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Mutations in the highly conserved SLQYLA motif of Vif in a simian–human immunodeficiency virus result in a less pathogenic virus and are associated with G-to-A mutations in the viral genome

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ABSTRACT

The simian–human immunodeficiency virus (SHIV)/macaque model for human immunodeficiency virus type 1 has become a useful tool to assess the role of accessory genes in lentiviral pathogenesis. In this study, we introduced two amino acid changes in the highly conserved SLQYLA domain (to AAQYLA) of the SIV Vif protein. The resulting virus, $SHIV_{VifAAQYLA}$, was used to infect three macaques, which were followed for over six months. Plasma viral loads and circulating $CD4^+$ T cell levels were assessed during the course of infection. The three macaques inoculated with $SHIV_{VifAAQYLA}$ did not develop significant $CD4^+$ T cell loss over the course of their infection, had plasma viral RNA loads that were over 100-fold lower than macaques inoculated with parental $SHIV_{KU\text{-}1bMC33}$, and developed no histological lesions in lymphoid tissues. DNA and RT-PCR analysis revealed that only a select number of tissues were infected with this virus. Sequence analysis indicates that the site-directed changes were stable during the first three weeks after inoculation but thereafter the S147A amino acid substitution changed to a threonine in two of three macaques. The L148A substitution remained stable in the *vif* amplified from the PBMC of all three macaques. Sequence analysis of *vif*, *vpu*, *env* and *nef* genes revealed G-to-A mutations in the genes amplified from macaques inoculated with $SHIV_{VifAAQYLA}$, which were higher than in a macaque inoculated with parental $SHIV_{KU\text{-}1bMC33}$. We found that the majority (>85%) of the G-to-A mutations were in the context of 5′-TC (minus strand) and not 5′-CC, suggestive that one or more of the rhesus APOBEC3 proteins may be responsible for the observed mutational patterns. The data also suggest that rhesus APOBEC3G probably accounted for a minority of the mutations since its GG-to-AG mutational pattern was infrequently detected. Finally, macaques inoculated with $SHIV_{VifAAQYLA}$ developed immunoprecipitating antibody responses against the virus. The results from this study provide the first *in vivo* evidence of the importance of the SLQYLA domain in viral pathogenesis and show that targeted mutations in *vif* can lead to a persistent infection with G-to-A changes accumulating in the viral genome.

Introduction

Human immunodeficiency virus type 1 (HIV-1) as well as other lentiviruses encode for a Vif protein, which has been shown to be essential for HIV-1 replication in certain cell types. The Vif protein of HIV-1 was first shown to interact with apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (APOBEC3G; hA3G) (Sheehy et al., 2002). This protein was found to provide cells with an innate intracellular anti-retroviral activity that is associated with hypermutation of the viral genome through cytidine deamination (Harris et al., 2003; Lecossier et al., 2003; Mangeat et al., 2003; Zhang et al., 2003). This hA3G-induced cytidine deamination results in cytidine to uridine changes during minus strand DNA synthesis, which ultimately leads to G-to-A mutations in the plus strand (Yu et al., 2004a). Several groups subsequently showed that the Vif protein can prevent hypermutation by binding to hA3G and targeting this protein for degradation via the proteasome (Conticello et al., 2003; Dussart et al., 2004; Kao et al., 2003; Liu et al., 2004; Mariani et al., 2003; Marin et al., 2003; Mehle et al., 2004; Sheehy et al., 2003; Stopak et al., 2003; Yu et al., 2003, 2004a, 2004b, 2004c). In addition to A3G, humans have six other A3 genes: hA3A, hA3B, hA3C, hA3DE, hA3F, and hA3H (Jarmuz et al., 2002). The members of this family either have one (hA3A, hA3C, and hA3H) or two (hA3B, hA3DE, hA3F and hA3G) Zn^{+2} coordinating deaminase domains organized as H-x_1-E-$x_{25\text{-}31}$-C-$x_{2\text{-}4}$-C (with × being a non-conserved position) (Chiu and Greene, 2008). In addition to the

