Replication potentials of HIV-1/HSIV in PBMCs from northern pig-tailed macaque (*Macaca leonina*)

Ai-Hua LEI[1,2,#], Gao-Hong ZHANG[1,#], Ren-Rong TIAN[1,2], Jia-Wu ZHU[1,2], Hong-Yi ZHENG[1,3], Wei PANG[1,*], Yong-Tang ZHENG[1,4,*]

1. *Key Laboratory of Animal Models and Human Disease Mechanisms of Chinese Academy of Sciences & Yunnan Province*, *Kunming Institute of Zoology*, *Chinese Academy of Sciences*, *Kunming* 650223, *China*
2. *Kunming College of Life Sciences,University of Chinese Academy of Sciences*, *Kunming*, *Yunnan* 650204, *China*
3. *University of Science and Technology of China*, *Hefei* 230026, *China*
4. *Kunming Primate Research Center*, *Kunming Institute of Zoology, Chinese Academy of Sciences*, *Kunming* 650223, *China*

Abstract: The northern pig-tailed macaque (*Macaca leonina*) has been identified as an independent species of Old World monkey, and we previously found that PBMCs from *M. leonina* were susceptible to human immunodeficiency virus type 1 (HIV-1), which may be due to the absence of a TRIM5 protein restricting HIV-1 replication. Here we investigated the infection potentials of six laboratory adapted HIV-1 strains and three primary HIV-1 isolates in PBMCs from *M. leonina*. The results indicate that these strains are characterized by various but low replication levels, and among which, HIV-1_{NL4-3} shows the highest replication ability. Based on the abundant evidence of species-specific interactions between restriction factors APOBEC3 and HIV/SIV-derived Vif protein, we subsequently examined the replication potentials of *vif*-substituted HIV-1 (HSIV) in *M. leonina* PBMCs. Notably, HSIV-vif_{mac} and st$HIV\text{-}1_{SV}$ chimeras, two HIV-1_{NL4-3}-derived viruses encoding the viral infectivity factor (Vif) protein from SIV_{mac239}, replicated robustly in cells from *M. leonina*, which suggests that HSIV could effectively antagonize the antiviral activity of APOBEC3 proteins expressed in cells of *M. leonina*. Therefore, our data demonstrate that *M. leonina* has the potential to be developed into a promising animal model for human AIDS.

Keywords: HIV-1; HSIV; Replication; PBMC; Northern pig-tailed macaque (*Macaca leonina*)

The lack of animal models that can be efficiently infected by HIV-1 has been a major impediment to the study of AIDS, anti-HIV-1 drugs and vaccines (Hatziioannou & Evans, 2012; Zhang et al, 2007). Presently, the most widely used non-human primate models for HIV/AIDS research are rhesus (*Macaca mulatta*), pig-tailed and cynomolgus macaques (*M. fascicularis*) infected with simian immunodeficiency viruses (SIVs) or SIV/HIV chimeric viruses (SHIVs) encompassing the HIV-1 *env*, *tat*, *rev* and *vpu* genes or reverse transcriptase gene (Baroncelli et al, 2008; Hatziioannou & Evans, 2012). Although these models have offered us with abundant information on immunopathogenesis and antiretroviral strategies (Evans & Silvestri, 2013; Lackner & Veazey, 2007), they have limitations largely due to the significant genetic differences between HIV-1 and SIV (Ambrose et al, 2007). SHIVs contain certain HIV-1 genes, but the absence of other HIV-1 genes has restricted their functional evaluation in viral pathogenesis or as targets for antiretroviral therapies *in vivo*.

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*Corresponding authors, E-mails: zhengyt@mail.kiz.ac.cn; pangw@mail.kiz.ac.cn.
#Authors contributed equally to this work.

Among Old World monkeys, the pig-tailed macaque is the only primate that can be infected by HIV-1, though the infection is transient and limited (Agy et al, 1992; Bosch et al, 2000; Hu, 2005; Kent et al, 1995). Of note, some HIV-2 strains have been adapted in pig-tailed macaques and can result in persistent infection, $CD4^+$ T cell depletion, and AIDS (Kuller et al, 2001; Otten et al, 1999). Additionally, in contrast to rhesus macaques, the course of SIV infection in pig-tailed macaques more closely resemble that of HIV-1 infection in humans (Batten et al, 2006). Consequently, there has been increasing interest in the use of pig-tailed macaques in HIV/AIDS research (Lei et al, 2013). Based on morphological characteristics and phylogeographic studies, pig-tailed macaques have been classified into three species: Sunda pig-tailed macaques or Southern pig-tailed macaques (*M. nemestrina*), locating in Borneo, Bangka, Sumatra and the Malay peninsula; Northern pig-tailed macaques (*M. leonina*), mainly occurring in China (southwestern Yunnan), eastern Bangladesh, Cambodia, Laos, Myanmar, Thailand, southern Vietnam and India; and Mentawai macaques (*M. pagensis*), living in the Mentawai islands (Groves, 2001; Gippoliti, 2001; Kuang et al, 2009; Rosenblum et al, 1997). So far, the majority of pig-tailed macaques used in SIV/SHIV infection is *M. nemestrina*.

