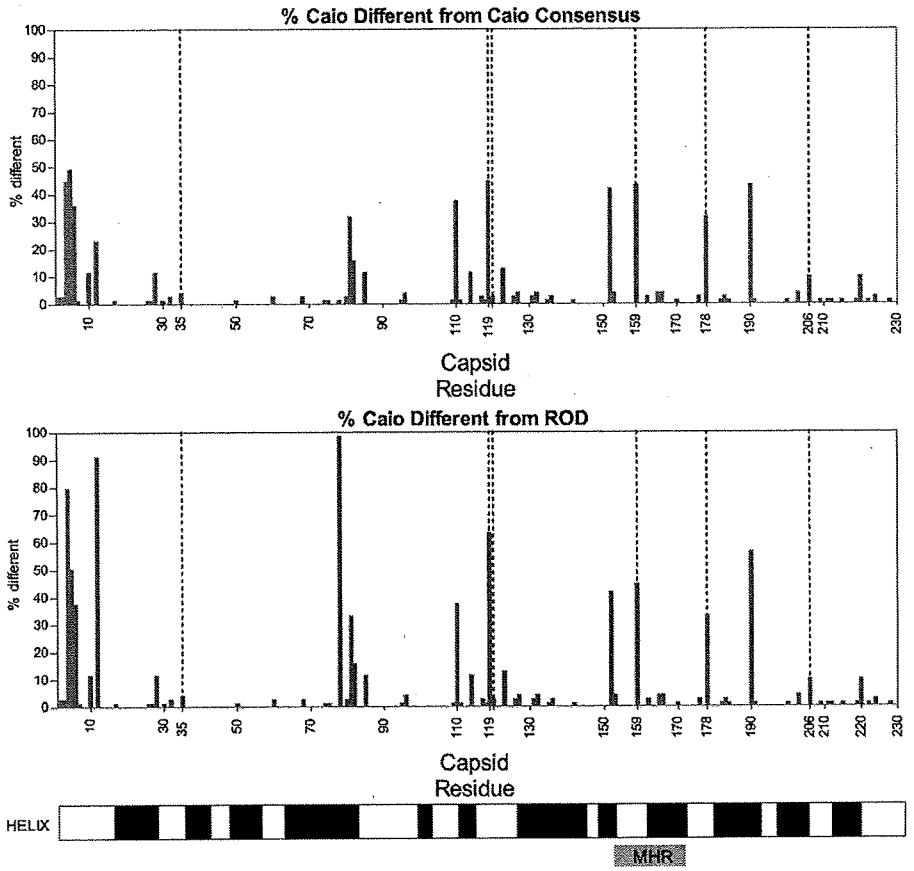

Fig. 1. Amino acid polymorphisms in the Caio HIV-2 p26. Polymorphisms in the p26 coding sequence are displayed as the percentage of the 69 sequences that differ at each position from either the Caio p26 consensus sequence (determined as the majority amino acid at each position, upper panel) or from the HIV-2_{ROD} p26 sequence (middle panel). Conserved alpha helices (in black) and the Major Homology Region (MHR) are indicated in the lower panel. Sites of variation that were associated with VL are indicated with dotted lines (Mann–Whitney test) ($p \leq 0.05$). [NB $p = 0.07$ for P178].

non-proine residues (Table 1). Three of the significantly varying positions (35, 120 and 206) were too infrequent for further study.

3.2. p26 CA variation correlating with VL

The association of proline 119, 159 and 178 with reduced VL becomes apparent when the log-transformed VL for each sample is plotted as a function of the total number of prolines at these three sites Fig. 2A. A Tobit regression analysis showed a clear relationship of increased VL with decreasing prolines in these three positions ($p = 0.003$).

To examine proline variation in more detail, CA types were grouped according the residue at each of the three positions using the code P (proline), N (not proline), or the wild card * (any amino acid) and ordered by increasing VL (Fig. 2B). Among the progression of median viral loads, there was a pattern of decreasing prolines, starting with the PPP group, through the intermediate forms to the complete non-proline NNN group (Fig. 2B). The two exceptions to this trend (the single proline NPN group had a lower median VL than the PNP and NPP groups) indicated that there may be interactions between specific combinations of these prolines in their effects on viral load but the number of examples was too small to demonstrate such effects statistically.

Considering the effect of single proline changes, proline 119 (P119) CAs (the P group) were isolated more frequently from patients with low VL with a 4.9-fold difference in the median VL of P** group compared to the N** group ($p = 0.0205$; Fig. 2B).

Proline 159 (P159) had a stronger effect on VL with at least 6.1-fold difference in the median VL of the *P* group compared to the *N* group ($p = 0.0075$; Fig. 2B). This site is of special interest being within the highly conserved MHR (Fig. 1) [20], essential for virion assembly.

Proline 178 (P178) showed modest variation with VL with only 3.5-fold difference in the median VL of the **P group compared to the **N group ($p = 0.0709$; Fig. 2B,). However, the presence of P178 was linked to the other two prolines: all CAs with both P119 and P159 had P178 (i.e. the PPP group = the PP* group, Fig. 2B). This may be due to a p26 folding requirement and/or genetic linkage.

Stronger associations were observed when the positions were analyzed in combination. The median VL in subjects with PP* viruses differed from NN* viruses by at least 13.6-fold ($p = 0.0028$, Fig. 2B). Importantly, median VL in subjects with viruses having all three prolines (PPP) compared to those lacking a proline at the sites (NNN) differed by at least 18.8-fold ($p = 0.0013$, Fig. 2B).

There are practical difficulties of sequencing circulating RNA genomes, especially from patients with <100 copies viral RNA per ml of plasma. Sixteen of the 69 sequences were derived from proviral DNA (listed as DNA in Table 1) because multiple attempts to obtain RNA sequence failed. To test if proline/VL associations were biased by the inclusion of these proviral sequences, the analysis was repeated after excluding the data from these 16 samples. The association of higher VL with non-proline residues remained significant at positions 119 ($p = 0.0123$) and 159 ($p = 0.0043$), and for the combined positions 119 + 159 ($p = 0.0012$) and 119 + 159 + 178

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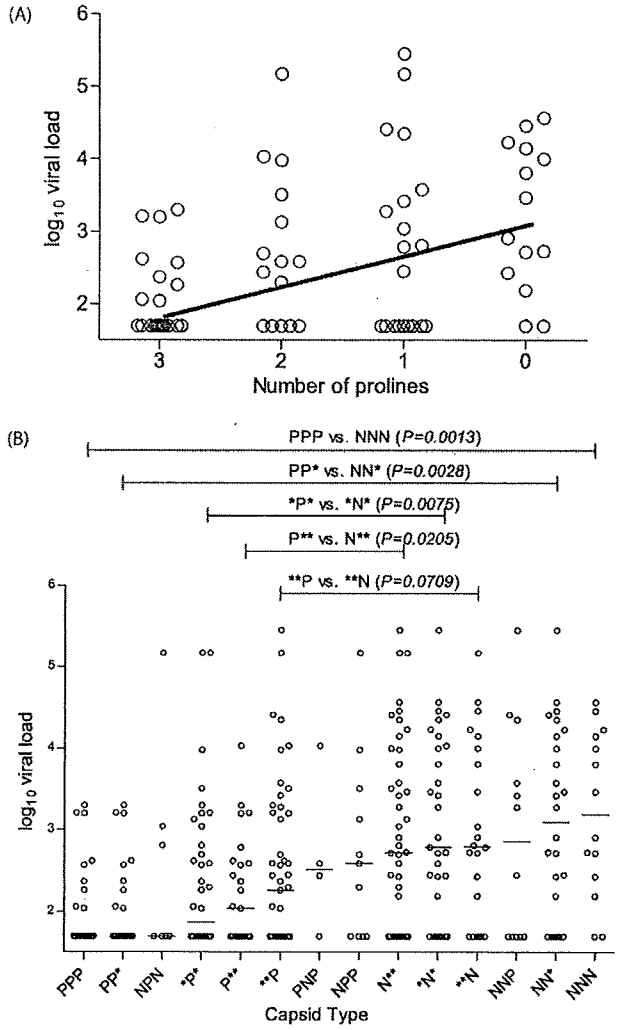

Fig. 2. HIV-2 VL correlates with amino acid variation at three p26 sites. (A) Relationship between VL and the number of prolines. Log-transformed VL for each sample was plotted as a function of the total number of prolines at p26 positions 119, 159 and 178. Tobit regression (Stata10, StatCorp TX, USA) was used to investigate the relationship between VL and the number of prolines at positions 119, 159 and 178. This form of regression is able to allow for censoring of viral loads below 100 in the dataset. The regression line was drawn with the equation $\log_{10}VL = 3.15 - 0.46 \times$ No. of prolines. (B) Relationship between VL and type of PPP motif. Log-transformed VL for each sample is plotted as a function of amino acid variation at p26 position 119, 159 and 178. Median VL are indicated by horizontal bars. The patients were stratified by the presence of proline (P) no proline residue (N) or any amino acid (*) at each of the positions 119, 159 and 178. For example, PPP = proline resides at positions 119, 159 and 178, NNN = no proline at the three positions. Comparisons of plasma VLs for different amino acid polymorphisms of particular interest were made using the non-parametric Mann–Whitney test (GraphPad Prism 5). *p*-values for these are shown above the figure.