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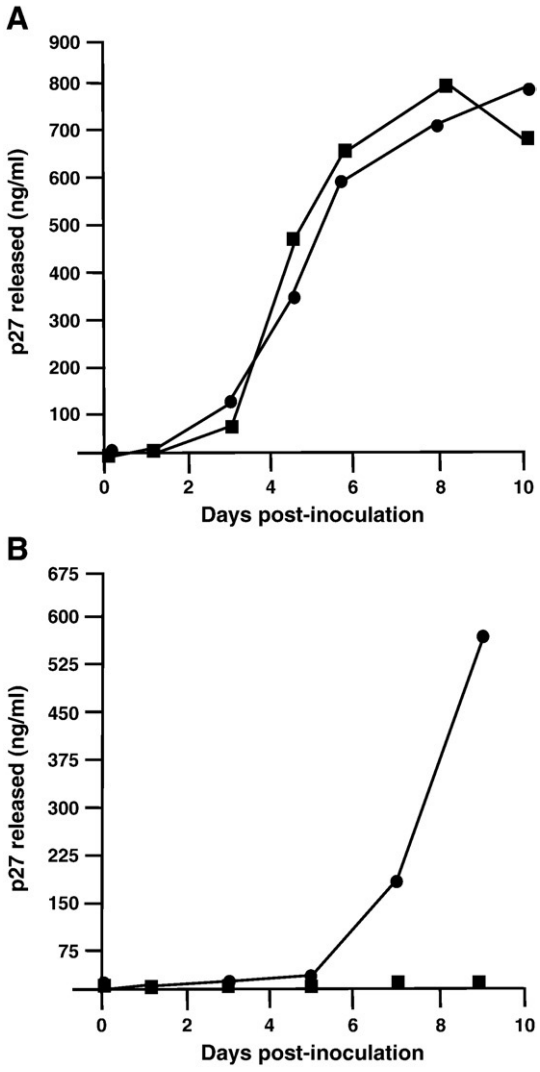

Fig. 1. Replication of $SHIV_{KU\text{-}1bMC33}$ and $SHIV_{VifAAQYLA}$ in APOBEC3G positive (CEM) and negative (CEM-SS) cell lines. Cells were inoculated with equal amounts of each virus and levels of p27 in the culture supernatants determined at various times post-inoculation. (Panel A) Replication of $SHIV_{KU\text{-}1bMC33}$ and $SHIV_{VifAAQYLA}$ in CEM-SS cells. (Panel B) Replication of $SHIV_{KU\text{-}1bMC33}$ and $SHIV_{VifAAQYLA}$ in CEM cells. (●) $SHIV_{KU\text{-}1bMC33}$; (■) $SHIV_{VifAAQYLA}$.

well studied hA3G, other family members such as hA3B, hA3DE, hA3F, and hA3H have been shown to also inhibit the replication of Δ*vif*HIV-1 (Dang et al., 2007; Doehle et al., 2005; Wiegand et al., 2004; Yang et al., 2007; Yu et al., 2004a, 2004b, 2004c; Zheng et al., 2004). Moreover, Δ*vif*SIV_{mac}239 is potently restricted by hA3G, hA3F, and hA3H and to a lesser extent by A3B and A3C and A3DE (Dang et al., 2007; 2008; Mariani et al., 2003; Virgen and Hatziianou, 2007; Yu et al., 2004a, 2004b, 2004c). While fewer studies have been performed on the APOBEC3 family members in macaques, one study has also shown that rhesus macaque rA3G and rA3F inhibit the replication of Δ*vif*SIVmac (Zennou and Bieniasz, 2006; Virgen and Hatziianou, 2007).

