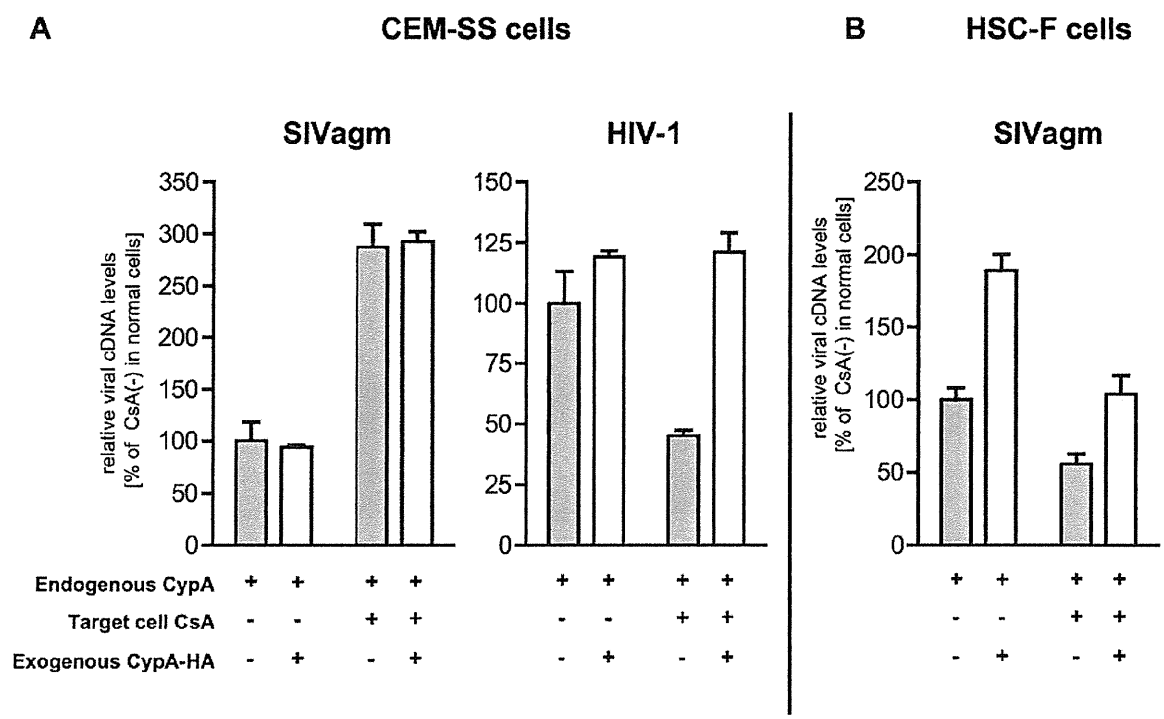

Figure 8 Effect of exogenous CypA expression on SIV and HIV-1 replication. (A) The amounts of viral cDNA synthesized after SIVagm (left panel) or HIV-1 (right panel) infection in human CEM-SS cells. Viruses produced from normal CEM-SS cells were used to infect CsA-untreated or CsA-treated target normal CEM-SS cells. Heat-inactivated virus was used as an infection control. Relative viral cDNA levels are shown as the ratio (%) of the viral cDNA levels to that of viruses produced from CsA-untreated CEM-SS in CsA-untreated CEM-SS cells. Mean values and standard deviations in three independent experiments are shown. (B) The amounts of viral cDNA after SIVagm infection in macaque HSC-F cells. SIVagm produced from normal HSC-F cells was used to infect CsA-untreated or CsA-treated target normal HSC-F cells. Heat-inactivated virus was used as an infection control. Relative viral cDNA levels are shown as the ratio (%) of the viral cDNA levels to that of viruses produced from CsA-untreated HSC-F in CsA-untreated HSC-F cells. Mean values and standard deviations in three independent experiments are shown.

Target human cell CypA was shown to be essential for HIV-1 but not for SIV infection. This may partially explain different sensitivities of HIV-1 and SIV replication to CsA treatment, although other unknown host factors could also be involved in the CsA-mediated enhancement of SIV replication in human T cells. Indeed, CsA treatment enhanced SIV replication even in CypA-KD cells (Figure 4B). We also attempted to examine the effect of cyclophilin B (CypB), another CsA-sensitive PPIase, on SIV replication by knock-down of CypB (Figure 5). CypB knock-down showed no significant effect on HIV-1 replication but enhanced SIV replication in human T cells, suggesting a possible involvement of CypB in SIV replication. Overall SIV replication was enhanced in CypB-KD cells (Figure 5B, SIVagm) and CsA treatment resulted in only slight enhancement of SIV replication (data not shown). As predicted, HIV-1 replication was not affected by the CypB knock-down (Figure 5B, HIV-1) but was inhibited by CsA treatment (data not shown). Thus, enhanced replication of SIV in CsA-treated human cells as shown in Figure 1A might be best explained by the neutralization of CypB's inhibitory effect. Previous reports showed that CypB can bind to both HIV-1 and SIV CA [14,35], but CypB incorporation into HIV-1/SIV virions was undetectable in the present study (data not shown). Regarding the cellular localization of CypB, association of CypB with the endoplasmic reticulum (ER) via an ER signal sequence has been reported [36]. In other reports [37-39], CypB has been shown to be associated with heparan sulfate proteoglycans (HSPG) on the cell surface. Overall, it remains unclear how CypB affects SIV infection directly or indirectly, because most CypB is believed to express in the endoplasmic reticulum but not in cytoplasm [40].

In contrast, CsA treatment inhibited SIV (SIVmac239 and SIVagm) replication in macaque T cells; CsA treatment of either virus producer or target cells resulted in suppression of SIV replication. This CsA effect on SIV replication in macaque T cells is similar to that on HIV-1 replication in human T cells. Although our attempts to

