TRIM5 genotypes in cynomolgus monkeys primarily influence inter-individual diversity in susceptibility to monkey-tropic human immunodeficiency virus type 1

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TRIM5α restricts human immunodeficiency virus type 1 (HIV-1) infection in cynomolgus monkey (CM) cells. We previously reported that a TRIMCyp allele expressing TRIM5–cyclophilin A fusion protein was frequently found in CMs. Here, we examined the influence of TRIM5 gene variation on the susceptibility of CMs to a monkey-tropic HIV-1 derivative (HIV-1mt) and found that TRIMCyp homozygotes were highly susceptible to HIV-1mt not only in vitro but also in vivo. These results provide important insights into the inter-individual differences in susceptibility of macaques to HIV-1mt.

Considering the global human immunodeficiency virus type 1 (HIV-1) epidemic, development of prophylactic vaccines is strongly desired. In order to evaluate the efficacy of the vaccines, a suitable animal model is also indispensable. However, HIV-1 does not grow in Old World Monkeys (OWMs) such as rhesus monkeys and cynomolgus monkeys (CMs). One of the restriction factors of OWMs is ApoB mRNA editing catalytic subunit 3G (APOBEC3G) (Sheehy et al., 2002). APOBEC3G modifies the minus-strand viral DNA during reverse transcription, resulting in impairment of HIV-1 replication. This activity can be counteracted by the viral protein Vif of simian immunodeficiency virus (SIV) but not by that of HIV-1 (Mariani et al., 2003). Another restriction factor is tripartite motif-containing protein 5α (TRIM5α), which recognizes the viral core and facilitates premature uncoating (Stremlau et al., 2004). To establish a feasible model of HIV-1 infection, monkey-tropic HIV-1 (HIV-1mt) clones were constructed, which were expected to escape from these restriction factors (Hatzioannou et al., 2006; Kamada et al., 2006). In CMs, we reported previously that a modified HIV-1mt, MN4-5S, in which vif and the loops of α-helices 4 and 5 (L4/5) and α-helices 6 and 7 of the capsid protein (CA) of HIV-1 were replaced with those of SIVmac239, a pathogenic molecular clone of rhesus macaque SIV, showed enhanced virus replication in vitro (Kuroishi et al., 2009) and in vivo (Saito et al., 2011).

Accumulating evidence indicates intra-species variations in human and macaque TRIM5 genes (Johnson & Sawyer, 2009). TRIMCyp is an alternatively spliced isoform of the TRIM5 gene in which the PRYSPRY domain of TRIM5α is
replaced with a retrotransposed cyclophilin A (cypA) gene (Brennan et al., 2008; Liao et al., 2007; Newman et al., 2008). We recently reported that the frequency of TRIMCyp alleles was >0.8 in Philippine CMs, which is in contrast to the situation in Indochina CMs (Saito et al., 2012a, 2012b). CM TRIMCyp, also known as Mafa TRIMCyp2 (Ylinen et al., 2010), can restrict HIV-1, but fails to do so in SIVmac and HIV-1mt NL-DT52 with L4/5 derived from SIVmac (Saito et al., 2012a), as the CypA domain of CM TRIMCyp binds to L4/5 of HIV-1, but not that of SIVmac (Price et al., 2009; Ylinen et al., 2010).

We recently reported that a new proviral HIV-1mt construct, MN4Rh-3, carrying a glutamine-to-aspartic acid substitution at position 110 (Q110D) of CA in the parental HIV-1mt MN4-8S (Fig. 1), exhibited further enhanced growth properties in a macaque T-cell line (Nomaguchi et al., 2013a, b). In the present study, we investigated whether TRIMCyp alleles in CMs could influence the susceptibility to HIV-1mt infection.

