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EDITORIAL REVIEW

Impact of TRIM5α *in vivo*

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HIV type 1 (HIV-1) has a very narrow host range that is limited to humans and chimpanzees. HIV-1 cannot replicate well in Old World monkey cells such as rhesus and cynomolgus monkeys. Tripartite motif (TRIM)5α is a key molecule that confers potent resistance against HIV-1 infection and is composed of really interesting new gene, B-box2, coiled-coil and PRYSPRY domains. Interaction between TRIM5α PRYSPRY domains and HIV-1 capsid core triggers the anti-HIV-1 activity of TRIM5α. Analysis of natural HIV variants and extensive mutational experiments has revealed the presence of critical amino acid residues in both the PRYSPRY domain and HIV capsid for potent HIV suppression by TRIM5α. Genetic manipulation of the human *TRIM5* gene could establish human cells totally resistant to HIV-1, which may lead to a cure for HIV-1 infection in the future.

AIDS 2015, **29**:1733–1743

Keywords: capsid, HIV-1, HIV-2, simian immunodeficiency virus, single-nucleotide polymorphism, TRIM5α, TRIMCyp

Introduction

Four host restriction factors capable of suppressing HIV-1 replication have been reported to date. First, ApoB mRNA editing catalytic subunit (APOBEC) 3G was found to modify the minus strand of HIV-1 DNA during reverse transcription [1–3], but this activity could be counteracted by the viral Vif protein [4–6]. Tetherin, also known as BST2 or CD317 [7,8], is an interferon-inducible membrane protein that inhibits the detachment of virus particles from infected cells. HIV-1 overcomes this restriction by expressing Vpu protein. The most recently identified host factor is SAMHD1 (a cellular protein sterile alpha motif and histidine/aspartic acid-domain containing protein), which is a dendritic and myeloid cell specific HIV-1 restriction factor counteracted by HIV-2/SIV Vpx [9,10]. These three factors are degraded by the proteasome and their antiviral activity is cancelled in the presence of viral proteins. In contrast, HIV accessory proteins are unable to counteract the fourth restriction factor tripartite motif (TRIM)5α. In this review, we will focus on the impact of TRIM5α and related proteins *in vivo*.

Identification of TRIM5α as a restriction factor against HIV-1 in old world monkey cells

HIV-1 major subtypes are thought to have been introduced into the human population from chimpanzees [11] and have a very narrow host range that is limited to humans and chimpanzees. Experimentally, HIV-1 fails to replicate in activated $CD4^+$ T lymphocytes obtained from Old World monkeys (OWMs), such as rhesus monkey (Rh) [12,13] and cynomolgus monkeys (CM) [14,15]. In contrast, other lentiviruses including the simian immunodeficiency virus isolated from sooty mangabeys (SIVsm) and the simian immunodeficiency virus isolated from African green monkeys (SIVagm) replicate in their natural hosts cells [16]. The SIV virus isolated from macaque monkeys (SIVmac), which evolved from SIVsm in captive macaques, was used as a simian AIDS model system in Rh [12,13].

Several earlier studies suggested that the block for HIV-1 replication in OWM cells occurs at a postentry step [12,13,17] and appears to result from failure to initiate

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Received: 2 June 2015; revised: 18 June 2015; accepted: 19 June 2015.

DOI:10.1097/QAD.0000000000000812

reverse transcription [13]. In 2004, Rh TRIM5α was identified as a factor that confers resistance to HIV-1 infection [18]. There are wide variations in the spectrum of viruses that TRIM5α from different monkey species can restrict. Rh and CM TRIM5α restrict HIV-1 infection but not SIVmac [18,19]. In contrast, human TRIM5α only weakly restricts HIV-1 and SIVmac, but potently restricts N-tropic murine leukaemia viruses (N-MLV). African green monkey TRIM5α restricts both HIV-1 and SIVmac but not SIVagm (reviewed in ref. [20]).

Structure of TRIM5α

TRIM5α is a member of the tripartite motif (TRIM) family of proteins with really interesting new gene (RING), B-box 2 and coiled-coil domains [21] (Fig. 1). Because proteins with RING domains possess E3 ubiquitin ligase activity [22], TRIM5α is thought to degrade the HIV-1 incoming core [23,24]. The coiled-coil domain of TRIM5α is important for the formation of homo oligomers [25–27], while the B-box 2 domain mediates higher-order self-association of TRIM5α oligomers [28–30] (Figs. 1 and 2).