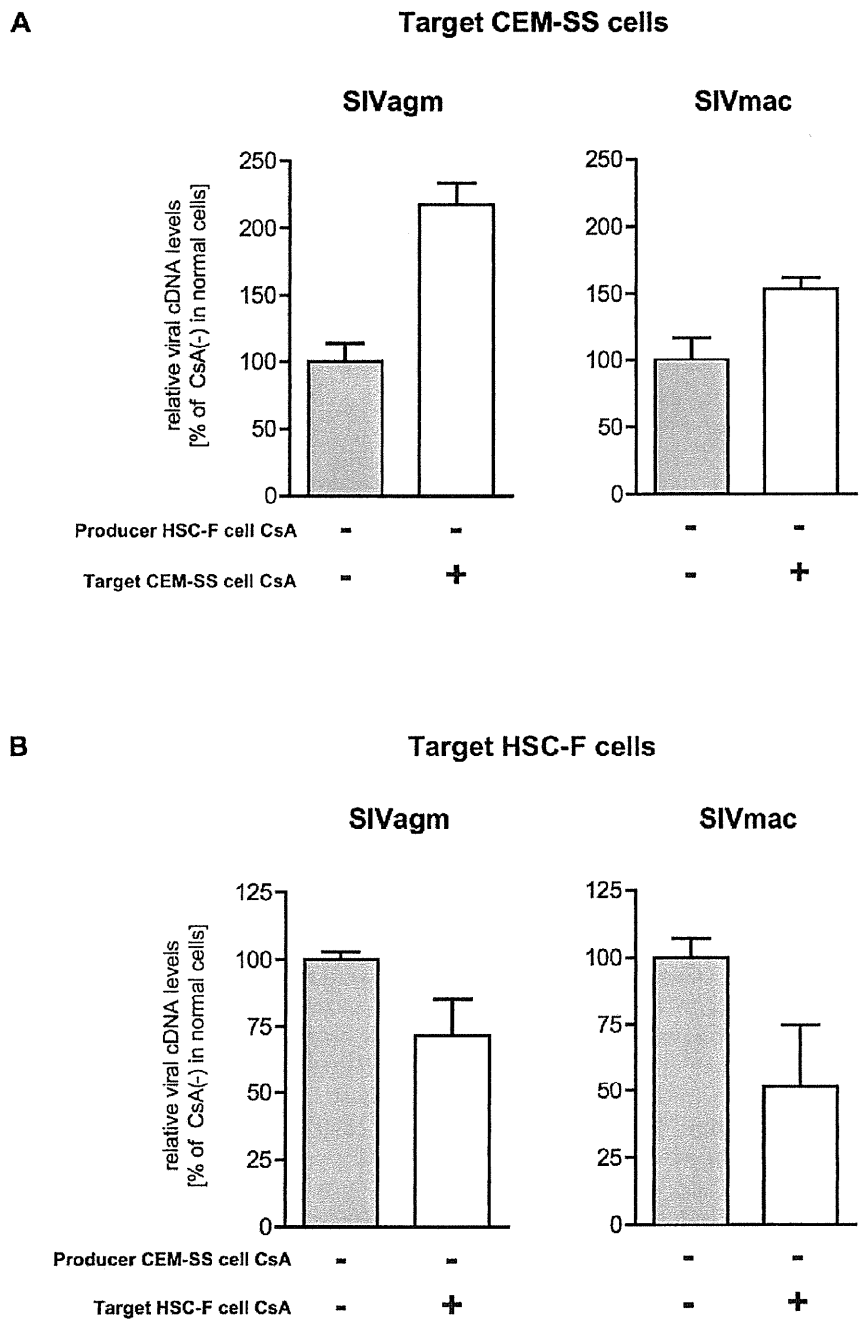

Figure 9 Infection of human and macaque cells with macaque- and human-derived SIV. (A) SIVagm and SIVmac produced from HSC-F cells in the absence or presence of CsA were used to infect CsA-untreated or CsA-treated target CEM-SS cells. Relative viral cDNA levels are shown as the ratio (%) of the viral cDNA levels to that of viruses produced from CsA-untreated HSC-F in CsA-untreated CEM-SS cells. Mean values and standard deviations in three independent experiments are shown. (B) SIVagm and SIVmac produced from CEM-SS cells in the absence or presence of CsA were used to infect CsA-untreated or CsA-treated target HSC-F cells. Relative viral cDNA levels are shown as the ratio (%) of the viral cDNA levels to that of viruses produced from CsA-untreated CEM-SS in CsA-untreated HSC-F cells. The relative viral cDNA levels synthesized were measured by real-time PCR. Mean values and standard deviations in three independent experiments are shown.

knock down CypA in macaque HSC-F cells were unsuccessful, we have obtained CypA knocked-down rhesus macaque kidney cell line: LLC-MK2 cells (Additional File 1). Analysis using these cells revealed that CypA is inhibitory for SIV replication in macaque cells and indicated that CypA dysfunction is likely to be largely involved in CsA-mediated reduction of SIV replication in macaque cells (Additional File 1). Taken together, the current study reveals that CsA treatment inhibited SIV replication in macaque T cells but enhanced SIV replication in human T cells, indicating a host species-specific effect of CsA on SIV replication. These data suggest that the effect of CsA on SIV infection seems to be general properties of SIV in human and macaque T cells, and cyclophilins may contribute to host-range control of certain lentiviruses.

Sequence analyses of CypA cDNA from human CEM-SS cells and macaque HSC-F, HSR-5.4, and Mn-3942 cells showed no difference in deduced amino acid sequences between human and macaque CypA (data not shown). Therefore, there may be a possible differential posttranslational modification of CypA such as acetylation [41,42] between human and macaque cells or there may be an additional host cell factor involved in the contribution of CypA to the determination of HIV-1 and SIV tropism by possibly affecting expression, localization, or function of CypA or viral capsid proteins.

CypA overexpression increased SIV infection in macaque T cells, suggesting that CypA may have different effects on SIV replication in human and macaque T cells. TRIM5α is known to restrict HIV-1 infection in macaque T cells, but this restriction has been shown to be relieved by CsA-mediated or small interfering RNA-mediated inhibition of CypA function, indicating involvement of CypA in TRIM5α-mediated restriction of HIV-1 infection in macaque cells [28,31,32]. Thus, CypA promotes HIV-1 infection in human cells but shows an inhibitory effect on HIV-1 infection in macaque cells. We attempted to examine the identification of macaque TRIM5α by PCR from macaque genomic DNA as reported previously [43] to investigate the possible effect of macaque TRIM5α on CsA-mediated reduction of SIV infection in macaque T cells, but macaque TRIM5α was identified in HSC-F cells but not in HSR-5.4 cells, suggesting that the effect of CsA on SIV replication in macaque T cells obtained from the present study (Figure 6) may be independent of macaque TRIM5α restriction (Additional File 2). In owl monkeys, which belong to new world monkeys, previous reports revealed the existence of a TRIM-CypA fusion protein (TRIMCyp) restricting HIV-1 infection, which was relieved by CsA treatment [44,45]. Recently, TRIMCyp has been found also in OWM, although OWM TRIMCyp did not restrict HIV-1 or SIVmac replication [43,46,47]. We also attempted to examine the identification of OWM TRIMCyp by PCR using primers on either side of the CypA insertion as previously reported [43] and the possibility of TRIMCyp expression by immunoblotting with anti-CypA antibody, but TRIMCyp expression was not detected in any of the macaque T-cell lines used in the present study (data not shown) although the CypA insertion was identified in the genome sequence of macaque T cells (Additional File 2).

Taken together, the present study reveals a species-specific effect of CsA on SIV replication. Our results suggest a contribution of CypA to efficient SIV replication in macaque cells. In contrast, analysis in human cells indicated that target cell CypA have no positive effect on SIV infection; rather, CypB was considered inhibitory for SIV replication. These results suggest possible involvement of cyclophilins in the determination of SIV tropism.

Conclusions

The present study revealed that CsA treatment enhances SIV replication in human T cells but abrogates SIV replication in macaque T cells, indicating a host cell species-specific effect of CsA on SIV replication. CypA knockdown or overexpression indicated a positive effect of CypA on SIV infection into macaque but not into human T cells. These results suggest possible contribution of CypA to the determination of SIV tropism.

Methods

Analysis of SIV and HIV-1 replication in human and macaque T cells

HeLa cells were propagated in Dulbecco modified Eagles medium containing 10% fetal bovine serum (FBS). The human CEM-SS and A3.01 T cell lines were cultured in RPMI 1640 containing 10% FBS. Macaque peripheral blood mononuclear cells (PBMCs) and three macaque T-cell lines, cynomolgus macaque-derived HSC-F, rhesus macaque-derived HSR-5.4, and pig-tailed macaque-derived Mn-3942 were cultured in RPMI 1640 containing 10% FBS, 10 mM HEPES buffer, 50 μM 2-mercaptoethanol, and 10 U IL-2 per ml [48]. Virus stocks of SIVagm, SIVmac, and HIV-1 used for analysis of viral replication were prepared by transfection of HeLa cells using LipofectAMINE LTX PLUS (Invitrogen Corp., Carlsbad, CA) with molecular clone DNAs of SIVagm9063 [49], SIVmac239 [50], and HIV-1_{NL4-3} [51], respectively. An *env*-defective variant of SIVagm9063 carrying an insertion of stop codon in the *env* gene (nucleotide position 10-15 from start of the *env* gene) was constructed by site-directed mutagenesis. Titers of the virus stocks were quantitated by SIV CA (p27) or HIV-1 CA (p24) enzyme-linked immunosorbent assay (ZeptMetrix Corporation, Buffalo, NY) and by determining the reverse transcriptase (RT) activity. CEM-SS cells (5×10^5) were exposed to 50 ng of SIV (p27) or 1 ng of HIV-1

(p24). Macaque T cells (5×10^5) were incubated with 5 ng of SIV (p27), and rhesus PBMCs (5×10^5) were incubated with 2 ng of SIV (p27). Virus production was monitored for 14 d post-infection by measuring RT activity in the culture supernatants as described previously [52]. In CsA treatment experiments, cells were cultured in the presence of 2.5 μM CsA (Sigma-Aldrich, Tokyo, Japan). Mean values in four independent experiments are shown.

Immunoblot analysis

Immunoblot analysis of cell lysates and viral pellets was performed as described previously [53]. Briefly, virus supernatants containing equal RT levels were concentrated by centrifugation through 20% sucrose. Pelleted viruses were analyzed by immunoblotting using anti-CypA or anti-CA antibodies. A polyclonal anti-SIVagm CA antibody provided by Vanessa Hirsch [54], plasma from a SIVmac239-infected rhesus macaque, and a monoclonal anti-HIV-1 p24 antibody (Abcam Inc, Cambridge, MA) were used to detect SIVagm, SIVmac, and HIV-1 CA, respectively. We used polyclonal anti-CypA antibody (BIOMOL Research Laboratories Inc., Plymouth Meeting, PA) and monoclonal anti-α-tubulin antibody (Sigma-Aldrich). A representative result from four independent experiments is shown in each figure.

Infectivity analysis

LuSIV cells which are derived from CEMx174 cells and contain a luciferase indicator gene under the control of the SIVmac239 LTR were maintained in RPMI 1640 medium containing 10% FBS and hygromycin B (300 μg/ml) [55]. LuSIV cells were cultured for 24 h after viral infection and lysed in 1 × reporter lysis buffer (Promega Corp., Madison, WI). To determine the luciferase activity, lysates were mixed with luciferase substrate (Promega Corp.) and light emission was measured in a luminometer (GloMax™ 96 Microplate Luminometer; Promega Corp.). Two sets of viruses were produced, and each was subjected to two sets of infection experiments. Thus, mean values of viral cDNA levels from four independent experiments are shown.

