Virological and molecular characterization of a simian human immunodeficiency virus (SHIV) encoding the envelope and reverse transcriptase genes from HIV-1

Ranajit Pal [a],*, Lindsey Galmin [a], Lara E. Pereira [b], Bin Li [b], Jining Zhang [b], Daniel Li [b], Jesse Francis [a], Janet M. McNicholl [b], Deborah E. Weiss [a], James M. Smith [b]

[a] Advanced BioScience Laboratories Inc., 9800 Medical Center Drive, Building D, Rockville, MD 20850, USA
[b] Centers for Disease Control and Prevention, Atlanta, GA, USA

A B S T R A C T

Simian–human immunodeficiency virus encoding both reverse transcriptase (RT) and envelope genes of HIV-1 (RT Env SHIV) is important for evaluating biomedical prevention modalities for HIV/AIDS. We describe virological characterization of a clade B RT Env SHIV following infection of macaques via multiple routes. *In vivo* passage of the RT Env SHIV through Indian rhesus macaque enhanced infectivity. Expanded virus had minimal envelope heterogeneity and was inhibited by NNRTIs and CCR5 antagonists. Infection of macaques with RT Env SHIV via mucosal or intravenous routes resulted in stable infection accompanied by peak plasma viremia of approximately 5×10^6 copies/ml that was controlled beyond set point. Molecular homogeneity of the virus was maintained following *in vivo* passage. Inhibition of RT Env SHIV by RT and entry inhibitors and ease of in *vivo* transmission make it a useful model for testing the efficacy of combinations of entry and RT inhibitors in nonhuman primates.

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Introduction

Mucosal transmission accounts for the majority of all HIV infections worldwide (UNAIDS, 2010). In the absence of an effective prophylactic HIV vaccine, antiretroviral prophylaxis strategies that are directed toward preventing sexual transmission of HIV-1 are urgently needed. Use of vaginal microbicides and/or oral pre-exposure prophylaxis (PrEP) to inhibit mucosal transmission of HIV-1 may represent important strategies in controlling the sexual transmission of HIV-1 (Cong et al., 2011; Curtis et al., 2011; D'Cruz and Uckun, 2004; Elias and Coggins, 1996; Garcia-Lerma et al., 2011; Garcia-Lerma et al., 2008; Garg et al., 2009; Grant et al., 2008; Klasse et al., 2008; Klasse et al., 2006; Lederman et al., 2006; McGowan, 2010; Ramjee et al., 2010; van de Wijgert and Shattock, 2007).

Drugs targeting RT have been used routinely to curb HIV-1 infection and have become an integral component of ARV therapy. Oral and topical PrEP regimens targeting RT have recently been shown to be at least partially effective in clinical trials (Abdool Karim et al., 2010; Grant et al., 2010; Jochmans, 2008; Network, 2011; Prajapati et al., 2009; Prevention, 2011). A number of RT inhibitors were shown to be more potent against HIV-1 RT than SIV RT (Buckheit et al., 2007; Buckheit et al., 2001; Kuritzkes, 2009). Therefore, efficacy of RT inhibitor-based PrEP in nonhuman primate models is often assessed against challenges with SHIV isolates encoding the RT gene of HIV-1. To this end, a number of RT SHIV isolates with varying degrees of pathogenicity have been constructed and used in challenge studies in nonhuman primates (Ambrose et al., 2004; Ambrose et al., 2007; Balzarini et al., 1997; Jiang et al., 2009; Pal et al., 2009; Soderberg et al., 2002). In recent years the development of new and effective antiretroviral therapies and PrEP has expanded rapidly beyond the original drugs targeting RT, and compounds designed to inhibit both binding and entry of HIV-1 to target cells are being investigated either alone or in combinations with RT inhibitors (Kuritzkes, 2009). Moreover, compounds with both entry and RT inhibitory activities are also being shown to be potent inhibitors of HIV-1 *in vitro* (Balzarini et al., 1996; Buckheit et al., 2008; Fletcher et al., 2005; Owen et al., 2004). To assess the *in vivo* efficacy of such compounds with dual inhibitory activities in the nonhuman primate model, SHIV encoding both an R5-tropic Clade B HIV-1 envelope and HIV-1 RT in an SIV background was recently constructed (Smith et al., 2010). This virus could infect rhesus macaques intravenously and intra-rectally, and was sensitive to selected RT and entry inhibitors. In this communication, we describe a more detailed characterization of this RT Env SHIV

construct by using a broader range and combinations of RT and entry inhibitors. Moreover, *in vivo* passage of this virus was evaluated in nonhuman primates via the intra-vaginal route, as well as via intravenous and intra-rectal routes. It is expected that this new SHIV recombinant virus with stable replication kinetics in macaques may find widespread application in assessing the efficacy of microbicide or PrEP prophylactic regimens with dual RT and entry inhibitory activities.

Results

In vivo passage and expansion of RT Env SHIV

In an attempt to enhance the infectivity of RT Env SHIV, the parental virus was successfully passaged serially *in vivo* through three Indian origin rhesus macaques, P237 (P1), P241 (P2) and P244 (P3). As illustrated in Fig. 1, blood and bone marrow were transfused once plasma viremia reached a peak load in each infected macaque. Macaque P244 (P3) showed a markedly higher peak plasma viral load of $10^{7.76}$ copies/ml on day 12 post infection, when compared to that observed in animals P237 ($10^{6.73}$ vRNA copies/ml) and P241 ($10^{6.65}$ vRNA copies/ml) at a similar time point (data not shown).