Sequence analysis of Vif proteins from different lentiviruses reveals that there is a highly conserved SLQ(Y/F)LA domain near the carboxyl terminus. The introduction of mutations in this domain results in decreased binding of Vif to Elongin C of the Cul5/Elongin B/C E3 ligase complex and increases A3G incorporation into virions resulting in G-to-A hypermutation (Kobayashi et al., 2005). However, no studies have assessed the role of this domain using a non-human primate model of HIV-1 pathogenesis. 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 a simian–human immunodeficiency virus (SHIV) with amino acid changes in the highly conserved SLQYLA ($SHIV_{VifAAQYLA}$) domain and assessed it for pathogenesis in macaques. Our results show for the first time that the SLQYLA domain has determinants that contribute to the pathogenicity of SHIV in macaques and that mutations in this domain result in the accumulation of G-to-A mutations in the viral genome.

Results

Replication of $SHIV_{VifAAQYLA}$ in APOBEC3G positive and negative cell lines

We performed assays to examine the replication of parental $SHIV_{KU\text{-}1bMC33}$ and $SHIV_{VifAAQYLA}$ in A3G/F positive (CEM) and negative (CEM-SS) cell lines. Cells were infected with each of the two viruses and the levels of p27 Gag released into the culture medium were quantified using a commercial antigen capture assay.

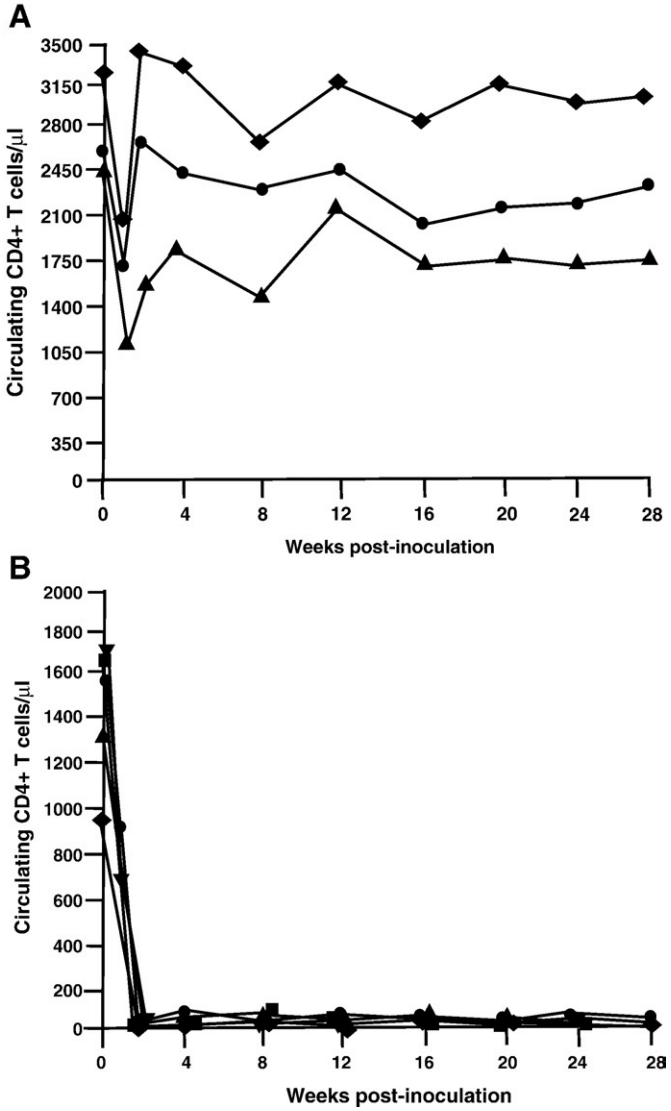

Fig. 2. Circulating $CD4^+$ T cell levels in macaques inoculated with $SHIV_{KU\text{-}1bMC33}$ and $SHIV_{VifAAQYLA}$. (Panel A) The levels of circulating $CD4^+$ T cells in three macaques (RAK10, ●; RCS10, ◆; and RPL10, ▲) following inoculation with $SHIV_{VifAAQYLA}$. (Panel B) The levels of circulating $CD4^+$T cells in four macaques (2000, ●; 2001, ■; CM4G, ▲; and CM4K, ◆; RRH10; ▼) following inoculation with $SHIV_{KU\text{-}1bMC33}$.