Measurement of viral cDNA levels after viral entry

Viruses were prepared from virus-infected CEM-SS or HSC-F cells. For infection, 5×10^5 target cells were incubated with a SIVagm containing 500 ng of p27, SIVmac containing 500 ng of p27, or HIV-1 containing 10 ng of p24 for 24 hr, and then total cellular DNA was extracted using a DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA). For CsA treatment of target cells, target cells were preincubated for 24 hr with 2.5 μM CsA (final concentration), a virus sample was added, and the infected cells (in 2.5 μM CsA, final concentration) were incubated for 24 hr. SIVagm, SIVmac, and HIV-1 inactivated by incubation at 65°C for 30 min were used as negative controls. For quantification of full-length viral cDNA levels by real-time PCR, primers 5'-GCTTCGGCCTCCATGATA-3' (nucleotides [nt] 1069-1086) and 5'-TGTTGCTACCGCTTCCTCTG-3' (nt 1231-1250) and probes 5'-TAGAACCAACAGGCTCGGAGGGCTTAAA-3' (nt 1141-1168) and 5'-AGTCTGTTCAATCTTGTGTGCGTGCTATTGC-3' (nt 1170-1202) were used for amplification and detection of *gag* region of the SIVagm9063 genome (GenBank accession number L40990); primers 5'-GATCTCTCGACGCAGGACT-3' (nt 680-698) and 5'-CCCTGGCCTTAACCGAAT-3' (nt 844-861) and probes 5'-AGGCTAGAAGGAGAGAGATGGGTGCGAG-3' (nt 773-800) and 5'-GCGTCGGTATTAAGCGGGGGAGAATTAG-3' (nt 802-829) for amplification and detection of *gag* region of the HIV-1_{NL4-3} genome (GenBank accession number M19921); primers 5'-GTAGTATGGGCAGCAAATGA-3' (nt 1408-1427) and 5'-TGTTCCTGTTTCCACCACTA-3' (nt 1631-1650) and probes 5'-GCATTCACGCAGAAGAGAAAGTGAAACA-3' (nt 1568-1595) and 5'-ACTGAGGAAGCAAAACAGATAGTGCAGAGA-3' (nt 1597-1626) for amplification and detection of *gag* region of the SIVmac239 genome (GenBank accession number M33262). For quantification of full-length viral cDNA levels by real-time PCR in CypA-KD human T cells, primers 5'-AGTGGGAGTTTGTCAATACC-3' (nt 3787-3806) and 5'-CTGATTTGTTGTGTCCGTTAG-3' (nt 3954-3974) and probes 5'-AGATGGGGCAGCCAATAGGGAAACTAAATT-3' (nt 3875-3904) and 5'-GGAAAAGCAGGATATGTAACTGACAGAGGAAGACAA-3' (nt 3906-3941) were used for amplification and detection of *pol* region of the HIV-1_{NL4-3} genome. As the control for standardization, TaqMan Endogenous Control kit for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used (Applied Biosystems, Inc., Foster City, CA). Real-time PCR was carried out in a LightCycler 2.0 instrument (Roche Diagnostics Corp., Indianapolis, IN). The ratios of viral cDNA levels to GAPDH DNA levels are shown.

Establishment of CypA and CypB knocked-down human T cell lines

Both human CEM-SS and A3.01 T cells were transduced with HIV-1-based vectors that confer puromycin resistance and express short hairpin RNAs (shRNA) targeting human CypA and CypB (Sigma-Aldrich). CypA and CypB knocked-down cell lines were obtained after selection with 1 μg/ml puromycin. Representative results obtained from experiments using three CypA and CypB knocked-down human CEM-SS cell lines are shown.

Exogenous expression of CypA in human and macaque T cells

CypA cDNAs amplified by PCR were inserted into the pMACS K^k.HA(C) vector (Miltenyi Biotec GmbH,

Bergisch Gladbach, Germany) to obtain pMACS K^k.HA (C)-CypA expressing HA-tagged CypA (CypA-HA) together with a cytoplasmic domain-truncated mouse major histocompatibility complex class I H-$2K^k$ protein, respectively. Human CEM-SS or macaque T cells (4×10^6 cells) suspended in 100 μl of nucleofection V solution with 2 μg of pMACS K^k.HA(c)-CypA (CypA-HA) or pMACS K^k.HA(c) (HA-control) plasmid vector DNAs were subjected to transfection (nucleofection) using a Nucleofector device. The nucleofection parameter was D-023 for CEM-SS cells and U-029 for HSC-F cells. After 1 day of culture, transfected cells were labeled with anti-H-$2K^k$ microbeads (MACSelect K^k MicroBeads) and enriched by separation using the MACS Separator (Miltenyi Biotec GmbH). The enrichment rate of transfected cells was determined by detection of H-$2K^k$ using MACSelect Control FITC Antibody (Miltenyi Biotec GmbH).

Additional material

Additional file 1: Effect of CypA knock-down on SIV infection in LLC-MK2 cells. (A) Immunoblot analysis of CypA expression. Lysates of LLC-MK2 (normal) and CypA-KD cells were subjected to the immunoblot analysis using anti-α-tubulin, anti-CypA and anti-CypB antibodies (Abcam Inc., Cambridge, MA) (left panel). The image of one representative blot is shown. (B) Effect of CypA knock-down on SIV infection in LLC-MK2 cells. Normal and CypA-KD LLC-MK2 cells were transfected with plasmid SIVmac239LTR-luc that contains a luciferase indicator gene under the control of the SIVmac239 LTR. After 24 h, transfected cells were used for VSVG-pseudotyped SIVagm env(-) virus infection. Infection was determined 24 h later by measuring the Tat-induced luciferase activity in the transfected cells. Luciferase activity induced by the virus in normal LLC-MK2 cells was defined as 100%. Mean values and standard deviations in three independent experiments are shown.

Additional file 2: Identification of a TRIM5α or a TRIMCyp in macaque T cells. Total DNA from macaque HSC-F and HSR-5.4 T cells was harvested. PCR primers on either side of the CypA insertion were used to detect both a TRIM5α and a TRIMCyp in macaque T cells as described [43]. H_2O denotes water control.

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Authors' contributions
HT conceived and coordinated the study, performed all analyses, and wrote the manuscript. HI assisted FACS analysis. NI and TK assisted infectious experiments. HA provided experimental tools. TM conceived and coordinated the study and was involved in writing the manuscript. All authors have read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References

1. Gautsch JW, Elder JH, Schindler J, Jensen FC, Lerner RA: **Structural markers on core protein p30 of murine leukemia virus: functional correlation with Fv-1 tropism.** *Proc Natl Acad Sci USA* 1978, **75**:4170-4174.
2. Rein A, Kashmiri SV, Bassin RH, Gerwin BL, Duran-Troise G: **Phenotypic mixing between N- and B-tropic murine leukemia viruses: infectious particles with dual sensitivity to Fv-1 restriction.** *Cell* 1976, **7**:373-379.
3. Towers G, Bock M, Martin S, Takeuchi Y, Stoye JP, Danos O: **A conserved mechanism of retrovirus restriction in mammals.** *Proc Natl Acad Sci USA* 2000, **97**:12295-12299.
4. Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J: **The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys.** *Nature* 2004, **427**:848-853.
5. Keckesova Z, Ylinen LM, Towers GJ: **The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities.** *Proc Natl Acad Sci USA* 2004, **101**:10780-10785.
6. Song B, Javanbakht H, Perron M, Park DH, Stremlau M, Sodroski J: **Retrovirus restriction by TRIM5alpha variants from Old World and New World primates.** *J Virol* 2005, **79**:3930-3937.
7. Yap MW, Nisole S, Lynch C, Stoye JP: **Trim5alpha protein restricts both HIV-1 and murine leukemia virus.** *Proc Natl Acad Sci USA* 2004, **101**:10786-10791.
8. Ylinen LM, Keckesova Z, Wilson SJ, Ranasinghe S, Towers GJ: **Differential restriction of human immunodeficiency virus type 2 and simian immunodeficiency virus SIVmac by TRIM5alpha alleles.** *J Virol* 2005, **79**:11580-11587.
9. Hatziioannou T, Perez-Caballero D, Yang A, Cowan S, Bieniasz PD: **Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha.** *Proc Natl Acad Sci USA* 2004, **101**:10774-10779.
10. Goff SP: **Genetic control of retrovirus susceptibility in mammalian cells.** *Annu Rev Genet* 2004, **38**:61-85.
11. 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.
12. Stremlau M, Perron M, Lee M, Li Y, Song B, Javanbakht H, Diaz-Griffero F, Anderson DJ, Sundquist WI, Sodroski J: **Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor.** *Proc Natl Acad Sci USA* 2006, **103**:5514-5519.
13. Thali M, Bukovsky A, Kondo E, Rosenwirth B, Walsh CT, Sodroski J, Gottlinger HG: **Functional association of cyclophilin A with HIV-1 virions.** *Nature* 1994, **372**:363-365.
14. Franke EK, Yuan HE, Luban J: **Specific incorporation of cyclophilin A into HIV-1 virions.** *Nature* 1994, **372**:359-362.
15. Towers GJ, Hatziioannou T, Cowan S, Goff SP, Luban J, Bieniasz PD: **Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors.** *Nat Med* 2003, **9**:1138-1143.
16. Braaten D, Luban J: **Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells.** *Embo J* 2001, **20**:1300-1309.
17. Braaten D, Franke EK, Luban J: **Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus SIV(CPZ)GAB but not group O HIV-1 or other primate immunodeficiency viruses.** *J Virol* 1996, **70**:4220-4227.
18. Franke EK, Luban J: **Inhibition of HIV-1 replication by cyclosporine A or related compounds correlates with the ability to disrupt the Gag-cyclophilin A interaction.** *Virology* 1996, **222**:279-282.
19. Hatziioannou T, Perez-Caballero D, Cowan S, Bieniasz PD: **Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells.** *J Virol* 2005, **79**:176-183.
20. Braaten D, Franke EK, Luban J: **Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription.** *J Virol* 1996, **70**:3551-3560.
21. Braaten D, Aberham C, Franke EK, Yin L, Phares W, Luban J: **Cyclosporine A-resistant human immunodeficiency virus type 1 mutants demonstrate**