The C terminal PRYSPRY domain is specific for the α-isoform of TRIM5-splicing variants. The amino acid sequences of the variable region 1 (V1) of TRIM5α PRYSPRY domain have been shown to determine the aforementioned species-specific restriction of retrovirus infection [19,31–38] (Fig. 1b). The PRYSPRY domain recognizes the viral core proteins because TRIM5α lacking this domain does not show antiviral activity. Furthermore, overexpression of truncated TRIM5α lacking the PRYSPRY domain shows a dominant negative effect on antiviral activity of full-length TRIM5α [27,39]. Because the interaction between individual capsid (CA) monomers and TRIM5α is very weak, CA recognition by TRIM5α is thought to be a synergistic combination of direct binding interactions with the PRYSPRY domain and lattice-like higher-order assembly of TRIM5α [40] (Fig. 2). Although the precise three-dimensional crystal structure of the PRYSPRY V1 region has not been resolved due to flexibility of the V1 loop, it is speculated that the PRYSPRY domain interacts with more than one CA monomer within the assembled core spanning the gap between CA hexamers to destroy inter-hexamer interaction [41].

The impact of rhesus monkey TRIM5α on simian immunodeficiency virus infections

To elucidate the impact of TRIM5α *in vivo*, the polymorphism in Rh TRIM5α V1 region, threonine/phenylalanine/proline (TFP) to glutamine (Q) at position 339 [42], has been attracting attention. Wilson *et al.* [43] showed that Rh TRIM5α TFP restricted HIV-1 and

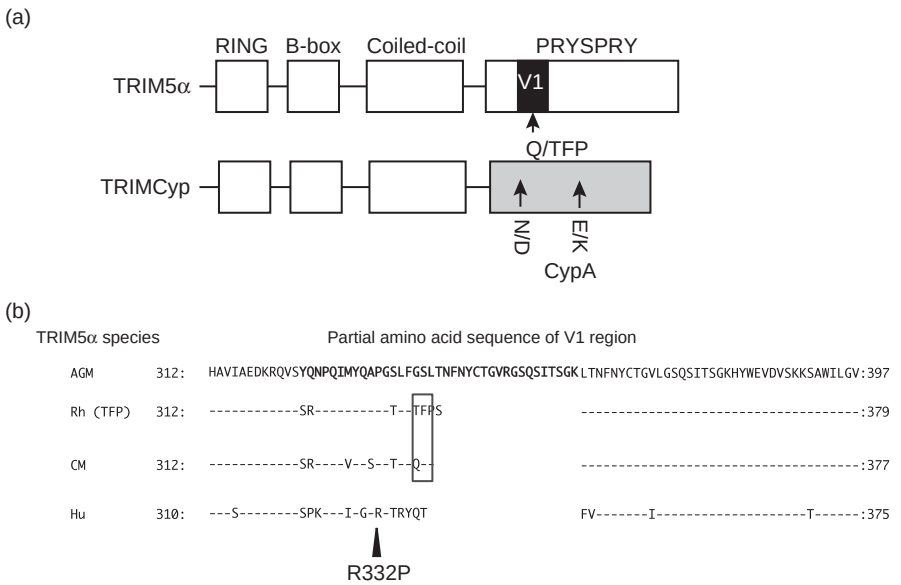

Fig. 1. Diversity of *TRIM5* genes. (a) The RING, B-box2, coiled-coil and PRYSPRY domains of TRIM5α and TRIMCyp are shown in boxes. CypA domains in TRIMCyp are shown as gray squares. V1 region is outlined. Polymorphisms are shown outside the boxes. (b) Alignment of partial amino acid sequences of V1 region of African green monkey (AGM), the TFP allele product of rhesus monkey (Rh), cynomolgus monkey (CM) and human (Hu) TRIM5α. A dash denotes the amino acid residue identical to those of AGM. A box indicates TFP and Q difference. Arrowhead shows the position of R332P substitution.

