Virology 404 (2010) 187–203

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Comparison of the replication and persistence of simian-human immunodeficiency viruses expressing Vif proteins with mutation of the SLQYLA or HCCH domains in macaques

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ARTICLE INFO

Article history:
Received 20 January 2010
Returned to author for revision
17 February 2010
Accepted 18 April 2010
Available online 3 June 2010

Keywords:
Vif
Simian-human immunodeficiency virus
SHIV
Rhesus macaques
APOBEC3G
APOBEC3F
Replication
BC box
Zn binding motif

ABSTRACT

The Vif protein of primate lentiviruses interacts with APOBEC3 proteins, which results in shunting of the APOBEC3-Vif complex to the proteosome for degradation. Using the simian-human immunodeficiency virus (SHIV)/macaque model, we compared the replication and pathogenicity of SHIVs that express a Vif protein in which the entire SLQYLA ($SHIV_{Vif5A}$) or HCCH ($SHIV_{VifHCCH(-)}$) domains were substituted with alanine residues. Each virus was inoculated into three macaques and various viral and immunological parameters followed for 6 months. All macaques maintained stable circulating $CD4^+$ T cells, developed low viral loads, maintained the engineered mutations, yielded no histological lesions, and developed immunoprecipitating antibodies early post-inoculation. Sequence analysis of *nef* and *vpu* from three lymphoid tissues revealed a high percentage of G-to-A-substitutions. Our results show that while the presence of HCCH and SLQYLA domains are critical *in vivo*, there may exist APOBEC3 negative reservoirs that allow for low levels of viral replication and persistence but not disease.

Introduction

Human immunodeficiency virus type 1 (HIV-1) and other lentiviruses encode for a Vif (virion infectivity factor) protein, which has been shown to be essential for HIV-1 replication in primary $CD4^+$ T cells and macrophages (Fan and Peden, 1992; Gabuzda et al., 1992; Blanc et al., 1993; Sakai et al., 1993; von Schwedler et al., 1993; Borman et al., 1995). The Vif protein interacts with apolipoprotein B mRNA editing enzyme catalytic peptide-like 3G (APOBEC3G; hA3G) promoting its accelerated degradation by the proteosome (Sheehy et al., 2003). APOBEC3G is a cytidine deaminase that, if packaged into HIV-1Δ*vif* virions, induces cytidine deamination of newly synthesized minus-strand viral DNA from cytosines to uracils, leading to G to A transitions in plus strand synthesis (Jarmuz et al., 2002; Harris et al., 2003; Mariani et al., 2003; Mangeat et al., 2003; Sheehy et al., 2003; Yu et al., 2004a). The RNA editing activity of the APOBEC3 family of proteins involves an active site characterized by a conserved zinc-binding motif, (Cys/His)-Xaa-Glu-Xaa_{23-28}-Pro-Cys-Xaa_{2-4}-Cys, containing a glutamate involved in proton shuttling during deamination (Jarmuz et al., 2002). In addition to A3G, humans have six other APOBEC3 genes; hA3A, hA3B, hA3C, hA3DE, hA3F, and hA3H (Jarmuz et al., 2002). Of those APOBEC3 genes, hA3B, hA3DE, hA3G, and hA3F, have been shown to inhibit the replication of HIV-1Δ*vif* (Dang et al., 2006, 2008; Doehle et al., 2005; Wiegand et al., 2004; Yang et al., 2007; Yu et al., 2004b; Zheng et al., 2004). SIV_{mac}239Δ*vif* has been shown to be restricted by hA3G, hA3F, hA3B, hA3C, hA3DE (Dang et al., 2006, 2008; Mariani et al., 2003; Yu et al., 2004b; Zennou and Bieniasz, 2006). The HIV-1 Vif has limited activity against rhesus and African green monkey A3 proteins while Vif from SIV_{mac}239 and SIV_{agm} have broader specificities. While less is presently known about the rhesus A3 proteins, it is known that HIV-1Δ*vif* can be inhibited by rhA3G, rhA3F, rhA3B, and to a lesser extent rhA3H and rhA3DE (Virgen and Hatziioannou, 2007). SIV_{mac}239Δ*vif* has been shown to be restricted by rhA3G, rhA3F, rhA3C, rhA3B and rhA3DE, and to a lesser extent rhA3H (Virgen and Hatziioannou, 2007; Zennou and Bieniasz, 2006).