($p = 0.0024$) (Table S1, lower panel). We conclude that independent of the origin of the sequences, there exists an association between low VL and proline residues at positions 119 and 159 independently, and with 119, 159 and 178 combined.

3.3. p26 CA variation influences susceptibility to TRIM5α

The TRIM5α was identified as a limit to cross-species retroviral infection [32–35]. This has led to a model of TRIM5α blocking retroviral infection by binding to the CA during entry, accelerating virus uncoating, and limiting subsequent steps in the infection process [32,34,36,37]. P119 was recently identified as a determinant of TRIM5α restriction [23] with HIV-2 CAs derived from TRIM5α sensitive viruses bearing a proline, and resistant strains having an alanine or glutamine at this site. It is possible that the reduced replication of the PPP viruses we observed in patients was part of the same phenomenon. Accordingly, the contribution of all three proline residues to TRIM5α restriction of replication was directly examined *in vitro*. Starting with the HIV-2 strain GH-123 as a PPP virus, P119, P159 plus P178, or all three prolines were altered to alanine or serine using site-directed mutagenesis. The growth of these variant viruses was compared in cells modified to express human TRIM5α; a parallel cell line expressing TRIM5α missing the SPRY domain, essential for p26 interaction, was used as a control to determine if these p26 changes altered virus replication independent of TRIM5α function [23]. Altering P119 (to produce APP) or P159 + P178 (to produce PSA) allowed 3-fold greater virus replication Fig. 3A. Alteration of all three prolines to ASA resulted in a 6-fold increase in virus replication. Thus the GH-123 variants displayed replication *in vitro* that closely mimic the behavior of the HIV-2 variants *in vivo*.

3.4. Phylogenetic analysis of p26 CA variation

One possible origin of the PPP form of p26 is that such a CA was encoded by a founder variant of HIV-2. The reduced growth of PPP viruses could be due to the p26 itself or to other shared and co-evolved features in these viruses. Alternately, changes at these three codons could occur more frequently. A phylogenetic analysis of Caio HIV-2 was performed to distinguish the PPP founder virus model from a multiple occurrence model. A founder effect with the appearance and spread of a PPP virus would appear as phylogenetic clustering of these variants. Ongoing selection for or against prolines in p26 would result in a phylogeny lacking PPP clustering. Because results derived from the 960 bp containing p26 coding could be dominated by variation in codons 119, 159 and 178, phylogeny was also inferred from a larger sequence spanning approximately 1300 bp of the envelope gene and including the highly variable V3 and V4 loops. The inferred phylogenies show that HIV-2 isolates encoding PPP p26 are distributed throughout the p26 and envelope trees (Fig. S1), supporting the conclusion that the occurrence of PPP p26 is not associated with a specific phylogenetic branch of Caio HIV-2 and is unlikely to be associated with a single occurrence of this CA motif. This conclusion is also supported by the high bootstrap values for some of the branches. These results are consistent with selection for and multiple appearance of the PPP p26 in the Caio population.

3.5. Modelling p26 CA sequence variation on structure and dimer formation

The bulky and constrained structure of proline strongly influences protein secondary structure; proline is inimical to alpha-helices and can kink otherwise flexible loops [38]. Thus polymorphisms involving prolines residues could alter the p26 structure. Using homology modelling, three-dimensional structures of six of the Caio HIV-2 p26 molecules (2 PPP, 2 ASA and 2 intermediate forms, APP and ASP) were constructed. The thermodynamically optimized 3-D structure models showed that the HIV-2 p26 consists of two packed core structures of N-terminal and C-terminal domains, a similar conformation to HIV-1 p24 [39]. Superimposition of the six HIV-2 p26 models showed that the overall 3-D structures of the variants were very similar with an exception: the amino acid substitution at position 119 from proline to alanine induced marked changes in the configuration of the loop between helices 6 and 7, as found previously with HIV-2 p26 N-terminal domain model [23]. In contrast, the substitution

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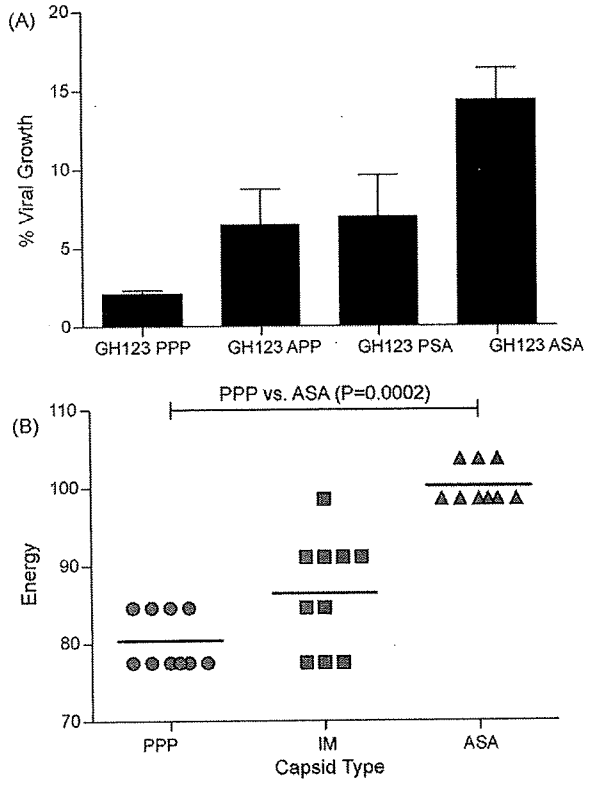

Fig. 3. HIV-2 capsid changes alter TRIM5α susceptibility and capsid stability. (A) Growth of HIV-2 GH123 (PPP) and its mutant viruses HIV-2 GH123/119A (APP), HIV-2 GH123/159S-178A (PSA), and HIV-2 GH123/119A-159S-178A (ASA) in the presence of human TRIM5α. MT4 cells (10^5) were infected with Hu-TRIM5α-SeV or CM-SPRY(-)-SeV at a multiplicity of infection of 10 plaque forming units per cell. 9 h after infection, the cells were superinfected with 20 ng of p26 of HIV-2 GH123, HIV-2 GH123/119A, HIV-2 GH123/159S-178A, or HIV-2 GH123/119A-159S-178A viruses. The culture supernatants were collected 6 days after infection, and the level of p26 was measured by using a RETROtek antigen ELISA kit (ZeptoMetrix Corp., Buffalo, NY). Ratios of HIV-2 CA levels of Hu-TRIM5α-SeV-infected cells to those of CM-SPRY(-)-SeV-infected cells are shown as percent growth. Error bars denote standard deviations in quadruplicate samples. A Kruskal–Wallis test for the entire data set clearly detected the difference of viral growth among those four viruses ($p = 0.006$). Furthermore, comparison of each mutant virus with GH123(PPP) using the Dunnett test also showed statistically significant differences of PPP vs. APP $p < 0.05$, PPP vs. PSA $p < 0.05$, and PPP vs. ASA $p < 0.01$. (B) Dimer binding energies as a function of capsid type. Samples were grouped into PPP, IM (intermediate, with proline at one or two of the three sites) or ASA, based on the amino acid at position 119, 159 and 178. The capsid dimer binding energies (absolute value) for each sequence were determined by homology modelling (see Section 2); the mean values for each group are indicated. Comparison between the PPP and the ASA group using the non-parametric Mann–Whitney test (GraphPad Prism 5) provided the indicated p-value.

at positions 159 or 178 induced no major changes in the main-chain backbone of HIV-2 CA (data not shown), suggesting that the structure of the CA can accommodate alternative residues at these sites.