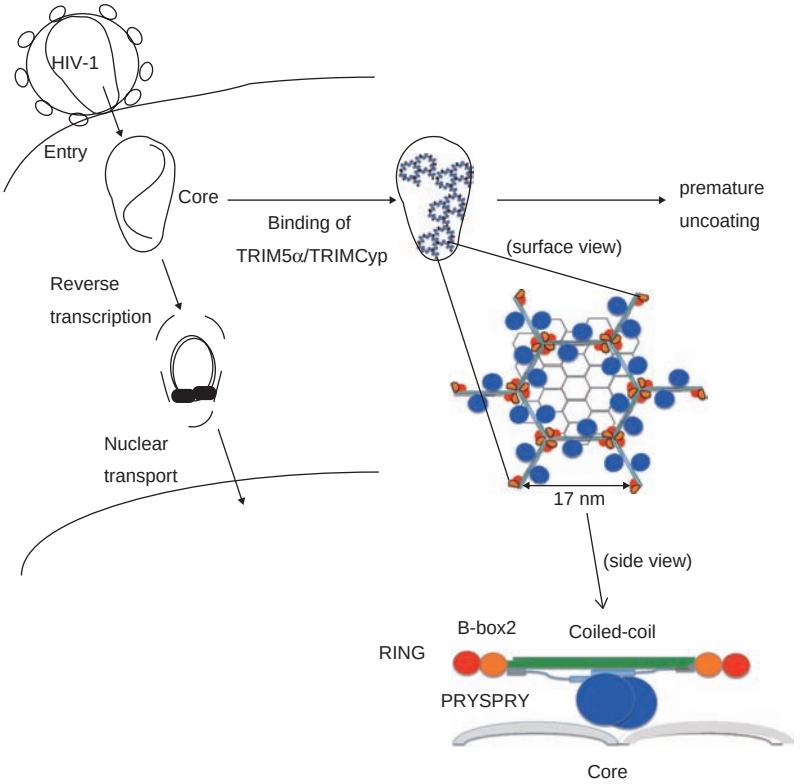

Fig. 2. Proposed models of TRIM5α/TRIMCyp restriction. Lattice-shaped oligomerized TRIM5α/TRIMCyp recognizes the incoming HIV-1 core. Subsequently, TRIM5α is poly-ubiquitinated, and ubiquitinated TRIM5α along with HIV-1 core complex is degraded. Red, orange and blue circles denote RING, B-box2 and PRYSPRY domains, respectively. Green bars denote coiled-coil regions.

HIV-2 but not SIVmac239, while Rh-TRIM5α Q restricted HIV-1 but not HIV-2 or SIVmac239 using TRIM5α-transduced cell lines. Furthermore, Kirmaier *et al.* [44] reported that the Rh-TRIM5α TFP restricted SIVsmE543 and SIVsmE041, although the Rh-TRIM5α Q did not show any anti-SIVsmE543 or anti-SIVsmE041 activity. It should be noted that the anti-HIV-1 activity of Rh-TRIM5α Q is still substantially stronger than the anti-SIVmac239 and SIVsmE543 activities of Rh-TRIM5α TFP [45]. SIVmac239 is a molecular clone of a highly adapted emergent Rh virus generated in the 1980s by experimental passage of SIV-positive plasma through several monkeys [46]. In contrast, SIVsmE041 is a primary isolate from a sooty mangabey and SIVsmE543 was cloned after experimental passage of SIVsm through two Rh individuals [47]. Comparison of SIVsmE543 CA amino acid sequence with that of SIVmac239 revealed an LPA-to-QQ change at positions 89–91 in the loop between α-helix 4 and 5 (L4/5) and an R-to-S change at position 97 in the α-helix 5 of CA, which are both critical for resistance against the Rh-TRIM5α TFP allele [48,49] (Fig. 3).

When SIVsmE543 was inoculated into Rh monkeys, viral replication was markedly diminished in Rh-TRIM5α $^{TFP/TFP}$ homozygotes compared with Rh-TRIM5α $^{Q/Q}$ homozygotes with a 2 to 3-log reduction after intravenous or intra-rectal infection; those findings are with the in-vitro results [44]. In low-dose repeated mucosal challenge experiments, two groups reported similar results using SIVsmE660, which has a CA sequence closely resembling that of SIVsmE543 [50,51]. In contrast to this clear effect of Rh TRIM5α genotypes on SIVsm infection, the effect of Rh-TRIM5α genotypes on SIVmac infection is subtle. Lim *et al.* retrospectively analysed the plasma viral load in Rh individuals after intravenous SIVmac251 challenge. They found that the Q allele was associated with higher levels of plasma viral RNA at the time when the levels of viral RNA stabilized after the period of acute infection (0.6 log median difference); this finding was associated with a rapid loss of central memory $CD4^+$ T cells, and a higher rate of progression to AIDS [45,52] compared with those animals with the TFP allele. These results were consistent with the in-vitro observations; however, it should be noted that the suppression of SIVsmE543 by Rh-TRIM5α TFP is more dramatic than that of SIVmac251. Fenizia *et al.* [53] did not detect any difference in susceptibility among Rh TRIM5 genotypes following repeated rectal challenge with SIVmac251.

In conclusion, it is absolutely necessary to determine the *TRIM5* genotype of a specific Rh monkey when SIVsm is used in experiments. It is also better to do so when SIVmac is used.

