Impact of TRIM5α in vivo

Emi E. Nakayama and Tatsuo Shioda

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 (TRIM5α) 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.

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, APOBEC3G, 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
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 leukemia 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 SIVagm.

---

**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.
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.
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 TRIM5α 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...
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 TRIM5α gene. This SNP is located in the RING domain and greatly reduces the ability of human TRIM5α to inhibit N-MLV infection [79]. 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
AIDS 2015, Vol 29 No 14

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 322 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 mangabe 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
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].

In addition, Lelignowicz et al. [109] reported that HLA-B/C3501 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 (CCA or CCG; underlines denote single nucleotide changes) at position 119 to escape from HLA-B/C3501 specific immune responses, even though this substitution caused the virus to become more sensitive to human TRIM5α. After transmission to individuals lacking HLA-B/C3501, viruses may have evolved from a P to an A (GCA or GCG) 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 (GGA or GGG) was derived from the A virus (GCA or GCC), 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

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

*aInconsistent with the in-vitro observations.*
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 Salpêtrière, 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.

References

1. Harris RS, Bishop KN, Sheehy AM, Craig HM, Petersen-Mahrt SK, Watt IN, et al. DNA deamination mediates innate immunity to retroviral infection. Cell 2003; 113:803–809.
2. Mangenot B, Turelli P, Caron G, Friedl M, Perrin L, Trono D. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature 2003; 424:99–103.
3. Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature 2002; 418:646–650.
4. Mariani R, Chen D, Schrofelbauer B, Navarro F, Konig R, Bollman B, et al. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 2003; 114:21–31.
5. Marin M, Rose KM, Koizak SL, Kabat D. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat Med 2003; 9:1396–1403.
6. Sheehy AM, Gaddis NC, Malim MH. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat Med 2003; 9:1404–1407.
7. Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature 2008; 451:425–430.
8. Van Damme N, Goff D, Katsura C, Jorgenson RL, Mitchell R, Johnson MC, et al. The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. Cell Host Microbe 2008; 3:245–252.
9. Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Segeral E, et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature 2011; 474:654–657.
10. Goldstone DC, Ennis-Adeniran V, Heddle J, Groom HC, Rice GI, Christodoulou E, et al. HIV-1 restriction factor SAMHD1 is a deoxynucleoside triphosphate triphosphohydrolase. Nature 2011; 480:379–382.
11. Gao F, Bailes E, Robertson DL, Chen Y, Rodenburg CM, Michael SF, et al. Origin of HIV-1 in the chimpanzee Pan troglodytes troglodytes. Nature 1999; 397:436–441.
12. Shibata R, Sakai H, Kawamura M, Tokunaga K, Adachi A. Early replication block of human immunodeficiency virus type 1 in monkey cells. J Gen Virol 1995; 76 (Pt 11):2723–2730.
13. Himathongkham S, Luciw PA. Restriction of HIV-1 (subtype B) replication at the entry step in rhesus macaque cells. Virology 1996; 219:485–488.
14. Akari H, Momi K, Terao K, Otani I, Fukazawa M, Mukai R, et al. In vitro immortalization of Old World monkey T lymphocytes with Herpesvirus saimiri: its susceptibility to infection with simian immunodeficiency viruses. Virology 1996; 218:382–388.
15. Akari H, Nam KH, Momi K, Otani I, Shibata H, Adachi A, et al. Effects of SIVmac infection on peripheral blood CD4+CD8+ T lymphocytes in cynomolgus macaques. Clin Immunol 1999; 91:321–329.
16. VandeWoude S, Apetrei C. Going wild: lessons from naturally occurring T-lymphotropic lentiviruses. Clin Microbiol Rev 2006; 19:728–762.
17. Chackerian B, Long EM, Luciw PA, Overbaugh J. Human immunodeficiency virus type 1 coreceptors participate in postentry stages in the virus replication cycle and function in simian immunodeficiency virus infection. J Virol 1997; 71:3932–3939.
18. Stremlau M, Owens CM, Perrin MJ, Kiessling M, Autissier P, Bessia C, Segeral E, et al. The tripartite motif family identifies cell compartments. Clin Immunol 2003; 127:148–159.
19. Nakayama EE, Miyoshi H, Nagai Y, Shioda T, Akari H, Nam KH, et al. Effect of SIVmac infection on peripheral blood CD4+CD8+ T lymphocytes in cynomolgus macaques. Clin Immunol 1999; 91:321–329.
20. VandeWoude S, Apetrei C. Going wild: lessons from naturally occurring T-lymphotropic lentiviruses. Clin Microbiol Rev 2006; 19:728–762.
21. Jackson PK, Eldridge AC, Freed E, Funstonthal L, Hsu YJ, Kaiser BK, et al. The lore of the RINGs: substrate recognition and catalysis by ubiquitin ligases. Trends Cell Biol 2000; 10:429–439.
22. Stremlau M, Perron M, Lee M, Li Y, Song B, Javanbakht H, et al. Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. Proc Natl Acad Sci U S A 2006; 103:5514–5519.
A dominant-negative effect of cytomolgous monkey tripartite motif protein TRIM5alpha on primate immunodeficiency virus restricts primate virus restrictive TRIM5alpha protein. PLoS Pathog 2010; 6:e1000357.

