental $SHIV_{KU\text{-}1bMC33}$. These results show for the first time that different Vpu proteins can influence the rate of $CD4^+$ T-cell loss in the SHIV/macaque model.

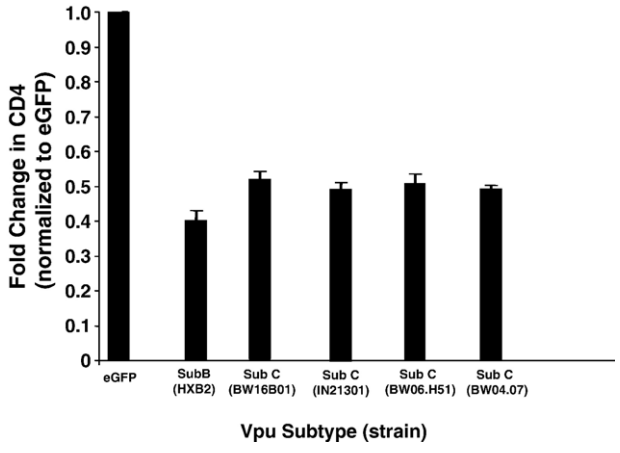

Fig. 2. The subtype C Vpu is less efficient at down-regulating surface CD4 than the subtype B Vpu protein. HeLa $CD4^+$ cells were transfected with plasmids pc*egfp*, pc*vpuegfp*, pcvpu_{SC}*egfp*1 or pcvpu_{SC}*egfp21301*, pcvpu_{SC}*egfpBW06.H51*, or pcvpu_{SC}*egfpBW04.07*. At 48 h, live cells were immunostained for CD4. Cells expressing EGFP or EGFP fusion proteins were assessed for CD4 down-regulation using flow cytometry.

Results

CD4 down-regulation by subtype B and C Vpu proteins

The sequence of the subtype B and C Vpu proteins analyzed in this study are shown in Fig. 1. Previously, we showed that fusion of the Vpu protein to enhanced green fluorescent protein (EGFP) still resulted in the ability to down-modulate cell surface CD4 (Hout et al., 2005, 2006; Singh et al., 2003). We analyzed the efficiency of cell surface CD4 down-regulation by the subtype B and C Vpu fusion proteins. HeLa $CD4^+$ cells were transfected with vectors expressing either the subtype B or C Vpu fusion proteins. At 48 h post-transfection, live cells were immunostained for CD4 and analyzed by flow cytometry to measure the intensity of cell surface CD4 expression. As shown in Fig. 2, the cells transfected with the vector expressing the subtype B fusion (VpuEGFP) consistently down-regulated CD4 more efficiently ($p < 0.008$) than cells transfected with the vector expressing the subtype C Vpu fusion protein (Vpu_{SC}EGFP1).

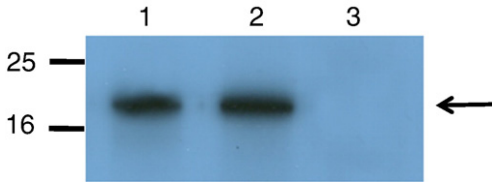

Fig. 3. The Vpu protein is expressed in C8166 cells infected with $SHIV_{SCVpu}$. C8166 cells were infected with $SHIV_{SCVpu}$ or $SHIV_{KU\text{-}1bMC33}$ for 5 days. The cells were starved for methionine/cysteine for 2 h and then radiolabeled for 12 h with ^{35}S-methionine/cysteine. Cell lysates were prepared as described in the text and Vpu proteins immunoprecipitated with an antisera generated against the cytoplasmic domain of the subtype B Vpu protein or the subtype C Vpu protein. (Lane 1) Vpu proteins immunoprecipitated from $SHIV_{KU\text{-}1bMC33}$-inoculated cultures. (Lane 2) Vpu proteins immunoprecipitated from $SHIV_{SCVpu}$-inoculated cultures. (Lane 3) Vpu proteins immunoprecipitated from uninfected cultures.