Viruses	Partial capsid sequences (90 100 120)	TRIM5α allele			TRIMCyp allele	
		Hu	Rh^{TFP}	Rh^{Q}/CM	$CM^{Cyp(DK)}$	Rh/PM/$CM^{Cyp(NE)}$
HIV-1 NL4-3	GP**IAP** GQMREP**R**GSDIAGTTSTLQEQIGWMT -**H**	No	Yes	Yes	Yes	No
HIV-2 GH123	GP**LPA**GQLRDP**R**GSDIAGTTSTVEEQIGWMYR**P**	Weak	Yes	Yes	No	Yes
HIV-2 UC1	GPLPAGQLRDP**R**GSDIAGTTSTVEEQIGWMYR**A**	No	Yes	No	No	Yes
HIV-2 UC2	GPLPAGQLRDP**R**GSDIAGTTSTVDEQIGWMYR**Q**	No	Yes	No	No	Yes
SIVsm	GPLPAGQLREP**R**GSDIAGTTSTVEEQIGWMYR**Q**	No	Yes	No	Unknown	Yes
SIVmac	AP**QQ-** GQLREP**S**GSDIAGTTSSVDEQIGWMYR**Q**	No	No	No	No	No

Yes: restricted , No: not restricted, Weak: weakly restricted

Fig. 3. HIV-2/simian immunodeficiency virus capsid sequence variations and restriction patterns of rhesus and cynomolgus monkey TRIM5α/TRIMCyp alleles. 'Yes' denotes restriction. 'Weak' denotes weak restriction. 'No' denotes no restriction. The unique QQ sequence at the 89th–90th positions of SIVmac is shown in purple. Arginine 97 at the base of the loop between helices 4 and 5 is shown in blue. The glutamine and alanine residues at position 120 of GH123 or analogous positions of other HIV-2 strains are shown in green. The proline residue at position 120 of GH123 is shown in red. $CM^{CypA(NE)}$ and $CM^{CypA(DK)}$ denote the minor and major alleles of cynomolgus monkey TRIMCyp, respectively.

TRIM5 and CypA fusion protein (TRIMCyp) in monkeys

TRIMCyp is a very interesting example of gain-of-function by retro-transposition in the *TRIM5* gene in several monkey species. In 2004, soon after the discovery of TRIM5α, analysis of *TRIM5* genes of owl monkeys in the New World monkey species identified a long interspersed nuclear element (LINE)-1 mediated retro-transposition of cyclophilin A (CypA) between exons 7 and 8, resulting in expression of a fusion protein designated TRIMCyp [54,55]. In 2008, another CypA insertion was found in Rh, CM and pig-tailed monkeys [56–59]. In these OWMs, the *CypA* gene is inserted at the 3′ end of the *TRIM5* gene, which is totally different from that of the owl monkey. This finding indicated that a CypA retro-transposition into the *TRIM5* gene in OWMs occurred independently from that in New World monkeys. A G-T transversion at the splicing acceptor of *TRIM5* exon 7 linked with CypA insertion causes alternative splicing [56] and the resultant mRNA lacks exons 7 and 8, and consequently, the PRYSPRY domain is replaced with CypA (Fig. 1a).

It would be reasonable to assume that the retro-transposition event occurred in a common ancestor of the three macaques, but there is considerable variation among the three monkey species in the frequency of CypA insertion and amino acid differences in the CypA domain of TRIMCyp resulting in a spectrum of antiviral activities. In pig-tailed monkeys, TRIM5α mRNA is absent. Pig-tailed monkey TRIMCyp restricted HIV-2 but not HIV-1 infection [56,60]. In Rh, the allele frequency of TRIMCyp is 25% in an Indian monkey population but completely absent from a Chinese population [59]. In the case of CM, however, it is a bit more complex. The TRIMCyp frequency in CM is apparently higher than that in Rh. TRIMCyp frequency tends to be higher in eastern than western Asia. There are major and minor haplotypes of CM TRIMCyp with single nucleotide polymorphisms in the CypA domain. The major haplotype of CM TRIMCyp bears aspartic acid (D) and lysine (K) at positions 369 and 446 [56,61], while the minor haplotype encodes asparagine (N) and glutamic acid (E) at these positions [62,63] (Fig. 1a). N369 and E446 are also found in pig-tailed monkeys and Rh TRIMCyps, and the CypA portion of the NE haplotype of CM TRIMCyp has the same amino acid sequence as that of Rh TRIMCyp. The major CM haplotype (DK haplotype) of TRIMCyp can suppress HIV-1 but not HIV-2, while the minor NE haplotype suppresses HIV-2 but not HIV-1, similar to pig-tailed monkeys and Rh TRIMCyp [63] (Fig. 3). It should be noted that so far, there is no polymorphism at amino acid position 339 of CM TRIM5α and all of the CM TRIM5α alleles carry Q at this position [19], while Rh TRIM5α has a Q-to-TFP polymorphism at position 339 [42]. Because the untranslated exon of both CM and Rh TRIMCyp alleles has Q at position 339, the Q allele may be an ancestor of these OWM *TRIM5* genes. After separation into Rh and CMs, selection pressure in CM might have driven amplification and diversification in TRIMCyp, while that in Rh might have driven diversification of the PRYSPRY domain of TRIM5α.