In recent years, it has been reported that the restriction factors, TRIM5α and APOBEC3, are the major barriers for HIV-1 to infect non-human primate cells (Huthoff & Towers, 2008; Liu et al, 2005; Thippeshappa et al, 2012). The TRIM5α protein mediates a post-entry block to retroviral infection by binding to incoming viral capsids through its C-terminal domain (Stremlau et al, 2004, 2006). The cytidine deaminases APOBEC3, especially APOBEC3G/3F, can be packaged into progeny virions, which can then inhibit viral replication largely by causing lethal hypermutations in viral genomes during reverse transcription. However, this restriction can be counteracted by HIV/ SIV-derived Vif protein in a species-specific manner (Mariani et al, 2003; Sheehy et al, 2002; Stopak et al, 2003; Thippeshappa et al, 2012; Zennou & Bieniasz, 2006). Interestingly, our laboratory previously found that *M. leonina* lacks a TRIM5α, and its novel TRIM5-Cyclophilin A (TRIM5-CypA) fusion protein is dysfunctional in blocking HIV-1 infection, which may explain why pig-tailed macaques are susceptible to HIV-1 (Kuang et al, 2009; Liao et al, 2007). These findings are consistent with previous studies in *M. nemestrina* (Brennan et al, 2007, 2008; Newman et al, 2008; Virgen et al, 2008). To overcome barriers imposed by APOBEC3, functional substitution of the *vif* gene with that from pathogenic SIV enables persistent infection of HIV-1 in *M. nemestrina* both *in vitro* and *in vivo* (Hatziioannou et al, 2009; Thippeshappa et al, 2011). Therefore, pig-tailed macaques appear to be a promising animal model for HIV-1 infection.

Here, to identify an isolate that can replicate efficiently in *M. leonina* cells, we investigated the replication potentials of six laboratory-adapted HIV-1 strains and three primary HIV-1 isolates in *M. leonina* peripheral blood mononuclear cells (PBMCs). The results showed that the replications in these HIV-1 strains are various and transient, whereas, constructed HSIV strains based on HIV-1_{NL4-3} with a substitutional *vif* gene from SIV_{mac239} replicate robustly. These results suggest that HSIV strains are resistant to APOBEC3G/3F proteins in *M. leonina* cells and can be applied to infect *M. leonina*, *in vivo*.

MATERIALS AND METHODS

HIV-1 strains and HSIV proviral plasmids

A panel of six lab-adapted subtype B HIV-1 strains, including HIV-1_{IIIB}, HIV-1_{RF}, HIV-1_{MN}, HIV-1_{SF2}, HIV-1_{NL4-3}, and HIV-1_{SF162}, were obtained from the NIH AIDS Research and Reference Reagent Program (USA) or MRC AIDS Research Project (UK). Primary isolates HIV-1_{KM018}, HIV-1_{TC2} and HIV-1_{WAN} were isolated from HIV-1 infected individual in Yunnan Province, China (Huang et al, 2013). All the above-mentioned HIV-1 strains are X4-tropic except that HIV-1_{SF162} and HIV-1_{KM018} are R5 tropism. Viral stocks were stored at −80 °C.

The infectious molecular clone of SIV_{mac239} (Shibata et al, 1991) has been previously described (Li et al, 2007). Proviral plasmids of HIV-1_{NL4-3} (Adachi et al, 1986), stHIV-1_{SV} (Hatziioannou et al, 2009) and HSIV-vif_{mac} were kindly contributed by Prof. Guang-Xia GAO of Institute of Biophysics from Chinese Academy of sciences (Figure 1). stHIV-1_{SV}, a simian-tropic(st) HIV-1, containing a macaque-adapted HIV-1 *env* gene (from $SHIV_{KB9}$) and its *vif* gene from SIV_{mac239} has been described in detail previously (Hatziioannou et al, 2009). HSIV-vif_{mac} chimera differs from HIV-1_{NL4-3} only in the *vif* gene.

Cell culture

H9 (human lymphoblastoma) and CEM×174 suspension cells were cultured in RPMI-1640 medium

(Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine. The adherent cell lines TZM-bl and 293T were maintained in complete DMEM containing 10% FBS.