Positions 159 and 178 are located in the C-terminal portion of p26 required for dimer formation as well as virion shell assembly [18]. Homology modelling of the p26 dimer was used to calculate binding energy for dimer formation. Since the C-terminal sequences used to prepare the initial six structures were common to a larger set of p26 sequences, dimer binding energies for 29 of the Caio CAs could be calculated. These calculations revealed that the PPP p26 dimers had weaker binding energies, the ASA dimers had stronger binding energies and the intermediate forms with only one or two of the prolines altered (IM) had intermediate binding energies (Fig. 3B). In addition, the viral loads of the 29 patients were modestly correlated with the absolute values of the binding energy of the viral CA ($r_s = 0.383$, $p = 0.040$). We conclude that the amino acid changes at positions 159 and 178 influence p26 dimer stability, with ASA CA dimers having a higher stability than PPP or APP CA dimers and the increased stability may be related to elevated VL in these patients.

4. Discussion

This study represents a detailed examination of HIV-2 p26 sequence variation within a set of 69 sequences isolated from subjects with both high and low VL. Disease progression after HIV-2 infection is highly dependent on VL [40] with subjects who control HIV-2 replication continuing to do this over a period of many years, suggesting that virus-host interactions result in a stable set-point of virus replication. The current study identified three sites of p26 variation correlating with VL. This study reveals a previously undescribed pattern of variation in the highly conserved p26 and indicates that the outcome of HIV-2 infection is partially predicted by the form of the p26 carried by the virus. In addition to confirming the importance of P119 as a determinant of TRIM5α restriction, the current study identified two additional amino acid positions (159 and 178) whose identities correlate with virus load. Although these associations were not significant after stringent adjustment for multiple comparisons, HIV-2 encoding p26 specifically modified at these three positions showed *in vitro* replication levels consistent with the *in vivo* VL data and further supported the conclusion that the three residues 119, 159 and 178 are important determinants of virus growth and influence TRIM5α restriction (Fig. 3).

Virus replication *in vivo* is influenced by a large number of host and viral factors and it would be naïve to conclude that these three residues are the sole determinants. That additional factors influence the course of infection is reflected in the VL data and the exceptions from the pattern (e.g. the three PPP viruses with greater than 10e3 VL and the 2 NNN viruses with undetectable VL). However, the power of such a population study is that patterns of HIV-2 behavior appear when large numbers of infection are monitored. As shown in Fig. 2, the substantial and statistically significant change in VL that accompanies the variation from PPP to NNN viruses strongly supports our conclusions that this p26 motif is an important determinant of the course of infection.

How might the PPP motif function? Our data support a destabilization of the CA by the three proline residues. P119 may directly form a recognition signal for TRIM5α binding, and the three proline residues may result in less tightly packed core that is more readily dismantled and processed after TRIM5α recognition. Our preliminary immunological studies show that patients with PPP virus mount stronger T cell responses to p26 and to the entire HIV-2 proteome (A.L., S.R.J. unpublished results) and this increased immune exposure might be a consequence of TRIM5α recognition and more efficient antigen presentation.

These results are consistent with TRIM5α restriction playing a direct role in limiting HIV-2 replication and a more indirect role in enhancing the immune response to the virus. The *in vitro* studies (Fig. 3A), although using manipulated cells and monitoring virus replication only over a short period of replication, demonstrated that variation in these three CA residues influence the susceptibility of HIV-2 replication to TRIM5α. *In vivo*, it is likely that TRIM5α effects are both manifested over multiple rounds of infection and TRIM5α may cooperate with other processes such as the adaptive immune response; *in vitro* cell culture conditions and growth in immortalized cell lines are unlikely to fully recreate these processes. We believe that our *in vivo* virus load data are the strongest support for the hypothesis that the PPP motif modulates VL.

If our hypothesis is correct, incident infections in Caio, infected patients that progress to require anti-retroviral therapy and moth-

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ers who infected their children should have a marked abundance of non-PPP forms. In fact, our phylogenetic analysis demonstrated no PPP virus clusters while there are some clusters of non-PPP viruses (Fig. S1), suggesting more frequent transmission of non-PPP viruses than PPP virus. We are currently examining these possibilities.

If the PPP p26 molecule is associated with a number of fitness-decreasing properties, what maintains this less fit gene in the population? One possibility is that there may be direct HLA selection for proline residues at these p26 positions. All three proline sites lie within or adjacent to known HLA epitopes. The presence of these key proline residues could either block the host immune recognition of these epitopes or interfere with processing to release the epitope. HIV-1 clearly adapts to its current host's HLA system by changing recognized epitopes [41–45] and it is likely that HIV-2 is subject to the same host HLA selection. Thus virus evolution may be driven by a shortsighted response to HLA selection resulting in PPP p26 that in the long term results in reduced viral replication. An abundance of HLA alleles in Caio that select for PPP p26 may be responsible for the high frequency of controlled HIV-2 infections in Caio. A cross-sectional study on HLA associations with p26 variation is underway. Adaptation to the current host's HLA haplotype has important consequences for the design of T cell based vaccines and could be exploited in vaccines to encourage the evolution of less aggressive variants.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.vaccine.2009.08.060.

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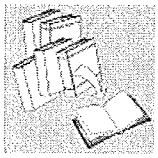

Anti-retroviral activity of TRIM5α

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SUMMARY

Human immunodeficiency virus type 1 (HIV-1) shows a very narrow host range limited to humans and chimpanzees. Experimentally, HIV-1 does not infect Old World monkeys, such as rhesus (Rh) and cynomolgus (CM) monkeys, and fails to replicate in activated CD4 positive T lymphocytes obtained from these monkeys. In contrast, simian immunodeficiency virus isolated from a macaque monkey (SIVmac) can replicate well in both Rh and CM. In 2004, tripartite motif 5α (TRIM5α) was identified as a host factor which plays an important role in the restricted host range of HIV-1. Rh and CM TRIM5α restrict HIV-1 infection but not SIVmac, while in comparison, anti-viral activity of human TRIM5α against those viruses is very weak. TRIM5α consists of the RING, B-box 2, coiled-coil and SPRY (B30.2) domains. The RING domain is frequently found in E3 ubiquitin ligase and TRIM5α is degraded via the ubiquitin-proteasome pathway during HIV-1 restriction. TRIM5α recognises the multimerised capsid (viral core) of an incoming virus by its α-isoform specific SPRY domain and is believed to be involved in innate immunity to control retroviral infection. Differences in amino acid sequences in the SPRY domain of TRIM5α of different monkey species were found to affect species-specific restriction of retrovirus infection, while differences in amino acid sequences in the viral capsid protein determine viral sensitivity to restriction. Accurate structural analysis of the binding surface between the viral capsid protein and TRIM5α SPRY is thus required for the development of new antiretroviral drugs that enhance anti-HIV-1 activity of human TRIM5α.

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INTRODUCTION

Human immunodeficiency virus type 1 (HIV-1), a major causative agent of acquired immunodeficiency syndrome (AIDS), belongs to the genera lentivirus of the family *Retroviridae*. HIV-1 is thought to have been introduced into the human population from chimpanzees [1] and has a very narrow host range limited to humans and chimpanzees. Experimentally, HIV-1 fails to replicate in activated CD4 positive T lymphocytes obtained from Old World monkey (OWM)s, such as rhesus (Rh) [2,3] and cynomolgus (CM) monkeys [4,5]. In contrast, another lentivirus simian immunodeficiency virus isolated from sooty mangabey (SIVsm) and simian immunodeficiency virus isolated from African green monkey (SIVagm) replicate in their natural hosts [6]. Simian immunodeficiency virus isolated from a macaque monkey (SIVmac), with a genome that has 55% nucleotide sequence homology to that of HIV-1, was evolved from SIVsm in macaques in captivity, and replicates efficiently in Rh [2,3] and CM [4,5]. The restricted host range of HIV-1 has greatly hampered its use in animal experiments, and, thus the development of prophylactic vaccines against HIV-1 infection. In 2004, tripartite motif 5α (TRIM5α) was identified as a host factor that plays an important role in the restricted host range of HIV-1. In this review

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Abbreviations used
A, alanine; AGM, African green monkey; AIDS; acquired immune deficiency syndrome; APOBEC, ApoB mRNA editing catalytic sub unit; CA, capsid protein; CM, cynomolgus monkey; CsA, cyclosporine A; CypA, cyclophilin A; HIV-1, human immunodeficiency virus type 1; HIV-2, human immunodeficiency virus type 2; L4/5, a loop between α-helices 4 and 5 of CA; L6/7, a loop between α-helices 6 and 7 of CA; NC, nucleocapsid protein; N-MLV, N-tropic murine leukemia viruses; OWM, Old World monkey; P; proline; Q, glutamine; R, arginine. Rh, rhesus monkey; RING, really interesting new gene; SPRY, a sequence repeat in the dual-specificity kinase splA and ryanodine receptors; SHIV, Chimeric virus between SIVmac and HIV-1; SIVmac, simian immunodeficiency virus isolated from macaque; TRIM5α, tripartite motif 5α; TRIMCyp, TRIM5 and CypA fusion protein; VL, viral load; VSV-G, vesicular stomatitis virus glycoprotein

article, recent findings in TRIM5α research are summarised and details of the molecular mechanisms of HIV-1 restriction by TRIM5α are discussed.

