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Analysis of the N-terminal positively charged residues of the simian immunodeficiency virus Vif reveals a critical amino acid required for the antagonism of rhesus APOBEC3D, G, and H

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ABSTRACT

Previous studies have shown that apolipoprotein B mRNA editing, enzyme catalytic, polypeptide G (APOBEC3G; hA3G) and F (APOBEC3F; hA3F) proteins interact with a nonlinear binding site located at the N-terminal region of the HIV-1 Vif protein. We have analyzed the role of 12 positively charged amino acids of the N-terminal region of the SIV Vif. Simian-human immunodeficiency viruses (SHIV) were constructed that expressed each of these amino acid substitutions. These viruses were examined for replication in the presence of rhesus macaque APOBEC3 proteins (rhA3A–rhA3H), incorporation of the different A3 proteins into virions, and replication in rhesus macaque PBMC. Similar to other studies, we found that K27 was essential for rhA3G activity and rhA3F but was not important for restriction of SHIVΔ*vif* by rhA3A, rhA3D or rhA3H. Our results identified the arginine at position 14 of the SIV Vif as a critical residue for virus restriction by rhA3D, rhA3G and rhA3H.

Introduction

Select members of the *apo*lipoprotein *B* mRNA *e*diting, enzyme *c*atalytic polypeptide 3 (APOBEC3; A3) gene family in primates represent an innate host defense system that can inhibit the replication of human immunodeficiency virus type 1 that does not express a Vif protein (Chiu and Greene, 2008; Goila-Gaur and Strebel, 2008; Harris et al., 2003; Jarmuz et al., 2002; Sheehy et al., 2002). The A3 family in humans and rhesus macaques consists seven members (A3A, A3B, A3C, A3D, A3F, A3G, and A3H) that are tandemly arranged on chromosome 22 or 10, respectively. All seven members either have one (A3A, A3C, A3H) or two (A3B, A3D, A3F, A3G) canonical cytidine deaminase motifs (H-x-E-x_{23-28}-P-C-x_{2-4}-C) (Betts et al., 1994; Dang et al., 2007; Jarmuz et al., 2002; Schmitt et al., 2011; Wedekind et al., 2003; Xie et al., 2004). Following the identification of A3G (formerly CEM15) as a potent inhibitor of the replication of Vif deficient HIV-1 (HIV-1Δ*vif*), it was found that in the absence of Vif A3G is incorporated and causes cytidine to uracil changes in the minus strand of single-stranded DNA during reverse transcription, ultimately leading to G-to-A mutations in the viral genome (Kao et al., 2003; Lecossier et al., 2003; Mangeat et al., 2003; Sheehy et al., 2002; Yu et al., 2004; Zhang et al., 2003). The Vif protein acts as an adapter that binds the APOBEC3 proteins to the Cul5/ElonginB/C/rbx E3 ligase complex for ubiquitination and degradation by the proteosome (Conticello et al., 2003; Dussart et al., 2004; Kobayashi et al., 2005; Liu et al., 2004; Marin et al., 2003; Mehle et al., 2004; Sheehy et al., 2003; Stopak et al., 2003; Yu et al., 2003, 2004). In addition to A3G, other A3 proteins have been shown to restrict the replication of HIV-1Δ*vif*. A3F is the most closely related protein to A3G at the amino acid level and has a tissue distribution similar to A3G (Wiegand et al., 2004; Zheng et al., 2004). When expressed exogenously, hA3F is incorporated into and restricts the replication of HIV-1Δ*vif* virions, although the level of deamination is approximately 10 times less than A3G (Holmes et al., 2007; Wang et al., 2007). A3F has been found to be expressed at lower levels in human CD4+ T cells than A3G, causes less cytidine deamination of Vif-deficient HIV-1 and has less or no effect on its restriction (Binka et al., 2012; Chaipan et al., 2013; Miyagi et al., 2010). A3B has been reported to have moderate activity against HIV-1Δ*vif* but is expressed at low levels in natural HIV-1 cellular targets, so its role in restriction of HIV-1 is uncertain (Doehle et al., 2005; Refsland et al., 2010; Yu et al., 2004). A3D was also identified to potently restrict HIV-1Δ*vif* (Dang et al., 2007; Liddament et al., 2004; Wiegand et al., 2004; Zheng et al., 2004). A3H has at least seven haplotypes with haplotype II being able to potently restrict

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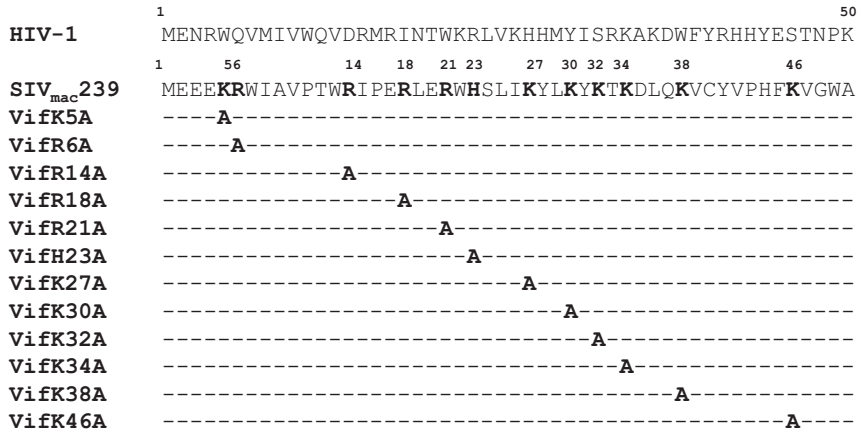

Fig. 1. Alignment of the amino terminal 50 amino acids of the HIV-1 and SIV Vif proteins. Underneath the SIV Vif sequence, the mutants analyzed in this study are indicated in bold.