34. Berthoux L, Sebastian S, Sayah DM, Luban J. TRIM5alpha modulates restriction to HIV-1 in rhesus macaques. PLoS Pathog 2010; 6:e1000738.

35. Daniel MD, Letvin NL, King NW, Kannagi M, Sehgal PK, Hunt RD, et al. Isolation of T-cell tropic HTLV-III-like retroviruses from macaques. Science 1985; 228:1201–1204.

36. Reynolds MR, Sacha JB, Weiler AM, Borchardt GJ, Gildden CE, Sheppard NC, et al. The TRIM5[alpha] genotype of rhesus macaques affects acquisition of simian immunodeficiency virus SIVmac660 infection after repeated limiting-dose intrarectal challenge. J Virol 2011; 85:9637–9640.

37. Yeh WW, Rao SS, Lim SY, Zhang J, Hraber PT, Brassard LM, et al. The TRIM5 gene promotes cross-species transmission of simian immunodeficiency virus in rhesus monkeys. J Virol 2011; 85:10389–10398.

38. Lim SY, Tran C, Gelman RS, Whitney JB, O’Brien KL, Barron DH, et al. Contributions of Mamu-A*001 status and TRIM5 allele expression, but not CCL3L1 copy number variation, to the control of SIVmac251 replication in Indian-origin rhesus monkeys. PLoS Genet 2010; 6:e1000997.

39. Fenizia C, Keele BF, Nichols D, Comarna S, Binello N, Vaccari M, et al. TRIM5alpha does not affect simian immunodeficiency virus SIV(mac251) replication in vaccinated or unvaccinated Indian rhesus macaques following intrarectal challenge exposure. J Virol 2011; 85:12399–12409.

40. Nisole S, Lynch C, Stoye JP, Yap MW. A Trim5-cyclophilin A fusion protein found in owl monkey kidney cells can restrict HIV-1. Proc Natl Acad Sci U S A 2004; 101:13324–13328.

41. Sayah DM, Sokolokajka E, Berthoux L, Luban J. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. Nature 2008; 456:567–571.

42. Brennen G, Kozzey Y, Hu SL. TRIMCyp expression in Old World primates Macaca nemestrina and Macaca fascicularis. Proc Natl Acad Sci U S A 2008; 105:3563–3568.

43. Newman RM, Hall LR, Morgan JS, O’Connor S, et al. TRIM5 suppresses cross-species transmission of a primate immunodeficiency virus and selects for emergence of resistant variants in the new species. PLoS Biol 2010; 8:e1000462.

44. Lim SY, Rogers T, Chan T, Whitney JB, Kim J, Sodroski J, et al. TRIM5alpha of Japanese macaques drives SIVsmm evolution in rhesus macaques. PLoS Pathog 2013; 9:e1003577.

45. Wu F, Kimera A, Goeken R, Ourmanov I, Hall L, Morgan JS, et al. TRIM5alpha drives SIVsmm evolution in rhesus macaques. PLoS Pathog 2013; 9:e1003577.

46. Daniel MD, Letvin NL, King NW, Kannagi M, Sehgal PK, Hunt RD, et al. Isolation of T-cell tropic HTLV-III-like retroviruses from macaques. Science 1985; 228:1201–1204.