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doi:10.1016/j.virol.2010.04.017

Sequence analysis of Vif proteins from different lentiviruses reveals that there are two highly conserved domains in the carboxyl terminus, the SLQ(Y/F)LA and Zn^{++} binding (HCCH) motifs. Previous cell culture studies with hA3 proteins showed that introduction of amino acid substitutions in the viral BC box (SLQ(Y/F)LA) resulted in decreased binding of Vif to Elongin C while substitutions in the HCCH domain prevent interactions with Cullin 5 of the Cul5/Elongin B/C/Rbx E3 ligase complex (Luo et al., 2005; Mehle et al., 2004a,b, 2006; Stopak et al., 2003; Yu et al., 2003, 2004c). These studies showed increased A3G incorporation into virions and G-to-A hypermutation (Mangeat et al., 2003; Shindo et al., 2003; Zhang et al., 2003). Our laboratory has been using the chimeric simian-human immunodeficiency (SHIV)/macaque model to study the role of Vpu and its various domains in $CD4^+$ T cell loss, virus release and pathogenesis (Stephens *et al.*, 2002; Singh *et al.*, 2003; Hout *et al.*, 2005; 2006; Hill et al., 2008). In this study, we constructed simian-human immunodeficiency viruses (SHIVs) in which five amino acids in the SLQYLA motif ($SHIV_{Vif5A}$) and four amino acids of the HCCH ($SHIV_{VifHCCH(-)}$) domain were changed to alanine residues. Our results indicate that rhA3F is stable in the presence of wild type and mutant Vif viruses and that rhA3G is more effective at cytidine deamination than rhA3F. Our results show that both the HCCH and SLQYLA domains were critical to Vif function *in vivo* but that production of viral RNA only persisted in macaques inoculated with the $SHIV_{VifHCCH(-)}$.

Results

Replication of $SHIV_{Vif5A}$ and $SHIV_{VifHCCH(-)}$ in APOBEC3 positive and negative cell lines

The sequence of the Vif mutants that were analyzed in this study are shown in Fig. 1. We performed assays to examine the replication of parental $SHIV_{KU\text{-}2MC4}$, $SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$, $SHIV_{VifAAQYLA}$ and $SHIV_{VifSTOP}$ in hA3G/F positive (C8166) and negative (SupT1) cell lines as well as rhesus PBMC (rhA3G/F+). We included $SHIV_{VifAAQYLA}$ in these growth curves for comparison as we previously reported on the replication of this mutant in tissue culture and in macaques (Schmitt et al., 2009). Cells were inoculated with equivalent amounts (25 ng of p27) each of the virus and the levels of p27 Gag released into the culture medium were quantified using a commercial antigen capture assay. All four mutant viruses ($SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$, $SHIV_{VifAAQYLA}$ and $SHIV_{VifSTOP}$) replicated in SupT1 cells to similar levels as parental $SHIV_{KU\text{-}2MC4}$ by day 15 post-inoculation, although the kinetics of replication were slower (Fig. 2A). Inoculation of equivalent amounts (25 ng p27) of $SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$, $SHIV_{VifAAQYLA}$, and $SHIV_{VifSTOP}$ into hA3G/F+C8166 cell cultures resulted in less than 0.01% of the p27 released compared to parental $SHIV_{KU\text{-}2MC4}$ (Fig. 2B). As shown in Fig. 2C, in rhesus PBMC $SHIV_{KU\text{-}2MC4}$ replicated to high levels (7.932 ng/ml) while $SHIV_{VifHCCH(-)}$ (0.119 ng/ml) and $SHIV_{Vif5A}$ (0.197 ng/ml), and $SHIV_{VifAAQYLA}$ (0.11 ng/ml) replicated to low but detectable levels. Replication was undetectable for $SHIV_{VifSTOP}$ in rhesus PBMC.