IDENTIFICATION OF TRIMα AS A RESTRICTION FACTOR AGAINST HIV-1 IN OWM CELLS

Several earlier studies have suggested that the block of HIV-1 replication in OWM cells occurs at a post-entry step [2,3,7] and appears to result from a failure to initiate reverse transcription [3]. Studies of HIV-1 and SIVmac chimera have suggested that restriction determinants lie within the HIV-1 p24 capsid protein (CA) [8–11]. The block was still observed in CD4-negative monkey cells infected with HIV-1 pseudotyped with vesicular stomatitis virus glycoprotein (VSV-G) (Figure 1) but was overridden by high-dose infection with VSV-G-pseudotyped virus or virus-like particles lacking genomic RNA [12–15]. Importantly, resistance against HIV-1 infection was shown to be dominant in heterokaryons between human and OWM cells, suggesting the presence of inhibitory factor(s) against HIV-1 infection in OWM cells [14].

In 2004, the screening of a Rh cDNA library identified TRIM5α, a component of cytoplasmic bodies, as a factor that confers resistance to HIV-1 infection [16]. Rh and CM TRIM5α restrict HIV-1 infection but not SIVmac [16,17]. In contrast, human TRIM5α is almost powerless to restrict the aforementioned viruses, but potently restricts N-tropic murine leukemia viruses (N-MLV), which belong to genera Gammaretrovirus. It was previously shown that human cells are resistant to infection with N-MLV and the presence in human

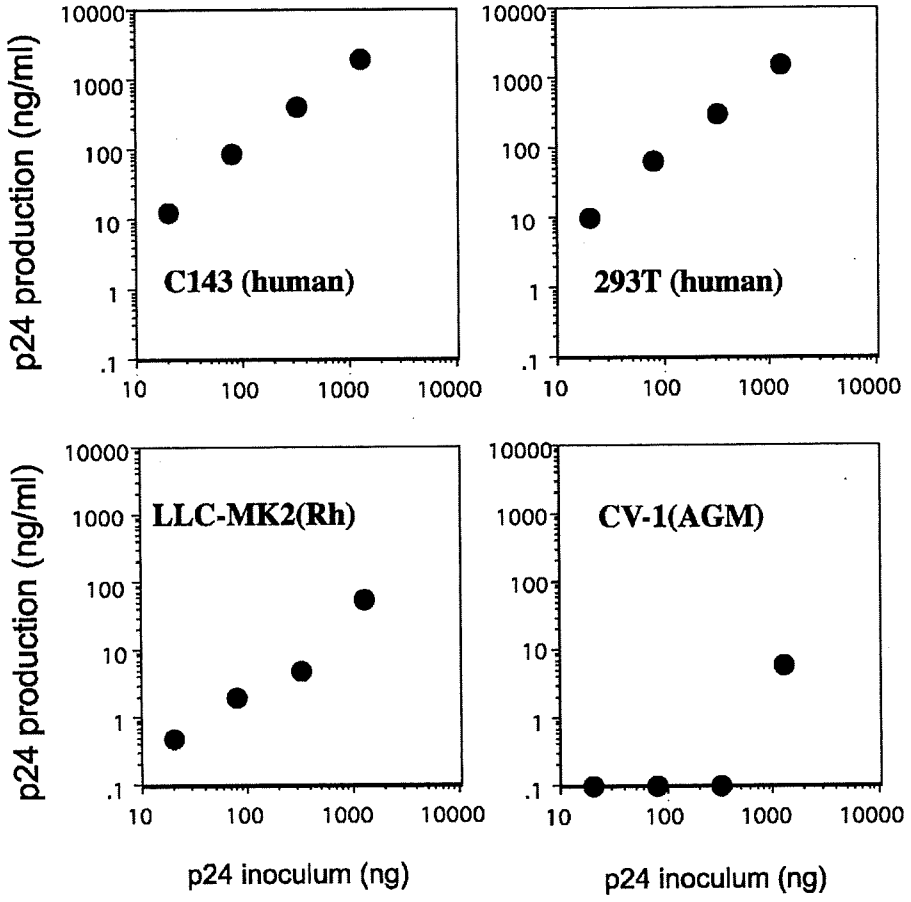


Figure 1. Old World monkey (OWM) cells are resistant to HIV-1 infection. Human C143 and 293T cell line were highly sensitive to vesicular stomatitis virus glycoprotein-pseudotyped HIV-1 infection, while rhesus monkey (Rh) LLC-MK2 and African green monkey (AGM) CV1 cell lines were resistant. When extremely high doses of virus were inoculated, cells became sensitive to infection, suggesting that the intrinsic restriction factor(s) were saturated with virions

cells of a virtual restriction factor known as Ref1 was posited. It is now widely accepted that the presence of human TRIM5α substantiates that of the restriction factor Ref1 [18–21]. On the other hand, African green monkey (AGM) cells have been shown to possess another factor, Lv1, which restricts both HIV-1 and SIVmac infection and we and others identified the factor as AGM TRIM5α [17,18]. AGM TRIM5α fails to restrict SIVagm. Unlike humans and other OWMs, pig-tailed monkeys lack expression of TRIM5α, but instead express TRIM5θ and TRIM5η, which lack anti-HIV-1 activity [22]. It is now known that type I interferons up-regulate the transcription of TRIM5α in human [23] and monkey cells [24] and this in turn enhances restriction activity against N-MLV [24,25].

TRIPARTITE MOTIF OF TRIM5α

The human genome contains approximately 70 genes of the TRIM family, which characteristically encode a tripartite protein motif [26–29]. This tripartite motif consists of a really interesting new gene (RING) zinc-finger domain, one or two B-box zinc-finger domains and an α-helical coiled-coil domain. *TRIM* genes are scattered throughout the human genome, while the *TRIM5* locus lies in a small cluster of four related *TRIM* genes including *TRIM6*, *TRIM34* and *TRIM22* [30]. Although the functions of most TRIM family members are still unknown, several TRIM proteins including TRIM1, TRIM19 (PML), TRIM22 and TRIM32 reportedly have anti-viral effects (reviewed in Reference [26]). Especially, TRIM19 can suppress broad spectrum of viruses such as herpes simplex virus type 1 and lymphocytic choriomeningitis virus [26]. TRIM21 is a trimeric protein that binds IgG Fc via the C-terminal of the B30.2 domain [31]. Subcellular localisation of the TRIM proteins varies among members of the TRIM family [27]: TRIM19, 24 and 27 are associated with nuclear bodies and TRIM1 and 18 with microtubules. TRIM5α was first identified as a cytoplasmic body protein [27], but diffuse expression in cytoplasm has proved important for its anti-viral activity [32].

As shown in Figure 2, TRIM5α consists of RING, B-box 2, coiled-coil and SPRY (B30.2) domains [27]. The RING domain containing proteins possess E3 ubiquitin ligase activity [33] and the intact RING domain of TRIM5α was thought to be essential for retrovirus restriction (see below). The intact

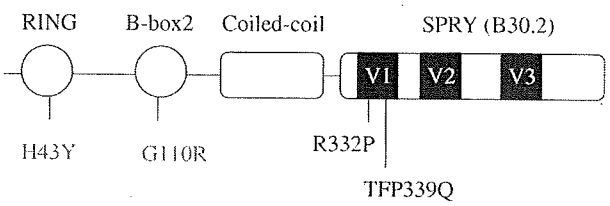


Figure 2. TRIM5α protein. TRIM5α protein contains RING, B-box 2, and coiled-coil domains, the three signature domains of the TRIM gene family. The α isoform possesses a SPRY domain sometimes referred to as a B30.2 domain. V1, V2 and V3 denote variable regions 1, 2 and 3 among monkey species, respectively. A histidine-to-tyrosine substitution at position 43 (H43Y) and a glycine-to-arginine substitution at position 110 (G110R) of human TRIM5 (shown in red) modulate anti-viral activity against retroviruses *in vitro*. Position 332 in human TRIM5α is arginine (R) and no polymorphism was reported in human genome. In contrast, proline (P)-to-glutamine (Q) substitution in rhesus monkey (Rh) and R-to-P or -Q substition in sooty mangabeys were found (R332P/Q). A 339th-TFP-341st to -Q polymorphism (TFP339Q) that reduces the anti-human immunodeficiency virus type 2 (HIV-2) activity was found in Rh TRIM5α