47. Hirsch V, Adger-Johnson D, Campbell B, Goldstein S, Brown C, Elkins WR, et al. A molecule cloned, pathogenic, neutralization-resistant rhesus macaque SIVmac, J Virol 1997; 71:1608–1620.

48. Wu F, Kimera A, Goeken R, Ourmanov I, Hall L, Morgan JS, et al. TRIM5alpha drives SIVsmm evolution in rhesus macaques. PLoS Pathog 2013; 9:e1003577.

49. Saito A, Kono K, Nomaguchi M, Yasutomi Y, Adachi A, Shioda T, et al. Geographic, genetic and functional diversity of antiretroviral host factor TRIM5alpha in cynomolgus macaques (Macaca fascicularis). J Gen Virol 2012; 93:594–602.

Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.
66. Hatziioannou T, Ambrose Z, Chung NP, Piatak M Jr, Yuan F, Trubey CM, et al. A macaque model of HIV-1 infection. Proc Natl Acad Sci U S A 2009; 106:4425–4429.

67. Hatziioannou T, Del Prete GQ, Keele BF, Estes JD, McNatt MW, Bitzegeio J, et al. HIV-1-induced AIDS in monkeys. Science 2014; 344:1401–1405.

68. Saito A, Nomaguchi M, Kono K, Iwatani Y, Yokoyama M, Nomaguchi M, Kono K, Iwatani Y, Yokoyama M, et al. Generation of rhesus macaque-tropic HIV-1 by restriction factor TRIM5alpha with human immunodeficiency virus type 1. J Gen Virol 2013; 94:1318–1324.

69. Nomaguchi M, Yokoyama M, Kono K, Nakayama EE, Shioda T, Saito A, et al. Gag-CA Q110D mutation elicits TRIMs-independent enhancement of HIV-1 replication in macaque cells. Microbes Infect 2013; 15:56–65.

70. Kono K, Song H, Yokoyama M, Sato H, Shioda T, Nakayama EE. Multiple sites in the N-terminal half of simian immunodeficiency virus Gag protein contribute to evasion from rhesus monkey TRIM5alpha-mediated restriction. Retrovirology 2010; 7:22.

71. Ylen LM, Keckesova Z, Wilson SJ, Ranasinghe S, Towers GI. Differential restriction of human immunodeficiency virus type 2 and simian immunodeficiency virus SIVmac by TRIM5alpha alleles. J Virol 2005; 79:11580–11587.

72. Lin TY, Emerman M. Determinants of cyclopillin A-dependent TRIM5 alpha restriction against HIV-1. Virology 2008; 379:335–341.

73. Pacheco B, Finzi A, Stremlau M, Sodroski J. Adaptation of HIV-1 to cells expressing rhesus monkey TRIM5alpha. Virology 2010; 408:204–212.

74. Ohkura S, Goldstone DC, Yap MW, Holdend-Yone K, Taylor IA, Stoye JP. Novel escape mutants suggest an extensive TRIM5alpha binding site spanning the entire outer surface of the murine leukemia virus capsid protein. PLoS Pathog 2011; 7:e1002011.

75. Hatziioannou T, Princiotta M, Piatak M Jr, Yuan F, Zhang F, Li J, et al. Generation of simian-tropic HIV-1 by restriction factor evasion. Science 2006; 314:95.

76. Nomaguchi M, Yokoyama M, Kono K, Nakayama EE, Shioda T, Doi N, et al. Generation of rhesus macaque-tropic HIV-1 clones that are resistant to major anti-HIV-1 restriction factors. J Virol 2013; 87:11447–11461.

77. Soll SJ, Wilson SJ, Kutfley SB, Hatziioannou T, Bieniaaz PD. Assisted evolution enables HIV-1 to overcome a high TRIM5alpha-imposed genetic barrier to rhesus macaque tropism. PLoS Pathog 2013; 9:e1003667.

78. Nomaguchi M, Nakayama EE, Yokoyama M, Doi N, Igarashi T, Shioda T, et al. Distinct combinations of amino acid substitutions in N-terminal domain of Gag-capsid afford HIV-1 resistance to rhesus TRIM5alpha. Microbes Infect 2014; 16:936–944.

79. Sawyer SL, Wu LL, Almy JM, Emerman M, Malik HS. High-frequency persistence of an impaired allele of the retroviral defense gene TRIM5alpha in humans. Curr Biol 2006; 16:95–100.