that Gag encodes the functional target of cyclophilin A. *J Virol* 1996, **70**:5170-5176.
22. Ott DE, Coren LV, Johnson DG, Sowder RC, Arthur LO, Henderson LE: **Analysis and localization of cyclophilin A found in the virions of human immunodeficiency virus type 1 MN strain.** *AIDS Res Hum Retroviruses* 1995, **11**:1003-1006.
23. Ackerson B, Rey O, Canon J, Krogstad P: **Cells with high cyclophilin A content support replication of human immunodeficiency virus type 1 Gag mutants with decreased ability to incorporate cyclophilin A.** *J Virol* 1998, **72**:303-308.
24. Bukovsky AA, Weimann A, Accola MA, Gottlinger HG: **Transfer of the HIV-1 cyclophilin-binding site to simian immunodeficiency virus from Macaca mulatta can confer both cyclosporin sensitivity and cyclosporin dependence.** *Proc Natl Acad Sci USA* 1997, **94**:10943-10948.
25. Sokolskaja E, Sayah DM, Luban J: **Target cell cyclophilin A modulates human immunodeficiency virus type 1 infectivity.** *J Virol* 2004, **78**:12800-12808.
26. Kootstra NA, Munk C, Tonnu N, Landau NR, Verma IM: **Abrogation of postentry restriction of HIV-1-based lentiviral vector transduction in simian cells.** *Proc Natl Acad Sci USA* 2003, **100**:1298-1303.
27. Takeuchi H, Buckler-White A, Goila-Gaur R, Miyagi E, Khan MA, Opi S, Kao S, Sokolskaja E, Pertel T, Luban J, Strebel K: **Vif counteracts a cyclophilin A-imposed inhibition of simian immunodeficiency viruses in human cells.** *J Virol* 2007, **81**:8080-8090.
28. Keckesova Z, Ylinen LM, Towers GJ: **Cyclophilin A renders human immunodeficiency virus type 1 sensitive to Old World monkey but not human TRIM5 alpha antiviral activity.** *J Virol* 2006, **80**:4683-4690.
29. Ikeda Y, Ylinen LM, Kahar-Bador M, Towers GJ: **Influence of gag on human immunodeficiency virus type 1 species-specific tropism.** *J Virol* 2004, **78**:11816-11822.
30. Sokolskaja E, Olivari S, Zufferey M, Strambio-De-Castillia C, Pizzato M, Luban J: **Cyclosporine blocks incorporation of HIV-1 envelope glycoprotein into virions.** *J Virol* 2010, **84**:4851-4855.
31. Berthoux L, Sebastian S, Sokolskaja E, Luban J: **Cyclophilin A is required for TRIM5{alpha}-mediated resistance to HIV-1 in Old World monkey cells.** *Proc Natl Acad Sci USA* 2005, **102**:14849-14853.
32. Stremlau M, Song B, Javanbakht H, Perron M, Sodroski J: **Cyclophilin A: an auxiliary but not necessary cofactor for TRIM5alpha restriction of HIV-1.** *Virology* 2006, **351**:112-120.
33. Noser JA, Towers GJ, Sakuma R, Dumont JM, Collins MK, Ikeda Y: **Cyclosporine increases human immunodeficiency virus type 1 vector transduction of primary mouse cells.** *J Virol* 2006, **80**:7769-7774.
34. Lin TY, Emerman M: **Cyclophilin A interacts with diverse lentiviral capsids.** *Retrovirology* 2006, **3**:70.
35. Luban J, Bossolt KL, Franke EK, Kalpana GV, Goff SP: **Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B.** *Cell* 1993, **73**:1067-1078.
36. Bram RJ, Hung DT, Martin PK, Schreiber SL, Crabtree GR: **Identification of the immunophilins capable of mediating inhibition of signal transduction by cyclosporin A and FK506: roles of calcineurin binding and cellular location.** *Mol Cell Biol* 1993, **13**:4760-4769.
37. Hanoulle X, Melchior A, Sibille N, Parent B, Denys A, Wieruszeski JM, Horvath D, Allain F, Lippens G, Landrieu I: **Structural and functional characterization of the interaction between cyclophilin B and a heparin-derived oligosaccharide.** *J Biol Chem* 2007, **282**:34148-34158.
38. Pakula R, Melchior A, Denys A, Vanpouille C, Mazurier J, Allain F: **Syndecan-1/CD147 association is essential for cyclophilin B-induced activation of p44/42 mitogen-activated protein kinases and promotion of cell adhesion and chemotaxis.** *Glycobiology* 2007, **17**:492-503.
39. Vanpouille C, Deligny A, Delehedde M, Denys A, Melchior A, Lienard X, Lyon M, Mazurier J, Fernig DG, Allain F: **The heparin/heparan sulfate sequence that interacts with cyclophilin B contains a 3-O-sulfated N-unsubstituted glucosamine residue.** *J Biol Chem* 2007, **282**:24416-24429.
40. Price ER, Zydowsky LD, Jin MJ, Baker CH, McKeon FD, Walsh CT: **Human cyclophilin B: a second cyclophilin gene encodes a peptidyl-prolyl isomerase with a signal sequence.** *Proc Natl Acad Sci USA* 1991, **88**:1903-1907.
41. Misumi S, Fuchigami T, Takamune N, Takahashi I, Takama M, Shoji S: **Three isoforms of cyclophilin A associated with human immunodeficiency virus type 1 were found by proteomics by using two-dimensional gel electrophoresis and matrix-assisted laser desorption ionization-time of flight mass spectrometry.** *J Virol* 2002, **76**:10000-10008.
42. Lammers M, Neumann H, Chin JW, James LC: **Acetylation regulates cyclophilin A catalysis, immunosuppression and HIV isomerization.** *Nat Chem Biol* 2010, **6**:331-337.
43. Wilson SJ, Webb BL, Ylinen LM, Verschoor E, Heeney JL, Towers GJ: **Independent evolution of an antiviral TRIMCyp in rhesus macaques.** *Proc Natl Acad Sci USA* 2008, **105**:3557-3562.
44. Sayah DM, Sokolskaja E, Berthoux L, Luban J: **Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1.** *Nature* 2004, **430**:569-573.
45. 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 USA* 2004, **101**:13324-13328.
46. Virgen CA, Kratovac Z, Bieniasz PD, Hatziioannou T: **Independent genesis of chimeric TRIM5-cyclophilin proteins in two primate species.** *Proc Natl Acad Sci USA* 2008, **105**:3563-3568.
47. Brennan G, Kozyrev Y, Hu SL: **TRIMCyp expression in Old World primates Macaca nemestrina and Macaca fascicularis.** *Proc Natl Acad Sci USA* 2008, **105**:3569-3574.
48. Akari H, Mori K, Terao K, Otani I, Fukasawa M, Mukai R, Yoshikawa Y: **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.
49. Hirsch VM, Dapolito G, Johnson PR, Elkins WR, London WT, Montali RJ, Goldstein S, Brown C: **Induction of AIDS by simian immunodeficiency virus from an African green monkey: species-specific variation in pathogenicity correlates with the extent of in vivo replication.** *J Virol* 1995, **69**:955-967.
50. Regier DA, Desrosiers RC: **The complete nucleotide sequence of a pathogenic molecular clone of simian immunodeficiency virus.** *AIDS Res Hum Retroviruses* 1990, **6**:1221-1231.
51. Adachi A, Gendelman HE, Koenig S, Folks T, Willey R, Rabson A, Martin MA: **Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone.** *J Virol* 1986, **59**:284-291.
52. Willey RL, Smith DH, Lasky LA, Theodore TS, Earl PL, Moss B, Capon DJ, Martin MA: **In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity.** *J Virol* 1988, **62**:139-147.
53. Takeuchi H, Kao S, Miyagi E, Khan MA, Buckler-White A, Plishka R, Strebel K: **Production of infectious SIVagm from human cells requires functional inactivation but not viral exclusion of human APOBEC3G.** *J Biol Chem* 2005, **280**:375-382.
54. Campbell BJ, Hirsch VM: **Vpr of simian immunodeficiency virus of African green monkeys is required for replication in macaque macrophages and lymphocytes.** *J Virol* 1997, **71**:5593-5602.
55. Roos JW, Maughan MF, Liao Z, Hildreth JE, Clements JE: **LuSIV cells: a reporter cell line for the detection and quantitation of a single cycle of HIV and SIV replication.** *Virology* 2000, **273**:307-315.