B-box 2 domain is also required for TRIM5α mediated antiviral activity since the restrictive activity of TRIM5α is diminished by several amino acid substitutions in the B-box 2 domain [34]. TRIM5α has been shown to form a trimer [35,36] or a dimer [37,38], while the B-box 2 domain mediates higher-order self-association of Rh TRIM5α oligomers. This self-association increases the efficiency of TRIM5α binding to the retroviral CA, thus potentiating restriction of retroviral infection [39,40]. The coiled-coil domain of TRIM5α has been identified as important for the formation of homo-oligomers [35], and homo-oligomerisation of TRIM5α as essential for antiviral activity [36,41]. The SPRY domain is specific for an α isoform among at least three splicing variants transcribed from the *TRIM5* gene. *TRIM5γ* and TRIM5δ lack the SPRY domain because of alternative splicing and their functions remain unknown. Exogenously expressed TRIM5γ is unstable [42] and over-expression of the TRIM5 protein lacking the SPRY domain dominant-negatively suppresses the anti-viral activity of the intact TRIM5α through hetero-oligomerisation [41,42].

SPRY (B30.2) DOMAIN OF TRIM5α

The C-terminal halves of TRIM family proteins are variable and half of them, including the TRIM5α, encode B30.2 (SPRY or PRYSPRY) domain [27]. Soon after identification of TRIM5α as a restriction

factor of Rh, many studies found that differences in the amino acid sequences in the TRIM5α SPRY domain of different monkey species affect the species-specific restriction of retrovirus infection [17,43–50].

Studies on human and Rh recombinant TRIM5αs have shown that the determinant of the species-specific restriction against HIV-1 infection resides in variable region 1 (V1) of the SPRY domain [43,44]. We found that 17-amino-acid residues and adjacent 20-amino-acid duplication in the V1 of AGM TRIM5α determined species-specific restriction against SIVmac [17]. Interestingly, a study comparing human and Rh TRIM5α showed that a single change from arginine (R) to proline (P) at the 332nd position in the V1 of human TRIM5α (R332P) conferred potent restriction ability against not only HIV-1 but also SIVmac239 [49,50]. In the case of human immunodeficiency virus type 2 (HIV-2) infection, we found that three amino acid residues of TFP at the 339th to 341st positions of Rh TRIM5α V1 are important for restricting particular HIV-2 strains which are still resistant to CM TRIM5α [45].

A study comparing orangutan and gorilla TRIM5αs showed that two amino acid residues at the 385th and 389th positions in the variable region 2 (V2) of SPRY domain of orangutan TRIM5α are important for restriction against HIV-1 and SIVmac [46]. We found that one amino acid residue at the 385th (baboon) or 383rd (CM) position in V2 of the SPRY domain of TRIM5α also affects its restriction ability against HIV-2 [47]. A computer-assisted 3-D model of the TRIM5α SPRY domain showed that V1 and V2 are located in the loops at the surface of the SPRY domain and the structure composed of the V1 and V2 regions is thought to be important for TRIM5α restriction (Figure 3).

Furthermore, a comparison of human and Rh TRIM5α restriction of N-MLV showed that the amino acid residues of human TRIM5α at the 409th and 410th positions in the variable region 3 (V3) of SPRY domain are important for restricting N-MLV [48].

Finally, biochemical studies have shown that TRIM5α associates with CA in detergent-stripped N-MLV virions [51] or with an artificially constituted HIV-1 core structure composed of the capsid-nucleocapsid (CA-NC) fusion protein in a SPRY domain dependent manner [52]. The SPRY domain is thus thought to recognise viral cores.

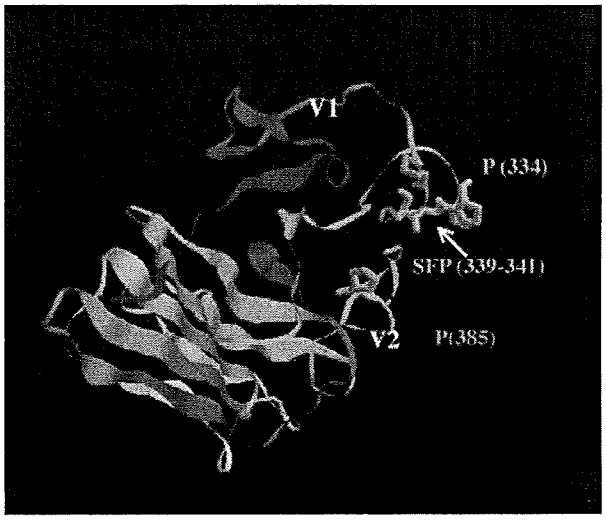


Figure 3. Structure of SPRY domain of TRIM5α. This 3-D model of the baboon SPRY domain was constructed with a homology-modeling technique based on mouse TRIM21 [131]. Position 332 in human TRIM5α is the same as position 334 in baboon TRIM5α. SFP motif in V1 and P residue at position 385 in V2 are shown

VIRAL DETERMINANT OF TRIM5α SENSITIVITY

To determine the CA region that interacts with TRIM5α, we focused on HIV-2, which strongly resembles SIVmac [53]. Previous studies have shown that HIV-2 strains vary widely in their ability to grow in OWM cells such as baboon, Rh and CM [54–58] and HIV-2 isolates with various growth capabilities in OWM cells were evaluated for their sensitivity to CM TRIM5α [59]. We found that viral sensitivity to CM TRIM5α inversely correlates with growth capability in OWM cells. Sequence analysis showed that the CM TRIM5α-sensitive viruses had proline (P) at the 119th or 120th position of CA, while the CM TRIM5α-resistant viruses had either alanine (A) or glutamine (Q) at the same position. Replacing the proline of a CM TRIM5α-sensitive HIV-2 molecular clone with either alanine or glutamine changed the phenotype from sensitive to resistant (Figure 4) and the mutant viruses replicated well in the presence of CM TRIM5α. The reverse was observed when the glutamine of a resistant SIVmac molecular clone was replaced with proline. Similar results, although to a lesser extent, were observed when human TRIM5α was used [59]. These results indicate that a single amino acid at the 119th or 120th