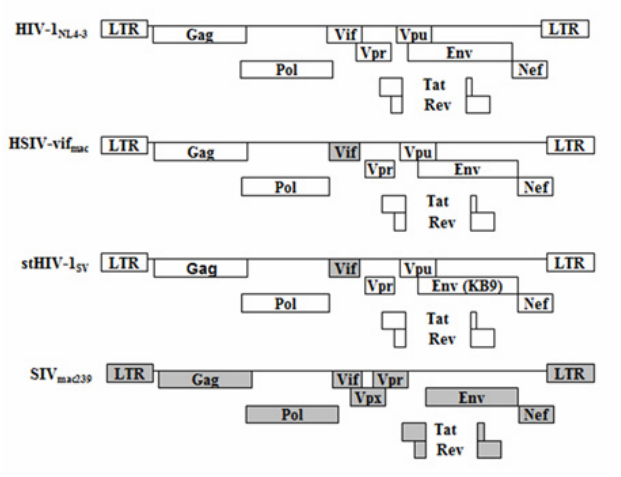

Figure 1 Schematic representation of viral clones used in this study

PBMCs were isolated from adult healthy *M. leonina*, Chinese rhesus macaques, or human peripheral blood by Ficoll-Paque gradient centrifugation as described previously (Dai et al, 2013). All PBMCs were cultured in RPMI-1640 medium with 10% FBS and 50 U/mL IL-2 (Sigma) before infection and thereafter. Human PBMCs were activated with 5 μg/mL phytohemagglutinin (PHA; Sigma) for 72 h. PBMCs from *M. leonina* and Chinese rhesus were activated for 72 h by 10 μg/mL and 5 μg/mL Concanavalin A (ConA; Sigma), respectively.

Transfection and replication assays

To obtain HIV-1/HSIV stocks, 293T cells in a 6-well plate were transfected with infectious molecular clones by using Lipofectamine 2000 (Invitrogen). After 48 h, culture supernatants were harvested and stored at −80 °C until use. Infectious virus titers were determined by serial 5-fold dilutions of the virus stock using TZM-bl reporter cells in a 96-well plate. After 48 h, cells were lysed and treated with Bright-Glo™ Reagent to read relative luminescence units (RLU) in the luminometer (Molecular Devices). $TCID_{50}$ of SIV_{mac239} was determined by infecting CEM×174 cells with serial dilutions of the stock as described previously (Aldovini & Walker, 1990).

For replication assays, 1×10^7 activated *M. leonina* PBMCs were infected with each of the nine HIV-1 strains (40 pg p24) mentioned above in duplicates for 3 h at 37 °C. Then the cells were rinsed three times with PBS to remove cell-free virus and resuspended in fresh medium. To monitor viral replication, supernatants were harvested and replaced every three days for p24 antigen quantification using an enzyme-linked immunosorbent assay kit (ELISA; ZeptoMetrix, Bufflaflo, NY). To compare $HIV\text{-}1_{NL4\text{-}3}$ and HSIV replication in H9 and stimulated PBMCs, viral stocks were mixed with 5×10^5 H9 cells or 1×10^6 activated PBMCs at a multiplicity of infection (MOI) of 0.01. Cells were rinsed three times after incubation, and supernatants were collected with the half of the medium being replaced at 3−4 day intervals postinfection for p24 analysis.

qRT-PCR assay

PBS-rinsed H9 and PBMCs (from human, *M. leonina* and Chinese rhesus macaque) were prepared for RNA isolation. Total RNA was extracted with TRIzol (Invitrogen) according to the manufacturer's protocols. cDNA was synthesized using the PrimeScript® RT reagent Kit with gDNA Eraser (Takara, Dalian, China). To examine TRIM5 and APOBEC3G/3F mRNA expression, qRT-PCR was performed in triplicate with SYBR® Premix Ex Taq™ II (Tli RNase H Plus) kit as described by the manufacturer (Takara, Dalian, China) in the ABI 7500 Fast Real-Time PCR System (Applied Biosystems). Human and macaque target gene expression were normalized to the endogenous GAPDH mRNA level and ribosomal protein L13A (RPL13A) mRNA level, respectively (Ahn et al, 2008). The mRNA expression levels of target genes in different cells are calculated using the $2^{-\triangle Ct}\times100\%$ ($\triangle Ct=Ct_{(Target\ gene)}-Ct_{(Internal\ reference\ gene)}$) method. Chinese rhesus macaque TRIM5α primers (Arhel et al, 2008) and other gene-specific primers used in our study are presented in Table 1.