First, we analysed the replication kinetics of HIV-1mt MN4Rh-3 in CD8+ cell-depleted PBMCs from 26 CMs comprising nine TRIM5α homozygotes, eight TRIM5α/ TRIMCyp heterozygotes and nine TRIMCyp homozygotes. Prior to this experiment, we confirmed the expression of TRIM5α and/or TRIMCyp in PBMCs from monkeys by reverse transcription-PCR (RT-PCR). We found that the mRNA expression was consistent with the TRIM5 genotype of each monkey, i.e. the TRIM5α or TRIMCyp homozygotes expressed the respective mRNA, and the heterozygotes expressed both TRIM5α and TRIMCyp mRNAs (Fig. S1, available in JGV Online). Virus stocks for infection experiments were prepared by transfecting HIV-1mt MN4Rh-3 and HIV-1mt MN4-8S clones into HEK293T cells (Saito et al., 2011). Preparation of CD8+ cell-depleted PBMCs and evaluation of viral growth were performed as described previously (Saito et al., 2011). In Fig. 2(a), representative viral kinetics in PBMCs from animals with each TRIM5 genotype are presented. For comparison, the replication kinetics of HIV-1mt MN4Rh-3 in cells from all 26 animals is shown at the bottom of the figure. Furthermore, the impact of each TRIM5 genotype on HIV-1mt MN4Rh-3 and MN4-8S replication was evaluated by plotting the peak p24 levels during the observation period (Fig. 2b). HIV-1mt MN4Rh-3 grew significantly better in the PBMCs from TRIMCyp homozygotes or heterozygotes than in those from TRIM5α homozygotes, whilst there was no significant difference between TRIMCyp homozygotes and heterozygotes (Fig. 2a, b). Our results on heterozygotes were consistent with previous findings that co-expression of TRIM5α variants with a distinct antiviral activity interferes with the antiviral activity of the wild-type TRIM5α (Javanbakht et al., 2005; Lim et al., 2010; Nakayama et al., 2006; Perez-Caballero et al., 2005; Stremlau et al., 2004). In addition, HIV-1mt MN4Rh-3 grew better in PBMCs of both TRIMCyp homozygotes and the heterozygotes than HIV-1mt MN4-8S (Fig. 2a, b), which was in agreement with our recent data obtained in a CM-derived T-cell line (Nomaguchi et al., 2013b). Of note, there was no significant difference between each TRIM5 genotype in the susceptibility to SIVmac239 infection (Fig. 2c), suggesting that the CM TRIM5 genotypes specifically influence susceptibility to HIV-1mt infection.

We finally investigated whether TRIM5 genotypes could influence the growth of HIV-1mt MN4Rh-3 in vivo. Healthy adult CMs seronegative for B virus and simian retrovirus were housed in individual isolators in a Biosafety Level 3 facility and maintained according to National Institute of Biomedical Innovation guidelines. All experiments were approved by the Animal Welfare and Animal Care Committee of the National Institute of Biomedical Innovation, as well as by Kyoto University. Bleeding and virus inoculation were performed under ketamine hydrochloride anaesthesia. Viral stocks propagated in CD8+ cell-depleted PBMCs were inoculated intravenously into TRIMCyp homozygotes (n=6) or TRIM5α homozygotes (n=3) at a dose of HIV-1mt corresponding to 10 ng CA per head. The profiles of plasma viral loads and anti-HIV-1 antibody responses were evaluated as described previously (Saito et al., 2011). We found that HIV-1mt MN4Rh-3 growth was readily observed in all

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**Fig. 1.** Structure of the HIV-1mt clones (MN4-8S and MN4Rh-3) used in this study. Open boxes denote HIV-1 (NL4-3) and hatched boxes denote SIVmac239 sequences. Black arrows show adaptive mutations that enhance viral growth potential in CM T-cell lines. Dotted arrows show the CA Q110D mutation.
(a) TRIMCyp homozygotes  TRIM5α/TRIMCyp heterozygotes  TRIM5α homozygotes