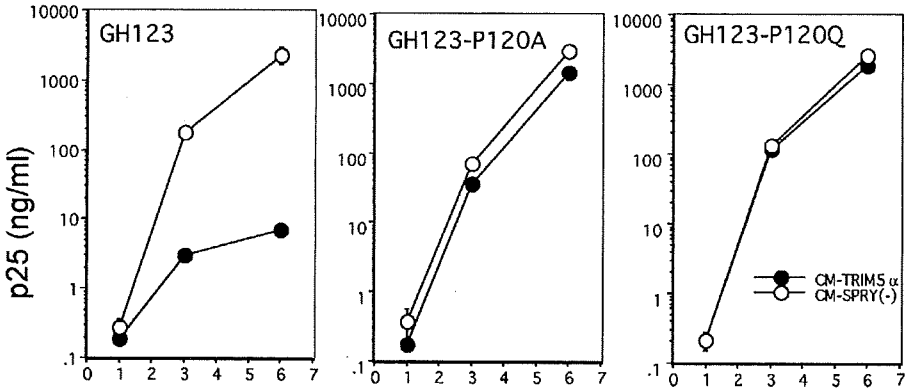


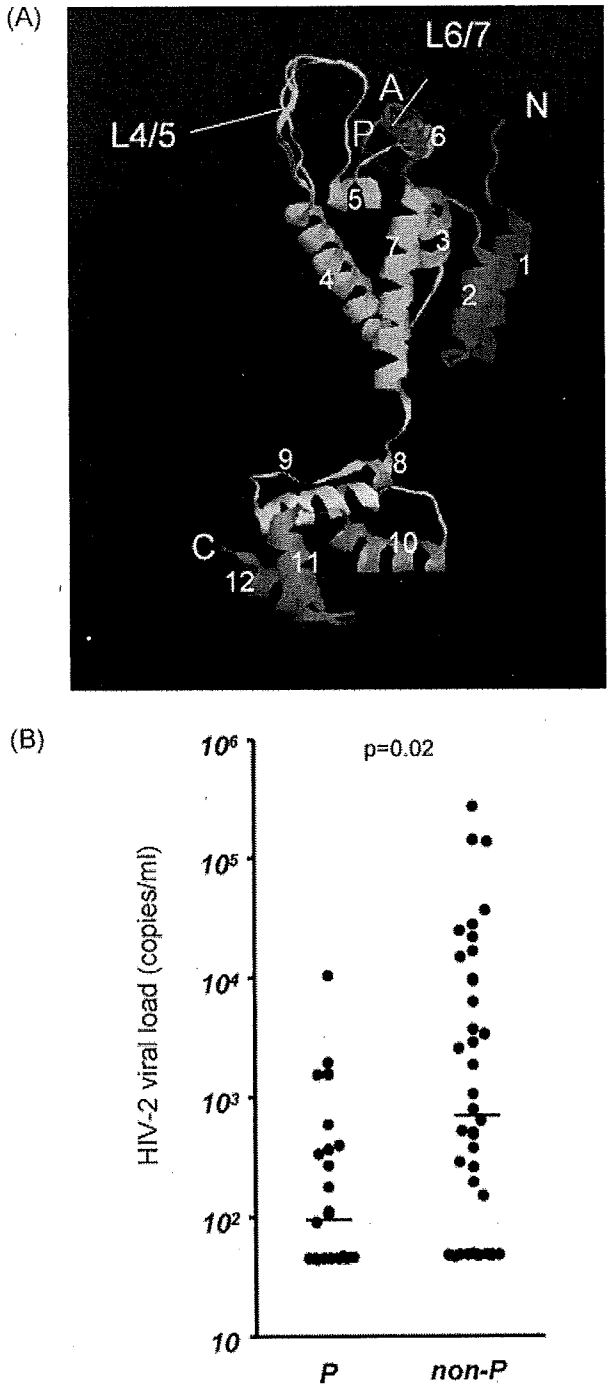
Figure 4. A single amino acid of HIV-2 capsid (CA) affects its replication in the presence of cynomolgus monkey (CM) TRIM5α. HIV-2 GH123 replication was restricted in CM-TRIM5α expressing human CD4 T cell line (CM-TRIM5α; black circles). The mutant GH123-P120A and GH123-P120Q viruses were generated by changing a single amino acid proline (P) at position 120 of GH123 CA to alanine (A) or glutamine (Q). These mutant viruses replicated in CM-TRIM5α expressing cells as efficiently as in cells lacking TRIM5α expression (CM-SPRY(-); white circles)

position of HIV-2 CA drastically affects viral sensitivity to TRIM5α.

A computer-assisted 3-D model of the HIV-2 CA showed that the 119th or 120th position is located in the loop between α-helices 6 and 7 (L6/7, Figure 5A). Previously, a single amino acid substitution at the 110th position of N-MLV CA had been shown to determine viral susceptibility to Fv1 [60], another restriction factor present in certain strains of mice [61] as well as to Ref1 (human TRIM5α) [18,20,21]. The recently published 3-D structure of MLV CA [62,63] 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. As mentioned above, V3 in the SPRY domain of human TRIM5α reportedly plays an important role in the restriction of N-MLV [48], whereas V1 in the SPRY domain of OWM TRIM5αs determines restriction specificity against HIV-1 and SIVmac [17,43,44,49,50]. These results indicate that the surface-exposed loop of CA is important for recognition by cellular restriction factors, even though critical amino acid residues in human TRIM5α for N-MLV restriction are different from those in CM TRIM5α for HIV-2.

HIV-2 is assumed to have originated from SIVsm as the result of zoonotic events involving monkeys and humans [53]. Almost all the SIV isolates in the Los Alamos database contain glutamine at the position corresponding to the 119th or 120th position of HIV-2 CA. In contrast, HIV-2 strains possess a mixture of glutamine, alanine and proline at the corresponding position. It is thus likely that glutamine-to-alanine or glutamine-to-proline substitutions occurred after the hypothesised zoonotic transfer of virus from monkeys to humans. According to this hypothesis, a single nucleotide change in the second position of the glutamine codons (CAA or CAG) would generate proline codons (CCN), and an additional single nucleotide change in the first position in CCN would generate alanine codons (GCN). Most likely, the glutamine residue first changed into proline residue in humans as a result of certain pressures from the human immune system in the absence of strong pressure from OWM TRIM5α. Since HIV-2 strains with proline residue show moderate sensitivity to human TRIM5α, an additional change would have to have occurred to generate alanine residue for better replication in human populations.

Does amino acid residue at the 119th or 120th position in HIV-2 CA affect HIV disease in infected individuals? It is known that HIV-1 and HIV-2 have distinct natural histories, levels of viremia, transmission rates and disease associations despite strong sequence homology between the two viruses [64]. Although some HIV-2-infected patients progress to AIDS, the infection is controlled in the majority of patients [65,66] and those with low viral load (VL) have a much longer survival [67]. A detailed sequence analysis of HIV-2 CA variations within a large community cohort



in Guinea Bissau comprising both high- and low-VL patients demonstrated that CA from viruses in low-VL patients had proline residues at the 119th or 120th position, but in patients with higher VL, the 119th or 120th position was frequently occupied by non-proline residues. Stratification of the subjects according to the presence or absence of proline at the 119th or 120th position showed a 3-fold difference in the median VL of the two groups (Figure 5B). These results demonstrate that HIV-2 replication in infected individuals can be linked to CA variation and human TRIM5α sensitivity [68].

In a case of Rh TRIM5α, Ylinen *et al.* mapped one of the determinants of TRIM5α sensitivity in a loop between α-helices 4 and 5 (L4/5) of HIV-2 [69]. They replaced the L4/5 of the SIVmac CA (CA 78 to CA97) with that of HIV-2 CA (CA78 to CA98) in the SIVmac backbone and found that the resultant mutant virus showed impaired growth ability in Rh cells compared with the parental SIVmac239. Although we could confirm this finding, the reciprocal virus with SIVmac239 CA L4/5 in the HIV-2 backbone did not gain resistance against Rh TRIM5α. However, we recently found that multiple components including L4/5 and L6/7 in the N-terminal half of SIVmac CA contributed to the escape of SIVmac from Rh TRIM5α (Kono *et al.* manuscript in preparation). Lin and Emerman also reported that SIVagmTAN with both HIV-1 L4/5 and L6/7 was susceptible to Rh TRIM5α restriction [70].

How do these regions in CA interact with specific regions in SPRY domain of TRIM5α? For this, it is necessary to obtain detailed and accurate structural information of the complex between viral CA

Figure 5. A single amino acid of HIV-2 CA affects 3-D structure of CA and its replication in infected individuals. (A) The 3-D models of six HIV-2 CAs were constructed with the homology-modeling technique. N and C indicate the amino termini and carboxyl termini, respectively. The ribbons represent the backbones of CA, and the 12 colour-coded-helices are numbered. P and A indicate a loop between α-helices 6 and 7 (L6/7) with a proline residue (in red) and an alanine residue (in blue), respectively, at position 119. (B) HIV-2 viral load (VL) correlates with amino acids variation at position 119 or 120 of CA. The differences in numbers were caused by the 1 amino acid insertion at the N-terminal part of CA in some HIV-2 isolates. Log-transformed VL for each patient in a West African community cohort is plotted. The patients were stratified according to the presence or absence of proline at position 119 or 120 (119P or non-P). Median VL of each group is indicated by a horizontal bar

and TRIM5α SPRY. Structural data on the binding surface between HIV-1 CA and TRIM5α SPRY of various primate species would also help to develop small molecular compounds that enhance anti-HIV-1 activity of human TRIM5α, and such compounds could be used as new therapeutic measures in HIV-1 infection.

ROLE OF CYCLOPHILIN A (CYPA)

The hydrophobic pocket of CypA makes direct contact with the proline residue at the 90th position and adjacent residues on the L4/5 of HIV-1 CA [71–73]. This interaction can be disrupted experimentally by mutations that alter CA proline 90 or adjacent residues [72], and by competitive inhibitors of the interaction including cyclosporine A (CsA) [74–76]. Disruption of CypA-CA interaction reduces HIV-1 susceptibility in human cells [72,74,76–80], with the block occurring early, at the time of reverse transcription [74]. These data have led to the hypothesis that, by binding to CA, CypA protects HIV-1 against antiviral restriction activity in human cells [81,82].