RESULTS

Replication of HIV-1 strains in *M. leonina* PBMCs

Our laboratory has previously reported that in contrast to Chinese rhesus macaque cells, *M. leonina* cells are susceptible to HIV-1, which may be due to the dysfunctional TRIM5-CypA in the TRIM5 locus (Kuang et al, 2009; Liao et al, 2007). To examine the replication potentials of different HIV-1 strains in *M. leonina* PBMCs, six lab-adapted HIV-1 strains and three primary HIV-1 isolates were used to infect *M. leonina* cells *in vitro*. Lab-adapted subtype B $HIV\text{-}1_{NL4\text{-}3}$ and $HIV\text{-}1_{IIIB}$ were chosen because of their ability in infecting T cells

Table 1 Primers used for qRT-PCR

Gene	Primer[a]	Sequence
Human-A3G	F	5' -CACGTGAGCCTGTGCATCTTC-3'
	R	5'-AAAGGTGTCCCAGCAGTGCTTA-3'
Human-A3F	F	5' -GTCCTGAAACCTGGAGCCT-3'
	R	5'-AGACGGTATTCCGACGAGA-3'
Human-TRIM5α	F	5' -ATGTCCGACGCTACTGGGTTGATGT-3'
	R	5' -TGTCTGGTATCTTGTCCCTCGTGCC-3'
Human-GAPDH	F	5'-GAAATCCCATCACCATCTTCCAGG-3'
	R	5' -GAGCCCCAGCCTTCTCCATG-3'
NPM[b]-A3G	F	5'-TACCACCCAGAGATGAGATT-3'
	R	5' -GTTTCCAGAAGTAGTAGAGG-3'
NPM-TRIM5-CypA	F	5' -CAAAGTCTGAAACGAAGATGGT-3'
	R	5'-GCGGCAGCGTCTCTAAACA-3'
Macaque-A3F	F	5'-CTTTAATAACAGACCCATCCTT-3'
	R	5' -GTTGCCACAGAACCGAGA-3'
ChRM[c]-A3G	F	5'-AACCTTGGGTCAGTGGACAGC-3'
	R	5'-TGGAGCCTGGTTGCGTAGA-3'
ChRM-A3F	F	5'-CTTTAATAACAGACCCATCCTT-3'
	R	5'-GTTGCCACAGAACCGAGA-3'
ChRM-TRIM5α	F	5'-TTGGATCCTGGGGGTATGTGCTGG-3'
	R	5'-TGATATTGAAGAATGAGACAGTGCAAG-3'
Macaque-RPL13A	F	5'-AAGGTGTTTGACGGCATCCC-3'
	R	5'-CTTCTCCTCCAAGGTGGCTGT-3'

[a]: The primers are presented as forward (F) and reverse (R); [b]: denotes northern pig-tailed macaque; [c]: denotes Chinese rhesus macaques.

of pig-tailed macaques in previous studies (Agy et al, 1992, 1997; Gartner et al, 1994). HIV-1_{MN}, HIV-1_{RF}, HIV-1_{SF162} and HIV-1_{SF2} were used due to their close genetic similarity with HIV-1_{NL4-3} and HIV-1_{IIIB}. The primary isolates HIV-1_{KM018} (Wang et al, 2011), HIV-1_{TC2} (Zhang et al, 2010) and HIV-1_{WAN}, which were often used in studies of anti-HIV-1 drugs in our lab, were also chosen to assess their replication potentials in *M. leonina* cells.

As shown in Figure 2, all the HIV-1 strains replicated transiently with different susceptibility in *M. leonina* PBMCs from four different donors and there was no significant increasing trend after day 3 post-infection. In lab-adapted HIV-1 strains, HIV-1_{NL4-3}, and to a lesser extent, HIV-1_{IIIB}, HIV-1_{MN} and HIV-1_{SF162} were all able to replicate productively in *M. leonina* cells. Nevertheless, the replication levels of HIV-1_{RF} and HIV-1_{SF2} were low, which suggested that they were not adapted well in *M. leonina* cells. Meanwhile, primary isolates HIV-1_{WAN} and HIV-1_{KM018} were unable to replicate productively in *M. leonina* PBMCs. In contrast, the replication level of clinical isolated HIV-1_{TC2} in *M. leonina* PBMCs was much higher than that of HIV-1_{WAN} and HIV-1_{KM018}, though was little lower than that of HIV-1_{NL4-3}. Taken together, our results indicate that although lab-adapted HIV-1 strains and primary HIV-1 isolates replicate differently in *M. leonina* cells, their replication levels are low.