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SIV replication in human cells

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Current human immunodeficiency virus type 1 pandemic is believed to originate from cross-species transmission of simian immunodeficiency virus (SIV) into human population. Such cross-species transmission, however, is not efficient in general, because viral replication is modulated by host cell factors, with the species-specificity of these factors affecting viral tropism. An understanding of those host cell factors that affect viral replication contributes to elucidation of the mechanism for determination of viral tropism. This review will focus an anti-viral effect of ApoB mRNA editing catalytic subunit, tripartite motif protein 5 alpha, and cyclophilins on SIV replication and provide insight into the mechanism of species-specific barriers against viral infection in human cells. It will then present our current understanding of the mechanism that may explain zoonotic transmission of retroviruses.

Keywords: HIV-1, SIV, APOBEC3G, TRIM5α, cyclophilin A, cyclophilin B

INTRODUCTION

There is significant evidence that the ongoing worldwide acquired immunodeficiency syndrome (AIDS) epidemic was caused by cross-species transmission of simian immunodeficiency viruses (SIVs) into the human population. Replication of primate lentiviruses in their natural hosts is generally non-pathogenic; however, cross-species transmission of these viruses can result in highly pathogenic phenotypes. How and when this transmission occurred is still debated but it is now generally accepted that HIV-2 originated from a sooty mangabeys strain of SIV (SIVsm; Hirsch et al., 1989; Chen et al., 1996) while HIV-1 appears to have originated from a chimpanzee strain of SIV (SIVcpz; Gao et al., 1999). Zoonotic transmission of SIVs, however, is not common and is controlled by host factors that generally prohibit SIV replication in human hosts and many human-derived cell lines.

Viral replication is modulated by host cell factors, with the species-specificity of these factors affecting viral tropism. Some of these host factors can restrict viral replication and the anti-viral systems mediated by such host restriction factors, termed intrinsic immunity, play an important role in determining species-specific barriers against viral infection. For instance, Fv-1 in mice is known to restrict replication of a murine leukemia virus (Rein et al., 1976; Gautsch et al., 1978; Towers et al., 2000) and tripartite interaction motif 5α (TRIM5α) recently has been found to be responsible for restricting HIV-1 but not SIV infection in Old World monkey (OWM) cells (Hatziioannou et al., 2004b; Keckesova et al., 2004; Stremlau et al., 2004; Yap et al., 2004; Song et al., 2005; Ylinen et al., 2005). Restriction of retroviral replication by these host cell factors takes place after viral entry, but before the integration step, and the viral determinants for this type of restriction have been mapped to the capsid (CA) protein (Gautsch et al., 1978; Kozak and Chakraborti, 1996; Towers et al., 2000; Goff, 2004; Stremlau et al., 2006). Two recent studies showed that the cellular protein SAMHD1 is myeloid-lineage cell-specific HIV-1 restriction factor counteracted by Vpx proteins from HIV-2 and SIVsm (Hrecka et al., 2011; Laguette et al., 2011). Restriction of lentivirus infection by SAMHD1 is likely to take place at the reverse transcription step. Another anti-retroviral protein, tetherin (also referred to as BST-2, CD317, or HM1.24) inhibits retrovirus release and is antagonized by HIV-1 Vpu protein, Nef protein of many SIVs, or Env protein of HIV-2 (Neil et al., 2008; Le Tortorec and Neil, 2009; Zhang et al., 2009). Understanding how host cell factors affect viral replication, positively or negatively, would contribute to elucidating the molecular mechanism that determines viral tropism. Here, we discuss an anti-viral effect of ApoB mRNA editing catalytic subunit (APOBEC), TRIM5α, and cyclophilins (Cyps) on SIV replication.

APOBEC: ENZYMATIC RESTRICTION FACTOR THAT TARGET RETROVIRUSES

Replication of HIV-1 in primary CD4+ T cells, monocyte, and some immortalized T cell lines depends on the presence of the HIV-1 accessory gene product, Vif (standing for virus infectivity factor; Fisher et al., 1987; Strebel et al., 1987), and it works in a host cell-specific manner. Vif is required for enhanced HIV-1 replication in some cell types called non-permissive cells. In contrast, HIV-1 replication is Vif-independent in permissive cells (Akari et al., 1992; Fan and Peden, 1992; Gabuzda et al., 1992; Blanc et al., 1993; Sakai et al., 1993; von Schwedler et al., 1993; Borman et al., 1995). Recently, some cytidine deaminases were identified as a new class of host restriction factors that target retroviruses such as HIV-1 or SIV (Harris and Liddament, 2004; Cullen, 2006). APOBEC3G (Apo3G), a member of the APOBEC family of cytidine deaminases, is the first identified enzymatic restriction factor and the determinant that makes cells permissive or non-permissive. Apo3G is also a host factor that restricts replication of human and simian lentiviruses in their respective target cells. Unlike TRIM5α or Fv-1, Apo3G does not exert its anti-viral activity by targeting the viral CA protein, but it has to be incorporated

into a newly synthesized virion during a production step, and then inhibits virus replication by targeting single-stranded viral cDNA during a subsequent infection step. HIV-1 counteracts Apo3G with Vif expression. During the production of progeny virions, Vif binds to Apo3G and induces Apo3G's proteasomal degradation, resulting in the decreased steady-state levels of human Apo3G (hApo3G; Yu et al., 2003).

There are several anti-retroviral mechanisms of Apo3G against HIV-1 infection. First, Apo3G-containing virus can accumulate in a large number of substitutions that register as cytidine (C) to deoxyuridine (dU) in a virus minus-strand during reverse transcription, resulting guanine (G) to adenine (A) mutations in a viral plus-strand, known as "G-to-A hypermutation" (Harris et al., 2003; Lecossier et al., 2003; Mangeat et al., 2003; Mariani et al., 2003; Zhang et al., 2003; Yu et al., 2004b). Second, Apo3G can inhibit tRNA annealing or tRNA processing during reverse transcription (Guo et al., 2006, 2007; Mbisa et al., 2007). Third, Apo3G inhibits DNA strand transfer or integration (Li et al., 2007; Luo et al., 2007; Mbisa et al., 2007). Although Apo3G has the most potent anti-HIV-1 activity among the APOBEC family of proteins, another member of the family, APOBEC3F (Apo3F) was shown to inhibit HIV-1 infection in the absence of Vif (Bishop et al., 2004a; Liddament et al., 2004; Wiegand et al., 2004; Zheng et al., 2004), whereas APOBEC3B (Apo3B) can inhibit HIV-1 infection in both the presence and absence of Vif (Bishop et al., 2004a; Doehle et al., 2005; Rose et al., 2005).

Although we can imagine the broad range of anti-retroviral activity of APOBEC family because APOBEC proteins from non-human species can also inhibit HIV-1 infection (Mariani et al., 2003; Bishop et al., 2004a,b; Wiegand et al., 2004; Cullen, 2006), the Vif-Apo3G interaction is thought to be species-specific (Simon et al., 1998; Mariani et al., 2003). Accordingly, hApo3G is insensitive to SIVagm Vif while African green monkey Apo3G (agmApo3G) is insensitive to HIV-1 Vif and the determinant of this species-specificity depends on amino acid 128 of hApo3G and agmApo3G (Mariani et al., 2003; Bogerd et al., 2004; Mangeat et al., 2004; Schrofelbauer et al., 2004; Xu et al., 2004).