HIV-1Δ*vif* and HTLV-I replication (Dang et al., 2008; OhAinle et al., 2008; Ooms et al., 2010, 2012). A3A is neither incorporated into the viral nucleocapsid nor does it restrict the replication of HIV-1Δ*vif* (Goila-Gaur et al., 2007). A3C is incorporated into the of HIV-1 virion and has been reported to cause limited cytidine deamination while other studies indicate that it has no effect on virus infectivity (Hultquist et al., 2011; Langlois et al., 2005).

Several domains have been identified in the N-terminal region of the HIV-1 Vif that are involved in the interaction with several human APOBEC3 proteins (Chen et al., 2009; Dang et al., 2007, 2010; Mehle et al., 2007; Pery et al., 2009; Russell and Pathak, 2007; Wichroski et al., 2005). Several studies that have used site-directed mutagenesis to identify amino acid residues that are critical for Vif neutralization of hA3G and hA3F. One study showed that deletion of amino acids 43–59 abolished interactions with A3G (Wichroski et al., 2005). In another study, the use of overlapping peptides found that a region from amino acids 33–88 formed a non-linear binding site for A3G (Mehle et al., 2007). Additionally, amino acid residues 14–17 and 40–44 were specifically found to effect the interactions with human A3F and A3G, respectively (Russell and Pathak, 2007). Other investigators showed that domains ^{23}SLVx4Yx9Y^{38}, ^{69}YXXL^{72}, ^{81}LGxGxxIxW^{89} and ^{171}EDRW^{174} were also involved in neutralizing hA3G and hA3F (Chen et al., 2009; Dang et al., 2010; Pery et al., 2009).

The simian immunodeficiency virus (SIV) and simian-human immunodeficiency virus (SHIV)/macaque models of infection have been used extensively to study different aspects of lentiviral pathogenesis and development of vaccines. Similar to the HIV-1 Vif, the SIV Vif has comparable YXXL, SLQYLA and HCCH domains (Luo et al., 2005; Pery et al., 2009; Schmitt et al., 2009, 2010; Yu et al., 2004). However, the N-terminal region of the SIV Vif (and other regions) have little sequence identity with the same region from the HIV-1 Vif, making it difficult to extrapolate findings to the Vif protein of SIV for testing *in vivo*. The SIV Vif has a region containing several positively charged amino acids that may be involved in electrostatic interactions with different rhesus macaque A3 proteins. In this study, we analyzed the importance of these positively charged residues on the exclusion of different rhesus macaque APOBEC3 proteins (rhA3A-H) from virions and on infectivity. We have identified a novel amino acid within the SIV Vif that is important for restriction by rhA3D, rhA3G, and rhA3H.

Results

Expression and stability of the Vif mutants

Comparison of the amino acid sequence of the HIV-1 and SIV$_{mac}$ Vif proteins show that there was 18% sequence identity at the first 50 amino acid positions (Fig. 1). To determine which positively charged amino acids at the N-terminus of the protein were critical to Vif function (i.e., virus replication in the presence of the rhesus A3 proteins), we substituted the positively charged residues at positions 5, 6, 14, 18, 21, 23, 27, 30, 32, 34, 38, and 46 to alanines (Fig. 1). First, we examined the stability of the Vif mutant proteins in cells to insure that any observed effects on virus restriction were not due to an unstable Vif protein. For these experiments, an SIV Vif (human codon-optimized) with an N-terminal 3X-Flag-tag was used. 293 cells were transfected with vectors expressing each Vif protein for 48 h. Cells were radiolabeled with ^{35}S-methionine and the radiolabel chased for 0 and 6 h. The cells were lysed and Flag-tagged proteins were immunoprecipitated using a rabbit polyclonal anti-Flag antibody. The results of this experiment showed that the twelve SIV Vif mutants were stable in 293 cells similar to the unmodified SIV Vif at the 6 h time point (Fig. 2).

Replication of SHIVs expressing mutant Vif proteins

We constructed SHIV viral genomes expressing Vif proteins with the amino acid substitutions in the N-terminal region as described above. The SHIV used, SHIV$_{KU\text{-}2MC4}$, has the *tat*, *rev*, *vpu* and *env* from HIV-1 (HXBc2) in the background of SIV$_{mac}$239 and was derived from passage in rhesus macaques (Liu et al., 1999). Virus stocks were prepared in SupT1 cells, which do not express A3 proteins. Growth curves (based on p27 release) were established for each virus in SupT1 cells and results indicate that all viruses replicated in the SupT1 cells (Fig. 3A and B).

Incorporation of macaque rhA3A-H into SHIV, SHIVΔvif and SHIV vif mutants into viral particles

To determine if the seven rhA3 proteins were incorporated into SHIVΔvif, parental SHIV$_{KU\text{-}2MC4}$, or SHIVs expressing the various Vif mutants, 293 cells were co-transfected with plasmids that expressed each of the tagged rhA3 proteins and each of the SHIV genomes. At 24 h, the cells were radiolabeled and the culture medium harvested at 36 h post-transfection. The virus was purified by ultracentrifugation as described in Material and methods section and immunoprecipitations were performed using an antibody against the HA-tag to determine if the A3 proteins were incorporated into virions. We chose radiolabeling and immunoprecipitations over immunoblots as they were more sensitive in detecting A3 proteins. Our results indicate that rhA3B but not rhA3C was incorporated into both SHIVΔ*vif* and SHIV$_{KU\text{-}2MC4}$ (Fig. 4A). Since rhA3B or rhA3C did not affect the replication of SHIVΔ*vif* and SHIV$_{KU\text{-}2MC4}$ (see below in Table 1, we did not