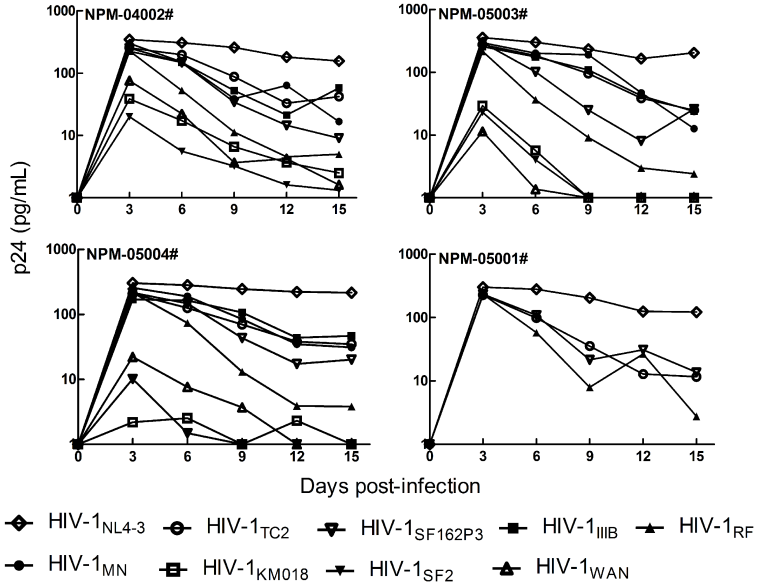

Figure 2 Replication of HIV-1 strains in *M. leonina* PBMCs

Infections of four different macaque donors are shown; Experiments were carried out with equal amounts of viruses (40 pg p24); Replication was monitored by determining the amount of p24 in culture supernatants every three days post-infection. NPM: northern pig-tailed macaque (*M. leonina*)

HIV-1 with *vif* substitution replicates robustly in *M. leonina* cells

The fact that HIV-1 replicates transiently in *M. leonina* cells despite the absence of a post-entry block to viral infection prompted us to consider other factors restricting HIV-1 replication. Several studies have demonstrated that APOBEC3 proteins in cells from rhesus macaque and African green macaque, which are

resistant to HIV-1 Vif protein, can effectively inhibit HIV-1 infection (Mariani et al, 2003; Virgen & Hatziioannou, 2007). Therefore, we determined the mRNA expressions of APOBEC3G/3F, the two potent antiviral proteins among APOBEC3 family members (Albin & Harris, 2010), in *M. leonina* PBMCs, human and Chinese rhesus macaque cells (Figure 3). As expected, TRIM5α mRNA was expressed in H9, human PBMCs and Chinese rhesus macaque PBMCs, whereas, *M. leonina* cells expressed TRIM5-CypA mRNA rather than TRIM5 α mRNA (Fiugre 3). Accordingly, we hypothesized that the reason why HIV-1 failed to infect M. leonina cells may be due to the potent anti-HIV-1 activity imposed by APOBEC3 proteins.

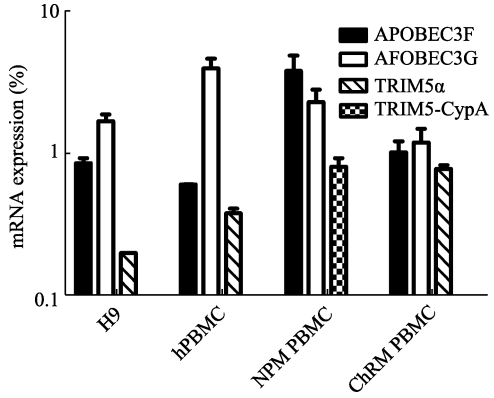

Figure 3 TRIM5 and APOBEC3G/3F mRNA expression in human and macaque cells

mRNA expression levels of human and macaque cells normalized to GAPDH and RPL13A mRNA using the $2^{-\Delta Ct}\times$ 100% method, respectively; Mean values of PBMCs isolations from three healthy donors are shown, and error bars represent standard errors of the means. hPBMC: human PBMCs; NPM: *M.leonina*; ChRM: Chinese rhesus macaque.