Both $SHIV_{Vif5A}$ and $SHIV_{VifHCCH(-)}$ incorporate rhA3G and rhA3F into virus particles

The above results suggested that hA3G and hA3F or rhA3G and rhA3F might be incorporated into maturing virus particles resulting in restriction of replication. As we are most interested in rhA3G and rhA3F, we transfected 293 cells with plasmids expressing an HA-tagged rhA3G or a V5-tagged rhA3F and the complete genomes of either $SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$, $SHIV_{VifSTOP}$, or parental $SHIV_{KU\text{-}2MC4}$. At 48 h post-transfection, the culture medium was collected, clarified, and the virus partially purified and concentrated by ultracentrifugation. Equal amounts of p27 (as determined by Western blot) were loaded and analyzed for the presence or absence of rhA3G or rhA3F. The results shown in Fig. 3A indicate that rhA3G was incorporated into $SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$, and $SHIV_{VifSTOP}$ virus particles but was excluded from $SHIV_{KU\text{-}2MC4}$ particles. However, we found that the rhA3F protein was incorporated into all four viruses (Fig. 3B). These results indicate that rhA3G was selectively incorporated only into the Vif mutants while rhA3F was incorporated into all viruses. The inability of $SIV_{mac}239$ Vif to degrade and prevent the incorporation of rhA3F has been previously reported (Virgen and Hatziioannou, 2007).

Rhesus A3F is stable in the presence of $SIV_{mac}239$ Vif

Since we observed that rhA3F was incorporated into virus particles, we determined the stability of rhA3F and rhA3G in the presence of the viral genome. 293 cells were co-transfected with vectors expressing rhA3G or rhA3F and the genomes of $SHIV_{KU\text{-}2MC4}$, $SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$, or $SHIV_{VifSTOP}$. Our results indicate that in the presence of the $SHIV_{KU\text{-}2MC4}$ genome, rhA3G was not stable whereas it was detected in the presence of the $SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$ or $SHIV_{VifSTOP}$ (Fig. 4). We also found that rhA3F appeared to be stable in the presence of both $SHIV_{KU\text{-}2MC4}$ and also $SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$, or $SHIV_{VifSTOP}$ genomes. However, it should be noted that we consistently found higher levels of rhA3F in the presence of the $SHIV_{VifSTOP}$ genome, indicating fundamental differences between the targeting site-directed Vif mutants and the absence of the Vif protein. The results obtained with rhA3F correlate well with the above experiment showing that rhA3F was incorporated into virus particles.

Rhesus A3G but not rhA3F causes significant G-to-A mutations in the nef gene of the SHIV genome

The results above indicated that significant levels of rhA3F were incorporated into virions and rhA3F was stable in cells expressing the wild type genome. We compared the level of G-to-A mutations of the SHIV genomes in the presence of either rhA3G or rhA3F. The results are shown in Fig. 5 and Table 1. Using viral genomes to express the Vif protein, we observed minimal G-to-A changes in $SHIV_{KU\text{-}2MC4}$ *nef* in the presence of either rhA3G or rhA3F. In the presence of rhA3G, we found that $SHIV_{Vif5A}$, $SHIV_{VifHCCH(-)}$ or $SHIV_{VifSTOP}$ *nef* had an increase in the number of G-to-A changes (from 2 to 24–29) or approximately 0.5% of the bases sequenced (Table 1). However, in the presence of

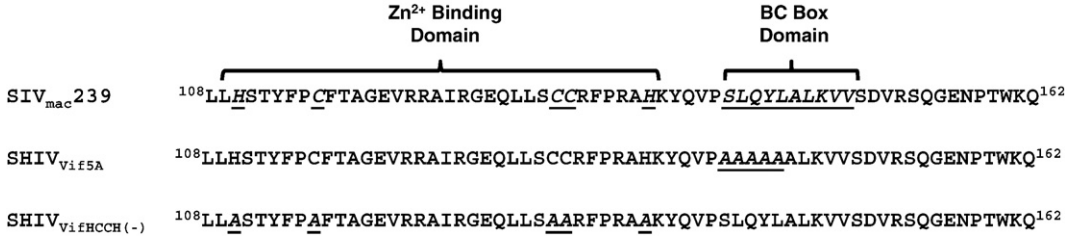

Fig. 1. Sequence of the wild type $SIV_{mac}239$ Vif protein and the two mutants analyzed in this study. The amino acids comprising the Zn^{+2} and BC box domains are italicized and underlined. The amino acid substitutions in the $SHIV_{Vif5A}$ and $SHIV_{VifHCCH(-)}$ are also underlined.