However, such species-specificity is not strictly controlled, for example, a report from the laboratory of Klaus Strebel demonstrated that SIVagm Vif supported replication of SIVagm virus in the hApo3G-positive human A3.01 T cell line (Takeuchi et al., 2005). Replication of *vif*-defective SIVagm in A3.01 cells was severely restricted, resulted in an accumulation of cytidine deaminase-induced G-to-A mutations in SIVagm genome (Takeuchi et al., 2005).

Moreover, two independent groups showed that the different APOBEC3 family members function to neutralize specific lentiviruses (Yu et al., 2004a; Dang et al., 2006). One report from the lab of Dr. Nathaniel R. Landau showed that APOBEC3B and APOBEC3C were potent inhibitors of SIV (Yu et al., 2004a). Both enzymes were efficiently encapsidated by HIV-1 and SIV. Another report from the lab of Dr. Yong-Hui Zheng demonstrated that APOBEC3DE blocked the replication of both HIV-1 and SIV but not that of MLV (Dang et al., 2006) and APOBEC3H inhibited the replication of HIV-1 by a cytidine deamination-independent mechanism (Dang et al., 2008). These findings raise the possibility that the various APOBEC3 family members protect against different lentiviruses and point to a possible role in the zoonotic transmission of SIV.

TRIM5α: FV-1-TYPE HOST FACTOR RESTRICTING HIV-1 IN PRIMATE CELLS

The host protein which dictates Ref1 activity was identified as an α-isoform of rhesus macaque TRIM5 protein by the laboratory of Dr. Joseph Sodroski (Stremlau et al., 2004). TRIM5 is a member of the TRIM family of proteins, and has RING, B-box 2, and coiled-coil as common and conserved domains among the family and B30.2 (PRYSPRY) domain on its C-terminal region (Nisole et al., 2005). Subsequently, the human and non-human primate homologs of TRIM5α were shown to restrict retroviruses, such as N-MLV, and equine infectious anemia virus (Hatziioannou et al., 2004b; Keckesova et al., 2004; Perron et al., 2004; Yap et al., 2004; Song et al., 2005; Ylinen et al., 2005; Si et al., 2006). Rhesus monkey TRIM5α (rhTRIM5α) has strong anti-HIV-1 activity but only modestly restricts SIV isolated from a macaque monkey (SIVmac) and does not block MLV infection, whereas its human homolog does not restrict HIV-1 infection.

TRIM5α recognizes incoming viral cores, but not a monomeric CA protein, thorough its B30.2 (PRYSPRY) domain. B-box 2 and coiled-coil domains are required for TRIM5α multimerization, and both coiled-coil and B30.2 (PRYSPRY) domains are essential for viral core binding (Reymond et al., 2001; Stremlau et al., 2006). TRIM5α captures HIV-1 core at a very early step(s) after infection, immediately after the release of the core into cytoplasm. To restrict HIV-1 infection and to recognize viral core, TRIM5α must oligomerize through its B-box 2 and coiled-coil domains (Mische et al., 2005; Li and Sodroski, 2008). Its RING domain has E3 ubiquitin ligase activity. It self-ubiquitination occurs TRIM5α is quickly degraded (Diaz-Griffero et al., 2006). This rapid degradation of TRIM5α is not required for post-entry restriction since replacement of TRIM5α RING domain with the corresponding domain of TRIM21, which has lower self-ubiquitination activity and a longer half-life than TRIM5α did not alter the anti-viral activity (Kar et al., 2008). Recently, the laboratory of Dr. Mark Yeager discussed a novel architecture made with dimers of TRIM5-21R. TRIM5α-21R forms a dimer through its B-box 2 and coiled-coil domains, and these dimers form six-sided rings on CA lattices to promote rapid core disassembly (Ganser-Pornillos et al., 2011). Overexpression of TRIM5α leads to the formation of cytoplasmic bodies and is believed to be required for its anti-viral activity (Stremlau et al., 2006; Campbell et al., 2008). During TRIM5α-mediated post-entry restriction, disassembly of viral cores is induced too quickly and the accumulation of viral RT-products is reduced (Stremlau et al., 2006). On the other hand, MG132 treatment inhibited quick-disassembly, yet HIV-1 infectivity was still restricted. Two reports showed that TRIM5α could block not only viral cDNA accumulation but also the nuclear import of viral cDNA (Berthoux et al., 2004; Wu et al., 2006). Thus, TRIM5α-mediated post-entry restriction is thought to have at least two phases: (i) TRIM5α induces rapid disassembly of viral core in a proteasome-dependent manner and (ii) TRIM5α degrades HIV-1 cDNAs in a proteasome-independent manner. The determinant of specificity and magnitude of the post-entry

restriction lies on B30.2 (PRYSPRY) domain. Previous report showed that TRIM5α alleles did not cluster by species between rhesus macaques and sooty mangabeys and none of the alleles from either species restricted SIV, suggesting that there is little effect of rhTRIM5α on transmission of SIVsm within species (Newman et al., 2006). Recently, Pacheco et al. (2010) reported that New World monkey (NWM) TRIM5α restricts foamy virus infection. Another consideration is the clinical significance of TRIM5α against AIDS in human. Moreover, several reports showed that the efficacy of TRIM5α-mediated suppression of HIV-1 replication might interfere with disease progression of AIDS in humans (van Manen et al., 2008; Cagliani et al., 2010; Takeuchi et al., 2012). Thus, TRIM5α-mediated restriction may be a multi-step process in retrovirus replication with the relationship between other host factor(s).

Recently, the lab of Dr. Yasuhiro Ikeda reported that rhesus macaque TRIM5α also inhibits HIV-1 production by inducing the degradation of a viral precursor Gag protein (Sakuma et al., 2007). To restrict HIV-1 production, amino acid residues in B-box 2 and coiled-coil domains dictated the specificity of the restriction. In the late restriction, the accumulation of HIV-1 RNA was not affected but the accumulation of precursor Gag was inhibited in an ubiquitin–proteasome-independent manner. This TRIM5α-mediated late-restriction is still controversial (Zhang et al., 2008), yet it is conceivable that TRIM5α restricts HIV-1 infection and production in two distinct mechanisms. Although TRIM5α restricts HIV-1 infection in a broad range of cells, its late restriction involved transient overexpression (Sakuma et al., 2007).

Here is another notable class of the TRIM family called TRIM-Cyp isolated from NWM. A report from the laboratory of Dr. Jeremy Luban demonstrated that owl monkey cells express TRIM-Cyp that restricts HIV-1 infection (Sayah et al., 2004). Although TRIM-Cyp has a cyclophilin A (CypA) sequence in its C-terminal region instead of B30.2 (PRYSPRY) domain that dictates the specificity and the magnitude of post-entry restriction in OWM TRIM5α-mediated post-entry restriction, it recognizes incoming core structure and restricts HIV-1 infection (Stremlau et al., 2006). Recently, TRIM-Cyp mRNA was also detected in a rhesus macaque cell, and overexpressed rhesus TRIM-Cyp restricts HIV-1 infection and production (Newman et al., 2006; Brennan et al., 2008; Wilson et al., 2008; Dietrich et al., 2010).

Unlike other restriction factors, there is no known accessory gene product of HIV-1 to antagonize TRIM5α-mediated restrictions. Indeed, human TRIM5α has only a modest restriction activity against HIV-1 infection. TRIM5 proteins from several NWM species restrict infection by SIVmac and SIVagm (Song et al., 2005). This suggests that TRIM5α could be a key molecule of the species-species barrier.

CYCLOPHILINS: HOST FACTORS INVOLVED IN RETROVIRUS REPLICATION

Cyclophilins are ubiquitous proteins and first identified as the target of cyclosporine A (CsA), an immunosuppressive reagent (Takahashi et al., 1989). CypA has proline-isomerase activity that catalyzes the *cis–trans* isomerization of proline residue (Fischer et al., 1989). The binding of CsA to CypA inhibits this isomerase activity (Takahashi et al., 1989). In retrovirus replication, CypA was found to bind HIV-1 CA in the yeast two-hybrid system (Luban et al., 1993). The sequence Ala88-Gly89-Pro90-Ile91 of CA protein is the major fragment bound to the active site of CypA (Franke et al., 1994; Gamble et al., 1996; Zhao et al., 1997). Interestingly, The peptidyl-prolyl bond between Gly89 and Pro90 of the CA fragment has a *trans* conformation, in contrast to the *cis* conformation observed in other known CypA–peptide complexes (Zhao et al., 1997; Bosco et al., 2002), and Gly89 preceding Pro90 has an unfavorable backbone formation usually only adopted by glycine, suggesting that special Gly89-Pro90 sequence but not other Gly-Pro motif is required for the binding of CA protein to CypA. Therefore, CypA might be likely to act as a molecular chaperone but not a *cis–trans* isomerase (Zhao et al., 1997). However, one report showed that CypA does not only bind CA protein but also catalyzes efficiently *cis–trans* isomerization of Gly89-Pro90 peptidyl-prolyl bond (Bosco et al., 2002). The relationship between the Gly89-Pro90 bond and catalysis of *cis–trans* isomerization by CypA remains unclear.