To overcome the antiretroviral activity of APOBEC3 in *M. leonina* cells, we subsequently investigated the replication potentials of two HIV-1-derived infectious clones in *M. leonina* PBMCs, stHIV-1_{SV} and HSIV-vif_{mac} chimeras containing the *vif* gene from SIV_{mac239} (Figure 1). Of note, stHIV-1_{SV}, encompassing a macaque-adapted HIV-1 *env* gene from $SHIV_{KB9}$, replicates efficiently in cells of *M. nemestrina* as described previously (Hatziioannou et al, 2009). Additionally, it has been previously reported that SIV_{mac}-derived Vif proteins could potently counteract the antiviral activity of APOBEC3 proteins in rhesus and pig-tailed macaques (Hatziioannou et al, 2009; Kamada et al, 2006). In our experiment, we used HIV-1_{NL4-3} and SIV_{mac239} to infect *M. leonina* PBMCs, meanwhile, took assays of HIV-1/HSIV infection in human T cell line H9, as well as human and Chinese rhesus macaque PBMCs as controls. We found that the replication levels of wild-type HIV-1_{NL4-3} and HSIV-vif_{mac} in H9 cell were higher than those of stHIV-1_{SV} (Figure 4A), suggesting that the inclusion of the *env* gene from $SHIV_{KB9}$ might, at least to some extent, affect HIV-1 infection. However, stHIV-1_{SV} and HSIV-vif_{mac} replicated as efficiently as HIV-1_{NL4-3} in human PBMCs from different donors (Figure 4A), demonstrating that the *vif* substitution could not influence HIV-1 infection. Notably, we observed that the replication levels of stHIV-1_{SV} and HSIV-vif_{mac} in *M. leonina* PBMCs were almost as high as those of SIV_{mac239} (Figure 4B), a pathogenic virus that can result in AIDS in pig-tailed macaques (Klatt et al, 2012). However, the replication level of HIV-1_{NL4-3} was much lower than that of HSIV, indicating that the *vif* replacement was sufficient for HIV-1 to robustly infect *M. leonina* cells *in vitro*. Meanwhile, stHIV-1_{SV} replicated better than HSIV-vif_{mac} in *M. leonina* cells, suggesting the incorporation of macaque-adapted HIV-1 envelope proteins might be conducive to *vif*-substituted HIV-1 replication in *M. leonina* cells. More importantly, based on the well-studied interactions between Vif protein and APOBEC3 proteins, HSIV-derived Vif protein might effectively antagonize the antiretroviral activity of APOBEC3G/3F proteins in *M. leonina* cells.

We next examined the replication potentials of HIV-1/HSIV in Chinese rhesus macaque PBMCs, which were resistent to HIV-1 infection as observed previously (Agy et al, 1992). As we expected, the replication level of HIV-1_{NL4-3} in Chinese rhesus macaque PBMCs was rather low compared with that of the pathogenic SIV_{mac239} (Figure 4C). *M. leonina* cells were more susceptible than Chinese rhesus macaque cells to HIV-1 (Figure 4B). Surprisingly, we found no difference between HIV-1_{NL4-3} and HSIV in their ability to infect Chinese rhesus macaque PBMCs, indicating that the *vif* substitution was insufficient for HIV-1 to replicate robustly in Chinese rhesus macaque PBMCs. Collectively, our results reveal that APOBEC3 proteins in *M. leonina* cells may function as an important barrier for HIV-1 infection and by replacing HIV-1 *vif* gene with that from pathogenic SIV can overcome this potent block.

DISCUSSION

According to primate taxonomy, *M. leonina* and *M. nemestrina* are two independent species in Old World monkeys (Groves, 2001; Malaivijitnond et al, 2012). Our