80. Javanbakht H, An P, Gold B, Petersen DC, O’Huigin C, Nelson GW, et al. Effects of human TRIM5alpha polymorphisms on antiretroviral function and susceptibility to human immunodeficiency virus infection. Virology 2006; 354:15–27.

81. Nakayama EE, Carpenter W, Costagliola D, Shioda T, Iwashimizu A, Debro P, et al. Wild-type and human 143Y variant of human TRIM5alpha show similar anti-human immunodeficiency virus type 1 activity both in vivo and in vitro. Immunogenetics 2007; 59:511–515.

82. Speelman EC, Livingston-Rosanoff D, Li SS, Vu Q, Bui J, Geraughty DR, et al. Genetic association of the antiviral restriction factor TRIM5alpha with human immunodeficiency virus type 1 infection. J Virol 2006; 80:2463–2471.

83. van Manen D, Rits MA, Beugeling C, van Dort K, Schuitmaker H, Kootstra NA. The contribution of rare human TRIM5alpha polymorphisms to the clinical course of HIV-1 infection. PLoS Pathog 2008; 4:e18.

84. Nakajima T, Nakayama EE, Kaur G, Terunuma H, Mimaya JI, Ohtani H, et al. Impact of novel TRIM5alpha variants, G1710A and G115C, on the anti-HIV-1 activity and the susceptibility to HIV-1 infection. AIDS 2009; 23:2091–2100.

85. Liu Fei, Qiu YQ, Li H, Kuang YQ, Tang X, Cao G, et al. An HIV-1 resistance polymorphism in TRIM5alpha gene among Chinese intravenous drug users. J Acquir Immune Defic Syndr 2011; 56:306–311.

86. Pertel T, Hausmann S, Morger D, Zuger S, Guerra J, Lascano J, et al. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. Nature 2011; 472:361–365.

87. Ovsyannikova IG, Dhimot H, Haralambieva IH, Vierkant RA, O’Byrne MM, Jacobson RM, et al. Rubella vaccine-induced cellular immunity: evidence of associations with polymorphisms in the Toll-like, vitamin A and D receptors, and innate immune response genes. Hum Genet 2010; 127:210–221.

88. Price H, Lapac P, Tuff J, Wachbn C, Kimani J, Ball TB, et al. A TRIM5alpha exon 2 polymorphism is associated with protection from HIV-1 infection in the Pumwani sex worker cohort. AIDS 2010; 24:1813–1821.

89. Nine H, Glaude D, Snocoe J, Martinez R, McLaren PJ, Ortiz M, et al. Susceptibility and adaptation to human TRIM5alpha alleles at positive selected sites in HIV-1 capsid. Virology 2013; 441:162–170.

90. Nakayama EE, Nakajima T, Kaur G, Mimaya JI, Terunuma H, Mehra N, et al. A naturally occurring single amino acid substitution in human TRIM5alpha linker region affects its anti-HIV-1 type 1 activity and susceptibility to HIV type 1 infection. AIDS Res Hum Retroviruses 2013; 29:819–824.

91. Harada S, Koyangi N, Yamamoto N. Infection of HTLV-I/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. Science 1985; 229:363–366.

92. Torimiro JN, Javanbakht H, Diaz-Griffero F, Kim J, Carr JK, Carrington M, et al. A rare null allele potentially encoding a dominant-negative TRIM5alpha protein in Baboon pygmys. Virology 2009; 391:140–147.

93. Castro BA, Barrett SW, Evans LA, Moreau J, Derouet K, Levy JA. Persistent infection of baboons and rhesus monkeys with different strains of HIV-2. Virology 1991; 184:219–226.

94. Fujita M, Yoshida A, Sakurai A, Tatsuji K, Ueno F, Akan H, et al. Susceptibility of HIV-2-immortalized lymphocytic HSC-C cells to various strains and mutants of HIV/SIV. Int J Mol Med 2003; 11:641–644.

95. Castro BA, Barnett SW, Evans LA, Moreau J, Derouet K, Levy JA. Biologic heterogeneity of human immunodeficiency virus type 2 (HIV-2) strains. Virology 1990; 178:527–534.