(b) **P = 0.0009**

(c) P = 0.9166

P = 0.7789

P = 0.8801
TRIMCyp homozygotes, with plasma viral loads reaching a peak at 2–4 weeks post-inoculation (p.i.) and ranging from $1.1 \times 10^4$ to $1.5 \times 10^5$ copies ml$^{-1}$ (mean $4.2 \times 10^4$ copies ml$^{-1}$; Fig. 3a). In contrast, HIV-1mt MN4Rh-3 scarcely replicated in TRIM5$\alpha$ homozygotes (mean $1.9 \times 10^5$ copies ml$^{-1}$; Fig. 3a). Accordingly, HIV-1-specific antibodies were also detected in plasma from 3 to 9 weeks p.i. in the TRIMCyp homozygotes but minimally in TRIM5$\alpha$ homozygotes (Fig. 3b), suggesting that the strength of antibody response reflected the level of virus replication. Notably, although TRIMCyp homozygotes had a higher viraemia compared with TRIM5$\alpha$ homozygotes, none developed persistent viraemia (Fig. 3a). As our present HIV-1mts were focused on evasion of TRIM5- and APOBEC3-mediated restrictions, it is reasonable to assume that additional modifications of the viral genome, especially in order to overcome bone marrow stromal antigen 2 (BST-2)-mediated (Jia et al., 2009; Neil et al., 2008; Van Damme et al., 2008) and SAM domain and HD domain-containing protein 1 (SAMHD1)-mediated restriction (Hrecka et al., 2011; Laguette et al., 2011), may be required to establish persistent viraemia in vivo. Moreover, Bitzegeio et al. (2013) recently suggested the existence of unidentiﬁed, type I interferon-inducible antiviral host factors in macaque PBMCs that inhibit HIV-1 replication.

In humans, several genetic factors related to HIV-1 susceptibility have been reported (reviewed by Chatterjee, 2010; Shioda & Nakayama, 2006). A polymorphism in the chemokine (C–C motif) receptor-5 (CCR5) gene is an eminent example; thus, individuals carrying a 32 bp deletion in CCR5 (CCR5-$\Delta$32) are resistant to CCR5-tropic HIV-1 infection and show delayed progression to AIDS (Dean et al., 1996; Samson et al., 1996). In addition to CCR5, polymorphisms in the genes encoding IL-4 and IL-10 (Shin et al., 2000) and human leukocyte antigen (Carrington & O’Brien, 2003), as well as TRIM5 (Sawyer et al., 2006), have also been suggested to affect disease progression in HIV-1-infected individuals. One of the single-nucleotide polymorphisms (SNPs) in human TRIM5 is a C127T nucleotide substitution, corresponding to an H43Y amino acid substitution in the RING domain. A correlation between this SNP and rapid disease progression has been suggested (van Manen et al., 2008), although this remains controversial (Nakayama et al., 2007; Speelmon et al., 2006). In macaques, an effect of polymorphisms in TRIM5 on SIV infection has been reported (Kirmaier et al., 2010; Lim et al., 2010); thus, rhesus macaques with TFP residues at positions 339–341 of TRIM5$\alpha$ show greater resistance to SIVsmE041 and SIVsmE543–3 compared with animals with a single glutamine residue at position 339 (Kirmaier et al., 2010). However, it remains elusive as to whether genetic diversity might affect HIV-1mt infection in macaques. In this study, we found for the first time that the TRIM5 genotypes of CMs primarily inﬂuenced inter-individual diversity in terms of susceptibility to HIV-1mt. Our results will provide an important insight into the divergent susceptibility of macaques to HIV-1mt. In particular, the finding that the TRIMCyp homozygotes exhibited a greater susceptibility to HIV-1mt infection will make it possible to identify the susceptibility of each CM by pre-screening for TRIM5 genotypes, which will be invaluable in establishing a pre-clinical non-human primate model of HIV-1mt infection using CMs. It is noteworthy that our result is consistent with the ﬁndings that pig-tailed macaques, a macaque species that is thought to possess TRIMCyp exclusively instead of TRIM5$\alpha$, shows higher susceptibility to HIV-1 infection (Agy et al., 1992). For this reason, pig-tailed macaques are expected to be a promising model animal for HIV-1mt infection. Indeed, it was reported previously that these macaques developed persistent viraemia following HIV-1mt challenge (Hatzioannou et al., 2009; Igarashi et al., 2007; Thippeshappa et al., 2011). Moreover, our findings, in which CM TRIM5 genotype was shown to inﬂuence susceptibility to retroviral infection, may imply that the marked geographical variation in the genotypes (Berry et al., 2012; Dietrich et al., 2011; Saito et al., 2012a; Saito et al., 2012b) is a consequence of selective pressures driven by some external factors. As both TRIM5$\alpha$ and TRIMCyp are thought to be associated with retrovirus replication, it is reasonable to speculate that a geographically diverse prevalence of some pathogen(s) such as exogenous or endogenous retroviruses might
Contribute to the variation in TRIM5 genotypes. We are now seeking to identify pathogen(s) that have played a critical role in the diversity of CM TRIM5 genotypes.

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