In OWM cell lines, however, CsA treatment has been reported to increase HIV-1 replication [15,83,84]. Concerning the relationship between CypA and TRIM5α, two groups reported that the simultaneous knock-down of CypA and TRIM5 in OWM cells caused minimal additional increase of HIV-1 infection compared with knock-down of TRIM5α alone, suggesting that CypA acts in trans to promote TRIM5α-mediated restriction of HIV-1 [85,86]. However, we demonstrated that exogenous expression of CypA suppresses HIV-1 infection in OWM cells in the absence of functional TRIM5α and that the inhibitory activity of CypA depends upon the interaction of CypA moiety with HIV-1 CA, while disruption of CypA-CA interaction by CsA treatment enhanced the HIV-1 susceptibility of OWM cells even in the absence of functional TRIM5α [41]. It is noteworthy that one of the two groups mentioned earlier observed slightly higher HIV-1 infection after simultaneous knock-down of CypA and TRIM5 than after knock-down of TRIM5 alone at the low-dose virus infection [85], suggesting that there is a TRIM5α-independent anti-HIV-1 activity mediated by CypA in OWM cells.

As already mentioned, L4/5 of SIVmac and HIV-2 CA is one of the determinants for Rh TRIM5α restriction, but it should be noted that SIVmac and HIV-2 CA L4/5s, unlike HIV-1 CA L4/5, do not bind to CypA [72].

CHIMERIC VIRUSES BETWEEN SIVMAC AND HIV-1 (SHIV)

In order to establish a monkey model of HIV-1/AIDS study, various SHIVs have been constructed and tested for their replicative capability in simian cells. The first SHIV was generated with a genetic backgbone of SIVmac containing HIV-1 *tat*, *rev*, *vpu* and *env* genes [11]. Although such an SHIV is useful for the analysis of humoral immune responses against Env protein [87–89], SHIVs containing other HIV-1 structural proteins, especially the Gag-Pol protein, were urgently needed since it is well known that the cellular immune response against Gag is important for disease control [90–92].

Construction of an SHIV with a minimal segment of SIVmac was reported by Kamada *et al.* [93]. This virus (NL-ScaVR, Figure 6A) contains the L4/5 of CA and the entire *vif* segment of SIVmac and was designed to escape from restrictions mediated by ApoB mRNA editing catalytic sub unit (APOBEC) 3G and CypA in OWM cells. APOBEC3G modifies the minus strand viral DNA during reverse transcription, resulting in impairment of viral replication [94–96], but this activity could be counteracted with the viral protein Vif [97–99]. Although HIV-1 Vif can potently suppress human APOBEC3G, it is not effective against Rh APOBEC3G, which explains at least partly the restriction of HIV-1 replication in monkey cells. As mentioned above, CypA directly binds to L4/5 of HIV-1 CA but not to SIVmac CA and augments HIV-1 infection in human cells but inhibits its replication in OWM cells [15,84,100].

NL-ScaVR could replicate in pig-tailed monkey primary CD4+ T cells as well as in the CM T cell line HSC-F. In both HSC-F and primary CD4+ T cells, NL-ScaVR grew to lower titers than did SIVmac [93], and inoculation into pig-tailed monkey with this SHIV did not cause CD4+ T cell depletion or any clinical symptoms [101]. In contrast, stHIV-1, another SHIV carrying 202 amino acid residues of SIVmac CA and *vif* generated by Hatziioannou *et al.*, could replicate more efficiently in Rh cells [102] and contains more of the SIVmac-derived CA segment than the virus reported by Kamada *et al.*, suggesting the presence outside of L4/5 of another determinant of intrinsic restriction factor(s) in CA.

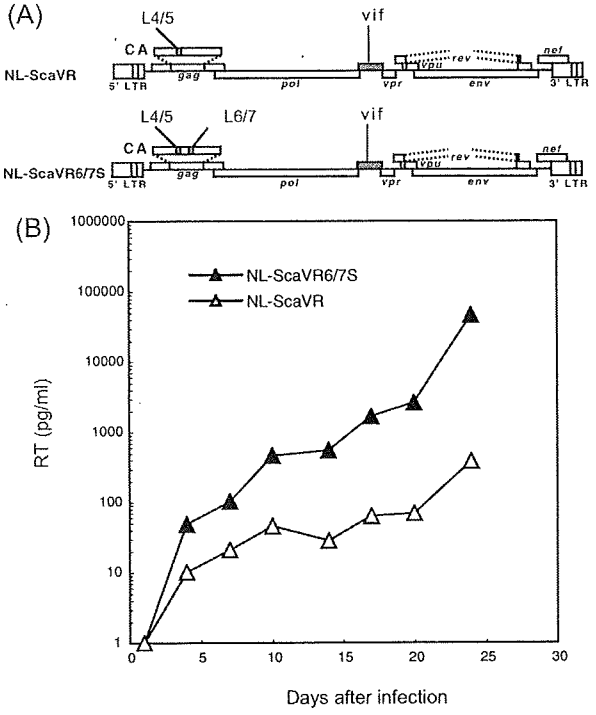


Figure 6. Modification of L6/7 of CA improves replication in CM cells of HIV-1 derivative. (A) Structure of chimeric viruses between simian immunodeficiency virus isolated from macaque (SIVmac) and HIV-1. White bars denote HIV-1 (NL4-3) and gray bars SIVmac239 sequences. (B) Equal amounts of NL-ScaVR (white triangles: virus with SIVmac L4/5 and *vif*), or NL-ScaVR6/7S (black triangles: virus with SIVmac L4/5, L6/7, and *vif*) were inoculated into CM CD4-positive T cell line HSC-F, and culture supernatants were collected periodically. The levels of reverse transcriptase (RT) in culture supernatants were measured by ELISA

As mentioned earlier, a single amino acid in L6/7 of HIV-2 CA was identified as a determinant of the susceptibility of HIV-2 to CM TRIM5α. Introduction of the entire SIVmac L6/7 of CA into the previously constructed version of HIV-1 derivatives with SIVmac L4/5 of CA and *vif* (NL-ScaVR6/7S, Figure 5A) caused only four amino acid changes in CA but showed improvement of replication capability of HIV-1 in CM cell line HSC-F (Figure 6B) and primary CD4+ T cells. While the high-dose inoculation of particles of wild type HIV-1 into monkey cells saturated endogenous TRIM5α and enhanced the subsequent infection with HIV-1, the introduction of both L4/5 and L6/7 of SIVmac greatly impaired the ability of HIV-1 particles to saturate TRIM5α [103]. These observations support the notion that TRIM5α recognises the overall structures composed of L4/5 and L6/7 of HIV-1 CA.

PROTEASOME-DEPENDENT AND PROTEASOME-INDEPENDENT MECHANISMS OF RESTRICTION

The RING domain containing proteins possess E3 ubiquitin ligase activity [33]. In fact, Rh TRIM5α was found to be poly-ubiquitinated and degraded rapidly via the ubiquitin-proteasome pathway [104], while disruption of the RING domain eliminated its auto-ubiquitination [104]. Furthermore, it was demonstrated that TRIM5α is degraded via the ubiquitin-proteasome pathway during HIV-1 restriction [105]. As for the antiviral activity of TRIM5α, however, deletion of the RING domain only partially attenuated its anti-HIV-1 activity [34,106], and modulation of E1 ubiquitin-activating enzyme expression did not affect TRIM5α-mediated restriction activity in a temperature dependent cell line [106]. Moreover, proteasome inhibitors did not affect TRIM5α mediated HIV-1 restriction but caused HIV-1 to generate late reverse transcripts during TRIM5α mediated restriction [107,108]. Proteasome-dependent and -independent pathways may therefore be involved in HIV-1 restriction by Rh TRIM5α (Figure 7). A proteasome-dependent HIV-1 restriction would mean co-degradation of TRIM5α with incoming viral capsids in proteasomes but the exact molecular mechanism of the proteasome-independent pathway is still unclear at present. It was previously shown that the incubation of *in vitro* assembled CA composed of recombinant HIV-1 CA-NC fusion protein with the purified TRIM5-21R protein containing the Rh TRIM5α B-box, coiled-coil and SPRY domains and the TRIM21 RING domain caused apparent breaks in the CA structure without any other cellular components [38]. It is thus likely that direct binding of Rh TRIM5α proteins to incoming HIV-1 CA proteins causes CA disassembly, which is observed as proteasome-independent restriction.