TRIM5 gene and HIV-1 variants capable of replicating in monkey cells

In order to establish a monkey model of HIV-1/AIDS, various SIVmac and HIV-1 chimeric viruses (SHIV) have been constructed and tested for their replicative capability in monkey cells. The first SHIV was generated in a genetic background of SIVmac with HIV-1 *tat*, *rev*, *vpu*

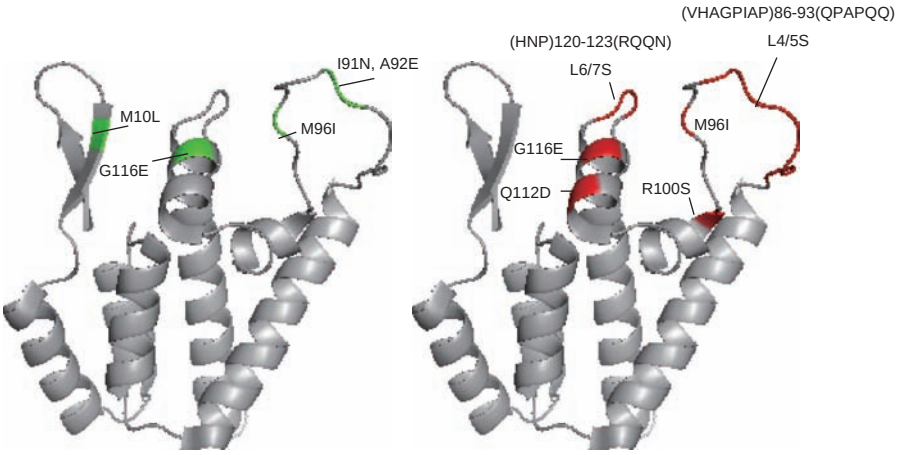

Fig. 4. Structure of the N-terminal half of HIV-1 capsid monomer. The ribbons represent the backbones of NL4-3. The upper side of the capsid monomer is supposed to be exposed to the outside of core structure. The positions of mutated amino acids of LNEIE and LSDQ viruses are shown in green and red, respectively.

and *env* genes in 1991 [64]. After the discovery of several host factors involved in HIV-1 restriction in OWM cells, the opposite approach was used to construct HIV-1 variants capable of replicating in monkey cells with a small segment of SIVmac that was necessary to counteract host restriction factors [65].

As mentioned above, there are considerable inter and intra-species variations in simian *TRIM5* genes. The most advanced monkey model of HIV-1 infection uses pig-tailed monkeys because it lacks expression of functional TRIM5α and pig-tailed monkey TRIMCyp fails to restrict HIV-1. Hatziioannou *et al.* [66] constructed a mutant HIV-1 that differs from the original HIV-1 only in the *vif* gene. This virus leads to the development of AIDS after several animal transfers with $CD8^+$ T cell knocked-down by anti-CD8 antibody injections [67]. Next to pig-tailed monkey, chronic and persistent infection was established in CM homozygous for the TRIMCyp allele infected with a mutant HIV-1 [68]. Although a marked increase in viral load was observed after injection of anti-CD8 antibody, the viral load decreased within months. This mutant HIV-1, MN4Rh-3, contains an additional mutation in CA that includes escape from CM TRIMCyp, and several mutations in the *integrase* and *envelope* genes, which lead to increased growth capability [69]. Although infected animals did not develop AIDS, this is a good model of the asymptomatic period of HIV-1 infection. It may be possible to use this model to examine factors that might trigger disease progression. In the case of Rh monkeys, multiple regions of CA, including the N-terminal region, L4/5 and amino acid at position 120, were shown to affect recognition by Rh TRIM5α [70–74]. Unfortunately, the replacement of whole CA with SIVmac was detrimental to viral growth [75]. Two research groups independently performed extensive mutagenesis of CA to obtain HIV-1 variants that escape from Rh TRIM5α mediated restriction. Although the mutant viruses designated LSDQ [76] and LNEIE [77] had different amino acid substitutions (Fig. 4), both variants were capable of replicating in the presence of Rh TRIM5α TFP allele products. However, levels of resistance to the Rh TRIM5α TFP allele of both HIV-1 variants were still lower than to CM TRIM5α/Rh TRIM5α Q allele products [78]. Therefore, further adaptation and/or genetic manipulation of HIV-1 variants is still required to establish an HIV-1 infection model in Rh.