The *in vitro* characterization of PHA- or ConA expanded RT Env SHIV from macaque P241 and P244 PBMC (Fig. 1) was also performed by evaluating SIV p27 content, RNA copies and infectious titers in rhesus macaque PBMC and TZM-bl cells. As shown in Table 1, the two virus stocks prepared from both ConA- or PHA-activated PBMC from macaque P244 (P3) had similar infectious titers and p27 content but these values were markedly higher than that observed with the virus expanded from P241 (P2) PBMC. This enhancement of infectivity of virus derived from P244 PBMC correlated well with the higher peak plasma viremia observed in macaque P244 which was approximately 10-fold higher than that observed in macaque P241.

Genomic heterogeneity of expanded RT Env SHIV stocks

In order to determine the degree of diversity in *env*, *gag*, *pol* and *nef* in the RT Env SHIV stocks following the three *in vivo* passages and *in vitro* expansion, a total of 20 amplicons per gene were obtained by SGA from virus stocks derived from PHA-activated and ConA-activated P244 (P3) PBMC. Since the V1–V5 region of *env* is highly variable, preliminary sequence analysis was focused on this segment of the *env* gene. The diversification of *in vitro*-expanded virus was first evaluated by comparing the genetic variation in ConA- and PHA-derived RT Env SHIV stocks. As shown by the Highlighter plot in Fig. 2A, the alignment of *env* V1–V5 sequences with the P244 (P3) plasma virus isolate as the reference sequence, indicates that 15 out of 20 amplicons (75%) from the ConA-derived stock were identical, with the remaining exhibiting 1–2 nucleotide substitutions per sequence (maximum diversity of 0.17%). In comparison, a lower fraction (40%) of amplicons from the PHA-derived stock exhibited identical *env* sequences, with the rest consisting of 1–6 nucleotide substitutions per sequence, suggesting a slightly higher level of genetic heterogeneity in this stock (maximum diversity of 0.5%). The ConA-derived virus stock was therefore selected for further analysis of *env* V1–V5, *gag*, *pol* and *nef* diversity. A comparison of these gene sequences to the original RT Env SHIV master stock by Highlighter analysis (Fig. 2B) revealed a low level of genetic variation, with the maximum diversity for each gene being less than 0.26%. The translation of *env* V1–V5 amplicon sequences to their corresponding protein sequences revealed minimal amino acid changes, with only a single amplicon exhibiting two substitutions (E256K and D355N). Similarly, minor amino acid substitutions were observed for Gag, Pol (drug resistance mutations in the RT and protease regions were not present) and Nef protein sequences, with two or fewer substitutions being observed per amplicon.

Characterization of RT Env SHIV in terms of sensitivity to RT and entry inhibitors

The function of HIV-1 reverse transcriptase and envelope genes in the RT Env SHIV was evaluated by determining the sensitivity of the ConA-derived expanded virus to RT and entry

Table 1
Characteristics of RT Env SHIV stocks expanded by *in vivo* passage through Indian rhesus macaques.

Isolate	Viral RNA copies/ml supernatant	p27 values (ng/ml)	TCID$_{50}$ in rPBMC	TCID$_{50}$ in TZM-bl
P241 (P2) PHA	2.36×10^9	73	2.05×10^2	2.86×10^4
P244 (P3) PHA	2.92×10^9	144	1.75×10^3	1.15×10^5
P244 (P3) ConA	2.37×10^9	182	2.02×10^3	1.15×10^5
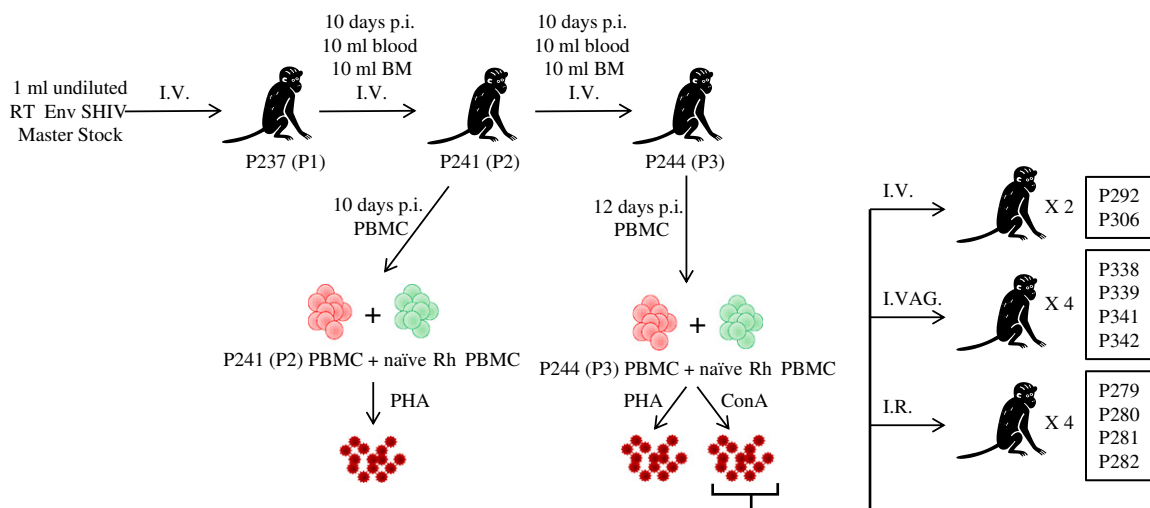
Fig. 1. Schematic representation of *in vitro* RT Env SHIV expansion and *in vivo* passage and infection of rhesus macaques with RT Env SHIV. The virus was passaged and expanded as described in the text.