96. Locher CP, Witt SA, Herndier BG, Abbey NW, Tenner-Racz K, Racz P, et al. Increased virus replication and virulence after serial passage of human immunodeficiency virus type 2 in baboons. J Virol 2003; 77:77–83.

97. Locher CP, Blackbourn DJ, Herndier BG, Reyes-Teran G, Barnett SW, Murphy KK, et al. Transient virus infection and pathogenesis of a new HIV type 2 isolate, UC12, in baboons. AIDS Res Hum Retroviruses 1998; 14:79–82.

98. Song H, Nakayama EE, Yokoyama M, Sato H, Levy JA, Shioda T. A single amino acid of the human immunodeficiency virus type 2 capsid affects its replication in the presence of cyclo-molgus monkey and human TRIM5alphas. J Virol 2007; 81:7280–7285.

99. Miyamoto T, Yokoyama M, Kono K, Shioda T, Sato H, Nakayama EE. A single amino acid of human immunodeficiency virus type 2 capsid protein affects conformation of two external loops and viral sensitivity to TRIM5alpha. PLoS One 2011; 6:e22779.

100. Hahn BH, Shaw GM, De Cock KM, Sharp PM. AIDS as a zoonosis: scientific and public health implications. Science 2000; 287:607–611.

101. Kozak CA, Chakraborti A. Single amino acid changes in the murine leukemia virus capsid protein gene define the target of Fv1 resistance. Virology 1996; 225:300–305.

102. Mortuza GB, Doddling MP, Goldstone DC, Haire LF, Stoye JP, Taylor JA. Structure of B-MuLV Gag capsid antigenic epitodal domain reveals key features of viral tropism, gag assembly and core formation. J Mol Biol 2008; 376:1493–1508.
103. Mortuza G, Haire LF, Stevens A, Smerdon SJ, Stoye JP, Taylor IA. High-resolution structure of a retroviral capsid hexameric amino-terminal domain. *Nature* 2004; 431:481–485.

104. Rowland-Jones SL, Whittle HC. Out of Africa: what can we learn from HIV-2 about protective immunity to HIV-1? *Nat Immunol* 2007; 8:329–331.

105. Poulsen AG, Kvinesdal B, Aaby P, Mølbak K, Frederiksen K, Dias F, et al. Prevalence of and mortality from human immunodeficiency virus type 2 in Bissau, West Africa. *Lancet* 1989; 1:827–831.

106. Berry N, Ariyoshi K, Balle P, Tedder R, Whittle H. Sequence specificity of the human immunodeficiency virus type 2 (hiv-2) long terminal repeat u3 region in vivo allows subtyping of the principal hiv-2 viral subtypes a and b. *AIDS Res Hum Retroviruses* 2001; 17:263–267.

107. Ariyoshi K, Jaffar S, Alabi AS, Berry N, Schim van der Loef M, Sabally S, et al. Plasma RNA viral load predicts the rate of CD4 T cell decline and death in HIV-2-infected patients in West Africa. *AIDS* 2000; 14:339–344.

108. Onyango CO, Leligdowicz A, Yokoyama M, Sato H, Song H, Nakayama EE, et al. HIV-2 capsids distinguish high and low virus load patients in a West African community cohort. *Vaccine* 2010; 28S2:860–867.

109. Leligdowicz A, Onyango C, Yindom LM, Peng Y, Cotten M, Jaye A, et al. Highly avid, oligoclonal, early-differentiated antigen-specific CD8+ T cells in chronic HIV-2 infection. *Eur J Immunol* 2010; 40:1963–1972.

110. Ibe S, Yokomaku Y, Shiino T, Tanaka R, Hattori J, Fujisaki S, et al. HIV-2 CRF01_AB: first circulating recombinant form of HIV-2. *J Acquir Immune Defic Syndr* 2010; 54:241–247.

111. Miyamoto T, Nakayama EE, Yokoyama M, Ibe S, Takehara S, Kono K, et al. The carboxyl-terminus of human immunodeficiency virus type 2 circulating recombinant form 01 AB capsid protein affects sensitivity to human TRIM5alpha. *PLoS One* 2012; 7:e47757.

112. Busnadiego I, Kane M, Rihn SJ, Preugschas HF, Hughes J, Blanco-Melo D, et al. Host and viral determinants of Mx2 antiretroviral activity. *J Virol* 2014; 88:7738–7752.