It has been well established that CypA promotes an early step of HIV-1 infection in human cells (Franke et al., 1994; Thali et al., 1994; Braaten et al., 1996a,c; Franke and Luban, 1996; Braaten and Luban, 2001; Sokolskaja et al., 2004; Hatziioannou et al., 2005). CypA is efficiently encapsidated into HIV-1 produced from infected cells through interaction with the CA domains of the Gag polyprotein and disruption of CypA incorporation into virions by CsA or HIV-1 Gag mutants caused a decrease in replication efficiency (Franke et al., 1994; Thali et al., 1994; Ott et al., 1995; Braaten et al., 1996a; Bukovsky et al., 1997; Ackerson et al., 1998; Braaten and Luban, 2001). It is still unclear how CypA is efficiently packaged into HIV-1 virion, but several reports showed that both dimerization of CA and multimerization of CypA are required for efficient interaction (Colgan et al., 1996; Javanbakht et al., 2007). Although CA-CypA interaction is required for infectivity, the important point is that CypA interacts with incoming HIV-1 cores in newly infected target cells rather than during HIV-1 budding from the virion producer cells, indicating that target cell CypA promotes HIV-1 infectivity (Kootstra et al., 2003; Towers et al., 2003; Sokolskaja et al., 2004).

CypA-dependent virus replication is only limited to retroviruses which encode CA that binds CypA. In fact, only those retroviruses are dependent upon CypA for replication (Luban et al., 1993; Franke et al., 1994; Thali et al., 1994; Braaten et al., 1996c; Franke and Luban, 1996). These observations suggested that CA–CypA interaction might contribute tropism determinants for retroviruses. HIV-1 infection in non-human primate cells is blocked prior to reverse transcription after virus entry (Shibata et al., 1995; Himathongkham and Luciw, 1996; Hofmann et al., 1999; Besnier et al., 2002; Cowan et al., 2002; Munk et al., 2002; Hatziioannou et al., 2003; Towers et al., 2003). This restriction is thought to be the same step in the retrovirus life cycle where CypA works (Braaten et al., 1996b). Indeed, analysis of CypA-binding region of CA with chimeric viruses of HIV-1 and SIV showed the viral determinant for species-specificity (Shibata et al., 1991, 1995; Dorfman and Gottlinger, 1996; Bukovsky et al., 1997;

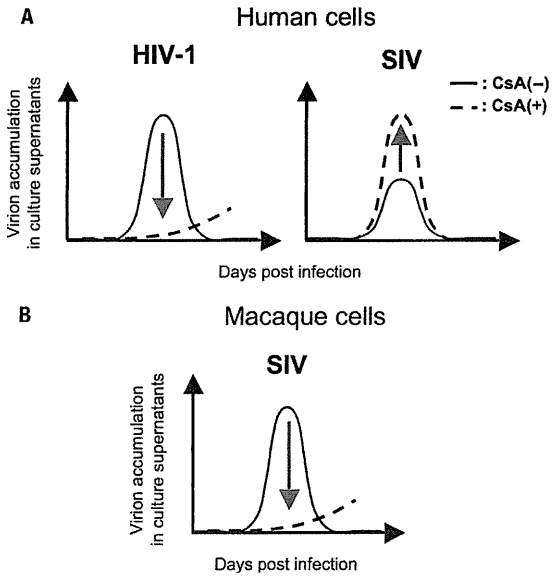

FIGURE 1 | A schema for the effect of CsA on HIV/SIV replication in human/macaque cells. (A) CsA treatment impairs the replication of HIV-1 (left panel) but enhances SIV replication (right panel) in human cells. **(B)** CsA treatment inhibits SIV replication in macaque cells. The solid line indicates virion accumulation of culture supernatant in the absence of CsA and the broken line indicates that of culture supernatant in the presence of CsA.

Cowan et al., 2002; Kootstra et al., 2003; Owens et al., 2003, 2004; Towers et al., 2003; Berthoux et al., 2004; Hatziioannou et al., 2004a, 2006; Ikeda et al., 2004; Sayah et al., 2004; Stremlau et al., 2004; Kamada et al., 2006).

Human CypA is required for efficient HIV-1 infection but not SIV. There is no known role for CypA in SIV infection in human cells. Recently, the first report from the laboratory of Klaus Strebel showed that human CypA acts as restriction factor against the infection of two SIVs (SIVmac and SIVagm) in human cells, and Vif protein of two SIVs counteracts a CypA-imposed inhibition against the infection of two SIV strains with exclusion of CypA from SIV virion (Takeuchi et al., 2007). This phenomenon is different from the function of SIVagm Vif against hApo3G previously reported from the same laboratory (Takeuchi et al., 2005) because they used human cells lacking detectable deaminase activity.

Moreover, a recent report showed a species-specific effect of CsA, a peptidyl-prolyl *cis–trans* isomerase (PPIase) inhibitor, on SIV replication, implying a possible contribution of Cyps to the determination of SIV tropism (**Figure 1**; Takeuchi et al., 2012). They demonstrated a host species-specific effect of CypA on SIV replication: CypA affects the replication of two SIVs (SIVmac and SIVagm) negatively in human cells but positively in macaque cells (**Figure 1**). Further analysis indicated that the infection of two SIVs was not significantly affected by CypA but inhibited by cyclophilin B (CypB), another PPIase, in human cells (**Figure 2A**; Takeuchi et al., 2012). In contrast, CypA is likely to have positive

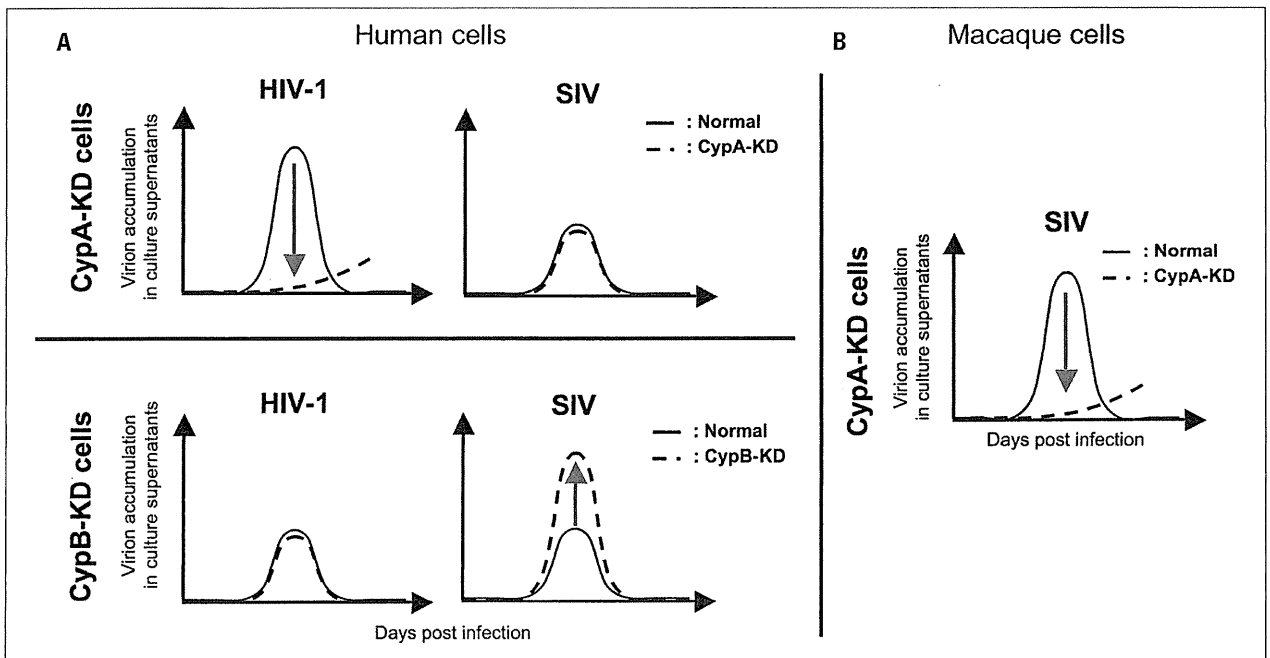

FIGURE 2 | A schema for the effect of cyclophilin A and cyclophilin B on HIV/SIV replication in human/macaque cells. (A) CypA knock-down (CypA-KD) impairs the replication of HIV-1 (upper left panel). In contrast, SIV replication is not reduced but rather enhanced by CypA knock-down (upper right panel). CypB knock-down (CypB-KD) shows no significant effect on HIV-1 replication (lower left panel) but enhances the replication of SIV (lower right panel). **(B)** CypA-KD inhibits SIV infection. The solid line indicates virion accumulation of culture supernatant produced from normal cells and the broken line indicates that of culture supernatant produced from CypA or CypB knock-down cells.

effects on the infection of two SIVs in macaque cells (**Figure 2B**; Takeuchi et al., 2012). These results suggest that Cyps might have a host species-specific effect of Cyps on SIV replication and provide insight into the mechanism of species-specific barriers against viral infection.