We recently found that the contribution of the RING domain to retrovirus restriction by TRIM5α differed among viral species. SIVmac completely escaped from attacks by RING mutants of AGM TRIM5α that could still moderately restrict HIV-1 infection. Addition of proteasome inhibitor MG132 had no effect at all on the anti-HIV-1 activity of AGM TRIM5α, but disrupted at

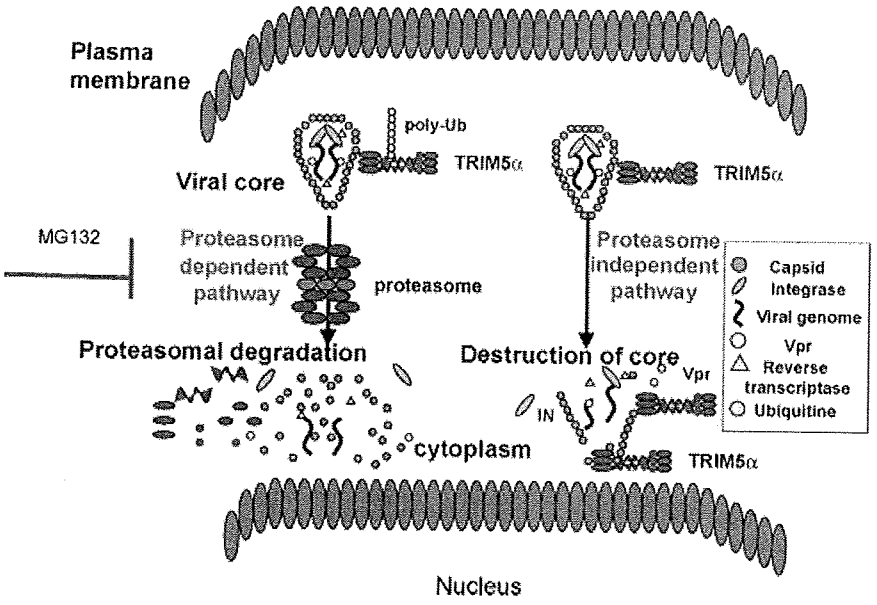


Figure 7. Current model of TRIM5α restriction pathways. (Left) A proteasome-dependent pathway. Oligomerized TRIM5α recognizes the incoming viral core through its SPRY domain and is poly-ubiquitinated in a RING-dependent manner. Ubiquitinated TRIM5α and the viral core complex are degraded in proteasome, while MG132 inhibits the activity of proteasome. (Right) A proteasome-independent pathway. Although RING mutant TRIM5α cannot ubiquitinate, HIV-1 CA and Rh or CM TRIM5α binding via the SPRY domain causes destruction of the viral core without any other cellular factors

least partly the anti-SIVmac activity of AGM *TRIM5α*. These results indicate that SIVmac is restricted by AGM TRIM5α mainly in a proteasome-dependent manner, whereas HIV-1 restriction by AGM, Rh and CM TRIM5α is both proteasome-dependent and -independent (Maegawa *et al.* submitted).

POLYMORPHISMS IN HUMAN TRIM5 GENE

HIV-1 infection in humans is generally characterised by a long-term, chronic disease course gradually progressing to AIDS. Polymorphisms in human *CCR5* and other genes reportedly affect the susceptibility to HIV-1 transmission and/or the rate of disease progression to AIDS [109,110]. Sawyer *et al.* reported a common histidine-to-tyrosine polymorphism at the 43rd amino acid residue (H43Y) of the human *TRIM5α* gene[111]. This single nucleotide polymorphism (SNP) is located in the RING domain (Figure 2) and was shown to greatly reduce the ability of TRIM5α to restrict N-MLV [111]. Several studies have reported that the anti-HIV-1 activity of TRIM5α with 43Y was lower than that with 43H *in vitro* [111,112] which was confirmed in a previous study of ours, although the difference in anti-HIV-1 activity was very small [113].

Associations of H43Y with the rate of progression to AIDS have been tested in several studies, but with inconsistent results [111–115]. Previously, we compared 43Y frequencies of HIV-1 infected long-term non-progresssors and standard progressors in France and Japan and failed to find any differences in 43Y frequency between the two groups in either country, indicating that this SNP does not affect disease progression [113]. In spite of the lower anti-HIV-1 activity of TRIM5α with 43Y, Javanbakht *et al.* have found a paradoxical protective effect of TRIM5α with 43Y against HIV-1 transmission in African-American [111,112]. Interestingly, we also found that the 43Y-allele was found less frequently in Japanese and Indian HIV-1 infected subjects than in the ethnic-matched controls [116]. The reasons for the discrepancy between the epidemiological and functional effects of H43Y remain unclear at present and further studies are required to clarify the impact of H43Y on susceptibility to HIV-1 transmission and/or rate of progression to AIDS.

In contrast to HIV-1, HIV-2 replication was moderately inhibited by the wild type human TRIM5α [59], but not at all by human TRIM5α with 43Y (Maegawa *et al.* submitted). This suggests that H43Y polymorphism has a stronger impact on HIV-2 than on HIV-1 in infected individuals. It would thus be of interest to investigate whether this allele affects the rate of disease progression in HIV-2-infected individuals.

In the B-box domain, we recently found in Japan a novel and rare glycine-to-arginine substitution at the 110th position of TRIM5α (G110R) (Figure 2) and this 110R allele was observed more frequently in HIV-1-infected subjects than in controls. As was observed epidemiologically, this substitution weakened the anti-HIV-1 and anti-HIV-2 activity *in vitro* [116]. These findings together indicate that anti-HIV-1 activity of human TRIM5α can hardly protect human beings from an HIV-1 pandemic, but it may affect HIV-1 transmission.

EVOLUTION OF *TRIM5* GENE

TRIM5 homologs have been found in the genomes of primates, mouse, rat, dog, cow and pig, but not in chicken [30,117]. *TRIM5* homolog genes are found in large numbers in cow, rat and mouse, but the human genome contains only a single *TRIM5* gene, and the dog's homolog was inactivated by a transposon [118]. No antiviral activity against eight retroviruses has been reported for the TRIM5 homologs of mouse (TRIM12 and TRIM30) [117] and mouse TRIM30 targets TAB2 (TAK1-binding protein 2) for degradation [119]. It is speculated that human TRIM5α also interact with a molecule of certain signal transduction cascade.

The *TRIM5* gene varies considerably among primate species. The distribution of positively selected amino acid site is located in the SPRY domain and coiled-coil domains [44,120,121]. It is thus not surprising that the very beginning of SPRY domain (V1) is highly variable since TRIM5α interacts with the retroviral core through this region as discussed above, while the main pressure for positive selection may be endogenous retroviruses [122]. In contrast to that for SPRY polymorphisms, the hypothesis to explain positive selection operating on the coiled-coil domain is too speculative, since the well-established function of the coiled-coil domains is only to promote TRIM5α oligomerisation [35]. Interestingly, in Rh, there is a 339th-TFP-341st to Q polymorphism in TRIM5α (Figure 2) [121], which reduces the anti-HIV-2 activity [45]. Position 332 in human TRIM5α is arginine (R) and there is no polymorphism in the human genome, but P/Q was found in Rh and R/P/Q polymorphism in sooty mangabeys [121,123] (Figure 2). H43Y polymorphism was frequently found in human but not in monkey species [118]. We recently found that H43Y mutation in AGM TRIM5α failed to affect its antiviral activity (Nakayama *et al.*). Since H43Y substitution seems to be neutral in monkey TRIM5α, there is no advantage in its accumulation in monkey population.

Among New World monkeys, owl monkeys possess CypA as a fusion protein with TRIM5 (TRIMCyp) as a result of LINE-1-mediated retrotranspositional insertion [124,125]. CypA can bind to the CA of HIV-1, so that the TRIMCyp expressed in owl monkey cells recognises the HIV-1 core and shows an anti-HIV-1 effect. Retrotransposition of *CypA* into the *TRIM5* gene also occurred independently in OWM Rh and pig-tailed monkeys [22,126–129]. Rh TRIMCyp restricts infection of HIV-2 and feline immunodeficiency virus but not HIV-1 [129], and TRIMCyp expressed in pig-tailed monkey also lacks anti-HIV-1 activity [22,126,127]. A lack of functional TRIM5α expression in pig-tailed monkey enabled Hatziioannou *et al.* to construct an SHIV strain that differs from HIV-1 only in the *vif* gene and can efficiently replicate in pig-tailed monkeys [130].

CONCLUSION

Within 5 years, many studies have emphasised the impact of TRIM5α on retroviral infection. The variable regions of the SPRY domain of TRIM5α evolved under the pressure of viral infection, and surface exposed loops of viral CA played a critical role in its escape from restriction of TRIM5α. Detailed and accurate structural analysis of the binding surface between viral CA and TRIM5α SPRY is thus needed for the development of new antiretroviral drugs that enhance anti-HIV-1 activity of human TRIM5α.

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