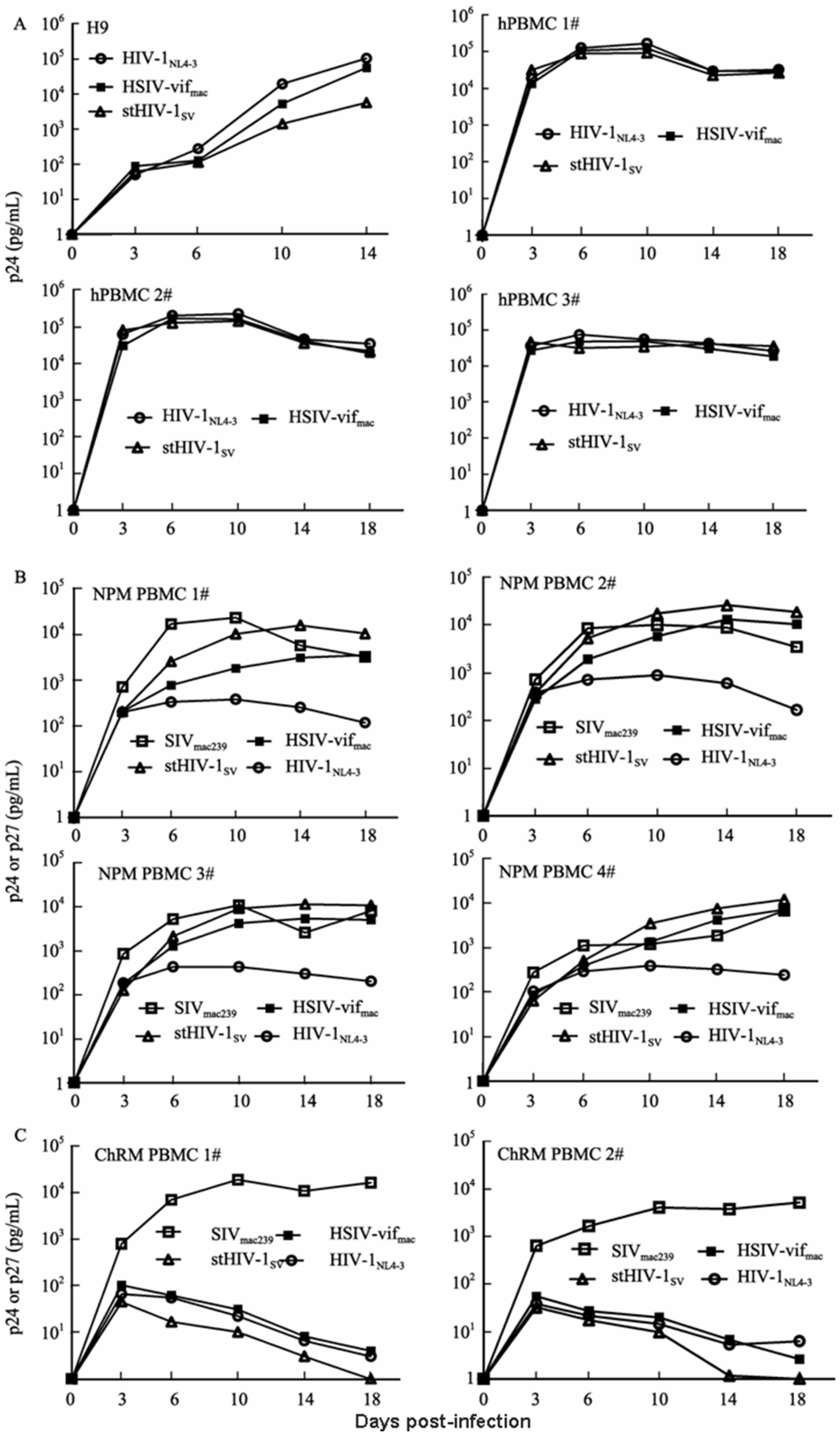

Figure 4 Replication of HIV and HSIV chimeras *in vitro*

A, B and C are replication of HIV and HSIV in H9 and human PBMCs (hPBMCs), *M. leonina* (NPM) PBMCs, and Chinese rhesus macaque (ChRM) PBMCs, respectively; Infections were carried out at an MOI of 0.01; Replication was monitored by determining the amount of p24 (for HIV/HSIV) or p27 (for SIV_{mac239}) per mL at 3- to 4-day intervals post-infection; Cells and donors are indicated at the top of each panel.

lab has previously reported that *M. leonina* cells are prone to HIV-1 infection, which may be due to the absence of a post-entry restriction imposed by a TRIM5 protein (Kuang et al, 2009; Liao et al, 2007). In this study, we showed that the replication levels of six lab-adapted HIV-1 strains and three primary HIV-1 isolates were various but low in *M. leonina* cells. Among the HIV-1 strains, HIV-1_{NL4-3} replicated best in *M. leonina* cells, which was consistent with the previous studies (Agy et al, 1997). However, HIV-1_{RF} and HIV-1_{SF2}, the two HIV-1 strains possessing similar biological properties with HIV-1_{NL4-3} (X4-tropic and lab-adapted subtype B virus), replicated poorly in *M. leonina* cells. Primary isolate HIV-1_{TC2}, rather than HIV-1_{KM018} and HIV-$1_{WAN,}$ could replicate in *M. leonina* cells. A similar phenomenon has also been reported regarding HIV-1 infection in cells of pig-tailed macaques, which suggested that the limited permissivity of macaque cells for HIV-1 may account for certain HIV-1 strains' failing in infecting pig-tailed macaque cells productively (Gartner et al, 1994). Moreover, a recent study suggested that the adaptation of HIV-1-derived envelope protein, which is responsible for viral entry, was necessary for the virus to infect cells from pig-tailed macaques (Humes & Overbaugh, 2011). However, whether the inability of certain HIV-1 isolates to infect *M. leonina* cells is related with viral entry or some other factors remains to be elucidated.