Polymorphisms in the human *TRIM5* gene and HIV-1 infection

Several single-nucleotide polymorphisms (SNPs) in the human *TRIM5* gene have been studied for their association with the rate of HIV-1 transmission and AIDS progression (Fig. 5), and only modest effects were observed. Sawyer *et al.* [79] reported an H-to-tyrosine (Y) polymorphism at amino acid position 43 (H43Y, rs3740996) of the human *TRIM5* gene. This SNP is located in the RING domain and greatly reduces the ability of human TRIM5α to inhibit N-MLV infection [79]. Several in-vitro studies have indicated that the anti-HIV-1 activity of human TRIM5α with 43Y was lower than that with 43H [79–81], although the difference in anti-HIV-1 activity was very small. The association of H43Y with the rate of progression to AIDS has been tested in several studies, but with inconsistent results [80–83]. Despite the lower anti-N-MLV and anti-HIV-1 activities of TRIM5α with 43Y [79], Javanbakht *et al.* [80] reported a paradoxical protective effect of TRIM5α with 43Y against HIV-1 transmission in African-Americans. Interestingly, we also observed that the 43Y-allele was found less frequently in Japanese and

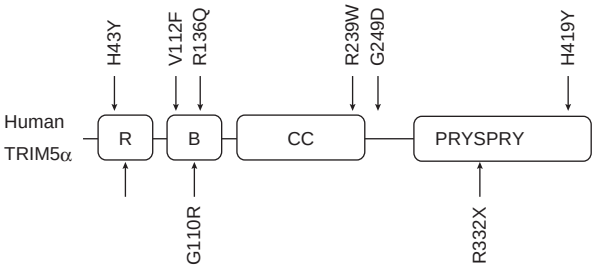

Fig. 5. Single nucleotide polymorphisms in human TRIM5α. The RING (R), B-box2 (B), coiled-coil (CC) and PRYSPRY domains of human TRIM5α are indicated by squares. Polymorphisms are shown outside the squares. Downward and upward arrows show common and rare SNPs, respectively. SNPs discussed in this review are shown in bold.

Indian HIV-1-infected individuals than in ethnicity-matched controls [84]. Furthermore, Liu *et al.* [85] reported that the frequency of H43Y homozygotes was higher in sero-negative intravenous drug users than in HIV-infected drug users. The reasons for this discrepancy between the epidemiological and functional effects of H43Y remain to be elucidated. Pertel *et al.* [86] reported that TRIM5α makes a major contribution to lipopolysaccharide signalling through Toll-like receptor 4. One possible explanation is that the lower activation of innate immunity by 43Y allele decreases the T-cell population in which that HIV-1 prefers to replicate. It is noteworthy here that an allelic dose-dependent decrease was observed between H43Y and tumour necrosis factor-alpha (TNF-α) secretion from peripheral blood mononuclear cells obtained from children who received rubella vaccination [87].

In Japan, we found a rare G-to-R substitution at position 110 of TRIM5α (G110R, rs146215995) in the B-box2 domain, and this 110R allele was observed more frequently in HIV-1-infected individuals than in non-infected individuals. Consistent with this epidemiological observation, this substitution weakened the anti-HIV-1 and anti-HIV-2 activity *in vitro* [84]. Price *et al.* [88] found that female Pumwani sex workers with the R136Q polymorphism (rs10838525) were less likely to seroconvert despite repeated heavy exposure to HIV-1. The B-box2 domain is important in higher-order oligomerization, which is required to form the hexagonal lattice-like structure to stabilize the interaction between TRIM5α and CA [40] (Fig. 2). It is likely that the R136Q substitution affects lattice formation of TRIM5α.

The G249D polymorphism in the linker region (rs11038628) is common in Asian and African populations but rare in whites. It was initially speculated that there was no functional effect of this SNP because it is located outside of any functional domains of human TRIM5α. Contrary to our expectation, however, we observed attenuation of anti-HIV-1 and anti-HIV-2 activity associated with this G-for-D substitution in both multiround replication and single-round infection assays. Rahm *et al.* [89] also reported reduced anti-HIV-1 activity of TRIM5α carrying this mutation. Furthermore, we investigated the presence of the G249D polymorphism in two ethnic populations, Japanese and Indian, and found that the TRIM5α 249D-allele was associated with an enhanced susceptibility to HIV-1 infection [90]. It is speculated that amino acid position 249 may affect the flexibility of the linker region and facilitate the mobility of PRYSPRY domain. CEM, HeLa, Jurkat and 293T cells were all homozygous for 249G, but MT4 cells established in Japan appeared to be homozygous for 249D. This may explain why MT4 cells are highly susceptible to HIV-1 infection [91].