It is well known that APOBEC3 proteins in macaque cells is a major impediment in HIV-1 replication in Old World monkeys and their antiviral activity can be abrogated by some SIV Vif proteins (Huthoff & Towers, 2008; Thippeshappa et al, 2012). Recently, some studies showed that SIV *vif* substitution is sufficient for HIV-1 to persistently infect *M.nemestrina* cells both *in vitro* and *in vivo* (Thippeshappa et al, 2012). Thus, we subsequently examined the replication potentials of stHIV-1_{SV} and HSIV-vif_{mac} (two chimeras encoding Vif protein from SIV_{mac239}) in PBMCs from human and *M. leonina*. Consistent with previous studies, we found that these two chimeras could replicate productively in human PBMCs, implying that SIV_{mac} Vif protein could inactivate human APOBEC3 proteins (Gaddis et al, 2004; Thippeshappa et al, 2011).

Importantly, we showed that stHIV-1_{SV} and HSIV-vif_{mac} are able to replicate robustly in *M. leonina* cells *in vitro*, suggesting that APOBEC3 proteins expressed by *M. leonina* cells are a major barrier to HIV-1 infection in this primate species. We also showed that the replication level of stHIV-1_{SV} expressing the $SHIV_{KB9}$-derived envelope protein is higher than that of HSIV-vif_{mac} in *M. leonina* PBMCs, implying that the modified *env* gene may be conducive for HSIV to infect *M. leonina* cells, which is consistent with previous reports that stHIV-1 can replicate efficiently in cells from *M. nemestrina* both *in vitro* and *in vivo* (Hatziioannou et al, 2009). A more recent study also showed that HSIV-vif, in which the HIV-1_{NL4-3} *vif* gene was functionally substituted by the *vif* gene from SIV_{mne027}, can replicate as efficiently as SIV_{mne027} in cells from *M. Nemestrina* (Thippeshappa et al, 2012). By contrast, HIV-$1_{NL-DT5R}$, a virus containing a part of the Gag CA sequence (corresponding to the HIV-1 CypA-binding loop) and a *vif* gene from SIV_{mac239}, is unable to achieve the replication level of SIV_{mac239} in pig-tailed macaque cells *in vitro* (Kamada et al, 2006), which may result in its transient infection in pig-tailed macaques (Igarashi et al, 2007). Although further modification or passaging *in vitro* of HIV-$1_{NL-DT5R}$ to some extent had enhanced its replication in cells from cynomolgus macaques, a macaque-tropic virus that can replicate as efficiently as SIV_{mac239} in macaque cells could not be obtained (Kamada et al, 2009; Kuroishi et al, 2009; Nomaguchi et al, 2013a, 2013b; Saito et al, 2011). In comparison, the HSIV-vif_{mac} we used seemed to replicate better than HIV-$1_{NL-DT5R}$ with SIV_{mac239} serving as a control, which may be either due to HIV-$1_{NL-DT5R}$ expressing modified Gag protein that was unnecessary for high- level of HIV-1 infection in cells of pig-tailed macaques or because the cells we used in this study was from *M. leonina* instead of *M. nemestrina*. However, whether there are significant differences between *M. nemestrina* and *M. leonina* after HSIV infection needs to be determined.

We also observed that HIV-1 and HSIV replication in Chinese rhesus macaque PBMCs were potently inhibited, which was consistent with a previous study (Hatziioannou et al, 2006). However, it has also been demonstrated that stHIV-1 containing both Gag CA (expressing viral capsid) sequence and a *vif* gene from SIV_{mac}, after serial passage *in vitro*, could replicate robustly in rhesus macaque lymphocytes, whereas, the replication of HIV(SCA) carrying only SIV_{mac} CA was low and transient (Hatziioannou et al, 2006). Additionally, HIV-$1_{NL-DT5R}$ as described above could also replicate in CD8-depleted PBMCs from a rhesus

macaque (Kamada et al, 2006). It is known that TRIM5α protein mediates the early block to HIV-1 infection in Old World monkey cells (Blanco-Melo et al, 2012). Thus, we conclude that TRIM5α, a potent retrovirus inhibitor, may function as a major impediment in Chinese rhesus macaque cells to HIV-1/HSIV replication. Comparatively, the absence of a TRIM5 blocking HIV-1 replication in *M. leonina* cells may partly explain why *M. leonina* cells are more sensitive than Chinese rhesus macaque cells to HIV-1 infection.

In summary, our results indicate that the abilities of HIV-1 strains to persistently infect *M. leonina* cells *in vitro* are various and limited. Notably, HSIV chimeras based on HIV-1_{NL4-3} encoding the SIV_{mac239} Vif protein can achieve the SIV_{mac239} replication level in *M. leonina* cells. Thus, HSIV infection in *M. leonina* may be developed into a promising animal model for human AIDS.

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