The artificial substitution of arginine (R) at position 322 of human TRIM5α to proline (P) conferred potent restriction ability against HIV-1 [37,38]. Position 332 is in the V1 region of the PRYSPRY domain (Fig. 1b) and, therefore, is supposed to be critical for species-specific recognition of viral CA by TRIM5α [37,38]. There is no equivalent human SNP in this position except for a rare null allele 332X, in which R332 is substituted with a stop codon in Baka pygmies at an allele frequency of 0.02. This rare allele encodes a truncated form of TRIM5α-lacking part of the PRYSPRY domain and shows a dominant negative effect against authentic TRIM5α *in vitro* [92].

Taken together, the anti-HIV-1 activity of human TRIM5α may affect HIV-1 transmission, although it is apparent that TRIM5α itself cannot protect humans from an HIV-1 pandemic. Table 1 summarizes characteristics of the genetic polymorphisms in human and monkey *TRIM5* genes.

Human TRIM5α and HIV-2 pathogenesis

In contrast to HIV-1, several HIV-2 strains showed an ability to grow in OWM cells such as baboon, Rh and CM cells [93–97]. We investigated viral sensitivity to CM TRIM5α and showed that the CM TRIM5α-sensitive viruses had proline (P) at position 119 of CA in the ROD strain or at position 120 in the GH123 strain, while the CM TRIM5α-resistant viruses had either alanine (A) or glutamine (Q) at the same position (Figs. 3 and 6). Replacing the P of a CM TRIM5α-sensitive HIV-2 molecular clone GH123 with A, Q or glycine (G) changed the phenotype from sensitive to completely resistant to CM TRIM5α [98,99]. Similar results, although to a lesser extent, were observed when human TRIM5α was used [98]. It has been speculated that HIV-2 might have been transferred to humans from a sooty mangabey infected with SIVsm as a result of a zoonotic event [100]. Almost all SIV isolates in the Los Alamos database contain Q at the position corresponding to

Table 1. Polymorphisms in human and monkey *TRIM5* gene.

Species	Mutation	Phenotypes associated with the mutation
Human	H43Y	Reduced anti-N-MLV activity Slightly reduced anti-HIV-1 activity Reduced risk of HIV-1 acquisitions in African-Americans[a] Reduced levels of TNF-α secretion after rubella vaccination
	R136Q	Reduced risk of HIV-1 acquisition in Pumwani, Kenya
	G249D	Reduced anti-HIV-1 and anti-HIV-2 activities Increased risk of HIV-1 acquisition in India
Rhesus monkey	TFP to Q	Increased sensitivity to SIVsm infection
	TFP to Cyp	Loss of anti-HIV-1 activity
Cynomolgus monkey	Q to Cyp	Increased sensitivity to monkey tropic HIV-1
	DK to NE in CypA	Loss of anti-HIV-1 activity

[a]Inconsistent with the in-vitro observations.

position 119 of HIV-2 CA. In contrast, HIV-2 strains possess a mixture of Q, A, P and G at the corresponding position. The 119th or 120th position is located in the loop between α-helices 6 and 7 (L6/7). Previously, a single amino acid substitution at the 110th position of N-MLV CA has been shown to determine viral susceptibility to mouse restriction factor, Fv1 [101]. The 3-D structure of MLV CA [102,103] revealed that the 110th position of N-MLV CA is located at a position in the surface-exposed loop analogous to the 119th or 120th position of HIV-2 CA.

HIV-1 and HIV-2 infections have distinct natural histories, levels of viremia, transmission rates and disease associations despite high levels of sequence homology between the two viruses [104]. Although some HIV-2-infected patients progress to AIDS as rapidly as HIV-1-infected patients, virus replication is controlled in the majority of HIV-2 patients [105,106] and those with low viral load achieve much longer survival than those with high viral load [107]. Detailed sequence analysis of HIV-2 CA variations within a large community cohort in Guinea-Bissau composed of both high and low viral load patients indicated that CA from viruses in low viral load patients had P residues at position 119, but in patients with higher viral load, position 119 was frequently occupied by Q, A or G residues. Stratification of the individuals according to the presence or absence of P at position 119 showed a three-fold difference in the median viral load of the two groups. These results indicate that HIV-2 replication in infected individuals can be linked to CA variation and human TRIM5α sensitivity [108].

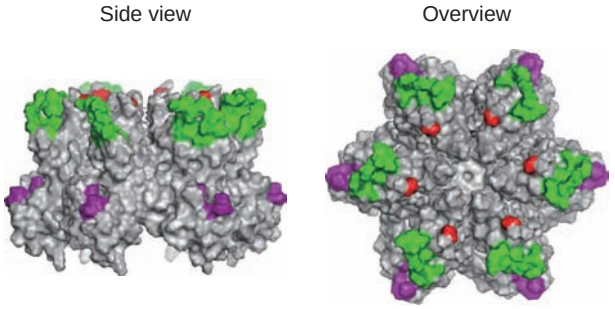

Fig. 6. Structure models of the HIV-2 GH123 CA hexamer. The space-filling model of CA hexamer from the side and the top is shown. Positions of HIV-2 CRF01_AB-specific amino acid substitutions, which are required for strong resistance against human TRIM5α, are shown in purple. Loops between helices 4 and 5 and position 120 are shown in green and red, respectively.

In addition, Lelignowicz *et al.* [109] reported that HLA-B*3501 was associated with HIV-2 with P at position 119 in the same community cohort as described above. The cytotoxic T-cell NY9-epitope (NPVPVGNIY) was located two amino acids downstream of position 119. It is thus possible that viruses were forced to change Q (coded as CAA or CAG) to P (C<u>C</u>A or C<u>C</u>G; underlines denote single nucleotide changes) at position 119 to escape from HLA-B*3501 specific immune responses, even though this substitution caused the virus to become more sensitive to human TRIM5α. After transmission to individuals lacking HLA-B*3501, viruses may have evolved from a P to an A (<u>G</u>CA or <u>G</u>CG) at position 119 to revert to being resistant to human TRIM5α.

Moreover, several patients with HIV-2 who had a high viral load and rapidly developed AIDS were identified in Japan. Sequence analysis of viruses isolated from these patients indicated that they carried G at position 119. These patients were infected with an A/B inter-group recombinant designated CRF01_AB [110]. Notably, HIV-2 CRF01_AB CA showed potent resistance to human TRIM5α. The nature of the genetic code suggests that the G virus (G<u>G</u>A or G<u>G</u>G) was derived from the A virus (G<u>C</u>A or G<u>C</u>G), implying that the viruses with G are highly adapted. The emergence of a possible highly pathogenic HIV-2 strain is an ongoing concern, given that retroviruses can easily evolve to evade host defenses. In addition to the previously identified role of amino acid 119 of the CA N-terminal domain, CRF01_AB-specific amino acid substitutions in the CA C-terminal domain (CTD) were also necessary for strong resistance to human TRIM5α [111]. It is interesting to note that this region of the CTD overlaps with the region that affects partial

resistance to another anti-HIV-1 host factor MxB [112]. These amino acid substitutions in the CA CTD may be exposed to and accessible from the outside of the viral core (Fig. 6).

Conclusion

The case of the 'Berlin patient' who was functionally cured of HIV-1 infection by receiving a haematopoietic stem cell transplant from a homozygote of CCR5 delta 32 allele presented an attractive strategy for curing HIV infection. Gene therapy including genome editing of the *CCR5* gene in $CD4^+$ T cells or haematopoietic stem cells to create HIV-1 resistant cells have both been tried. Although human TRIM5α does not block HIV-1 infection, it is possible that restriction can be acquired by modifying the human *TRIM5* gene through mutations in the V1 region or insertion of a CypA gene as found in monkeys. As described above, a study comparing human and Rh TRIM5α showed that a single change from R to P at position 332 of human TRIM5α (R332P) conferred potent restriction ability against not only HIV-1 but also SIVmac239 [37,38]. However, further studies are necessary to examine the feasibility of human TRIM5α manipulation in achieving a cure for HIV-1 infection.

Acknowledgements

We sincerely thank our collaborators: C. Onyango, M. Cotton, S. Rowland-Jones (Medical Research Council Laboratories, Gambia), K. Bozek, F.S. Domingues (Max Plank Institute for Informatics, Germany), M. Yokoyama, H. Sato (National Institute of Infectious Diseases, Japan), A. Saito, H. Akari (National Institute of Biomedical Innovation, Japan), A. Adachi, M. Nomaguchi (Tokushima University, Japan), T. Nakajima, A. Kimura (Tokyo Medical and Dental University, Japan), I. Theodorou, P. Debre (Hôpital Pitié Salpetrière, France), K. Yoshimura, S. Matsushita (Kumamoto University, Japan), A. Iwamoto (The University of Tokyo, Japan) and all members of our laboratory.

Conflicts of interest

There are no conflicts of interest.

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