CONCLUDING REMARKS

Viral replication is modulated by host cell factors. Many of these factors function in a species-specific manner. On the other hand, there exist host factors that restrict viral replication. The antiviral system mediated by some of these restriction factors, termed intrinsic immunity, which is distinguished from the conventional innate and adaptive immunity has been indicated to play an important role in making species-specific barriers against viral infection. As discussed in this review, we describe the current progress in understanding of such restriction factors against retroviral replication, especially focusing on TRIM5α and APOBEC whose anti-retroviral effects have recently been recognized. Additionally, we mentioned a host species-specific effect of Cyps including CypA and CypB on SIV replication. Such restriction factors would play an important role in determining species-specific barriers against viral infection.

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REFERENCES

Ackerson, B., Rey, O., Canon, J., and Krogstad, P. (1998). Cells with high cyclophilin A content support replication of human immunodeficiency virus type 1 Gag mutants with decreased ability to incorporate cyclophilin A. *J. Virol.* 72, 303–308.

Akari, H., Sakuragi, J., Takebe, Y., Tomonaga, K., Kawamura, M., Fukasawa, M., Miura, T., Shinjo, T., and Hayami, M. (1992). Biological characterization of human immunodeficiency virus type 1 and type 2 mutants in human peripheral blood mononuclear cells. *Arch. Virol.* 123, 157–167.

Berthoux, L., Sebastian, S., Sokolskaja, E., and Luban, J. (2004). Lv1 inhibition of human immunodeficiency virus type 1 is counteracted by factors that stimulate synthesis or nuclear translocation of viral cDNA. *J. Virol.* 78, 11739–11750.

Besnier, C., Takeuchi, Y., and Towers, G. (2002). Restriction of lentivirus in monkeys. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11920–11925.

Bishop, K. N., Holmes, R. K., Sheehy, A. M., Davidson, N. O., Cho, S. J., and Malim, M. H. (2004a). Cytidine deamination of retroviral DNA by diverse APOBEC proteins. *Curr. Biol.* 14, 1392–1396.

Bishop, K. N., Holmes, R. K., Sheehy, A. M., and Malim, M. H. (2004b). APOBEC-mediated editing of viral RNA. *Science* 305, 645.

Blanc, D., Patience, C., Schulz, T. F., Weiss, R., and Spire, B. (1993). Transcomplementation of VIF- HIV-1 mutants in CEM cells suggests that VIF affects late steps of the viral life cycle. *Virology* 193, 186–192.

Bogerd, H. P., Doehle, B. P., Wiegand, H. L., and Cullen, B. R. (2004). A single amino acid difference in the host APOBEC3G protein controls the primate species specificity of HIV type 1 virion infectivity factor. *Proc. Natl. Acad. Sci. U.S.A.* 101, 3770–3774.

Borman, A. M., Quillent, C., Charneau, P., Dauguet, C., and Clavel, F. (1995). Human immunodeficiency virus type 1 Vif- mutant particles from restrictive cells: role of Vif in correct particle assembly and infectivity. *J. Virol.* 69, 2058–2067.

Bosco, D. A., Eisenmesser, E. Z., Pochapsky, S., Sundquist, W. I., and Kern, D. (2002). Catalysis of *cis/trans* isomerization in native HIV-1 capsid by human cyclophilin A. *Proc. Natl. Acad. Sci. U.S.A.* 99, 5247–5252.

Braaten, D., Aberham, C., Franke, E. K., Yin, L., Phares, W., and Luban, J. (1996a). Cyclosporine A-resistant human immunodeficiency virus type 1 mutants demonstrate that Gag encodes the functional target of cyclophilin A. *J. Virol.* 70, 5170–5176.

Braaten, D., Franke, E. K., and Luban, J. (1996b). Cyclophilin A is required for an early step in the life cycle of human immunodeficiency virus type 1 before the initiation of reverse transcription. *J. Virol.* 70, 3551–3560.

Braaten, D., Franke, E. K., and Luban, J. (1996c). Cyclophilin A is required for the replication of group M human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus SIV(CPZ)GAB but not group O HIV-1 or other primate immunodeficiency viruses. *J. Virol.* 70, 4220–4227.

Braaten, D., and Luban, J. (2001). Cyclophilin A regulates HIV-1 infectivity, as demonstrated by gene targeting in human T cells. *EMBO J.* 20, 1300–1309.

Brennan, G., Kozyrev, Y., and Hu, S. L. (2008). TRIMCyp expression in Old World primates *Macaca nemestrina* and *Macaca fascicularis*. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3569–3574.

Bukovsky, A. A., Weimann, A., Accola, M. A., and Gottlinger, H. G. (1997). Transfer of the HIV-1 cyclophilin-binding site to simian immunodeficiency virus from *Macaca mulatta* can confer both cyclosporin sensitivity and cyclosporin dependence. *Proc. Natl. Acad. Sci. U.S.A.* 94, 10943–10948.

Cagliani, R., Fumagalli, M., Biasin, M., Piacentini, L., Riva, S., Pozzoli, U., Bonaglia, M. C., Bresolin, N., Clerici, M., and Sironi, M. (2010). Long-term balancing selection maintains *trans*-specific polymorphisms in the human TRIM5 gene. *Hum. Genet.* 128, 577–588.

Campbell, E. M., Perez, O., Anderson, J. L., and Hope, T. J. (2008). Visualization of a proteasome-independent intermediate during restriction of HIV-1 by rhesus TRIM5alpha. *J. Cell Biol.* 180, 549–561.

Chen, Z., Telfier, P., Gettie, A., Reed, P., Zhang, L., Ho, D. D., and Marx, P. A. (1996). Genetic characterization of new West African simian immunodeficiency virus SIVsm: geographic clustering of household-derived SIV strains with human immunodeficiency virus type 2 subtypes and genetically diverse viruses from a single feral sooty mangabey troop. *J. Virol.* 70, 3617–3627.

Colgan, J., Yuan, H. E., Franke, E. K., and Luban, J. (1996). Binding of the human immunodeficiency virus type 1 Gag polyprotein to cyclophilin A is mediated by the central region of capsid and requires Gag dimerization. *J. Virol.* 70, 4299–4310.

Cowan, S., Hatziioannou, T., Cunningham, T., Muesing, M. A., Gottlinger, H. G., and Bieniasz, P. D. (2002). Cellular inhibitors with Fv1-like activity restrict human and simian immunodeficiency virus tropism. *Proc. Natl. Acad. Sci. U.S.A.* 99, 11914–11919.

Cullen, B. R. (2006). Role and mechanism of action of the APOBEC3 family of antiretroviral resistance factors. *J. Virol.* 80, 1067–1076.

Dang, Y., Siew, L. M., Wang, X., Han, Y., Lampen, R., and Zheng, Y. H. (2008). Human cytidine deaminase APOBEC3H restricts HIV-1 replication. *J. Biol. Chem.* 283, 11606–11614.

Dang, Y., Wang, X., Esselman, W. J., and Zheng, Y. H. (2006). Identification of APOBEC3DE as another antiretroviral factor from the human APOBEC family. *J. Virol.* 80, 10522–10533.

Diaz-Griffero, F., Li, X., Javanbakht, H., Song, B., Welikala, S., Stremlau, M., and Sodroski, J. (2006). Rapid turnover and polyubiquitylation of the retroviral restriction factor TRIM5. *Virology* 349, 300–315.

Dietrich, E. A., Jones-Engel, L., and Hu, S. L. (2010). Evolution of the antiretroviral restriction factor TRIMCyp in Old World primates. *PLoS ONE* 5, e14019. doi: 10.1371/journal.pone.0014019

Doehle, B. P., Schafer, A., and Cullen, B. R. (2005). Human APOBEC3B is a potent inhibitor of HIV-1 infectivity and is resistant to HIV-1 Vif. *Virology* 339, 281–288.

Dorfman, T., and Gottlinger, H. G. (1996). The human immunodeficiency virus type 1 capsid p2 domain confers sensitivity to the cyclophilin-binding drug SDZ NIM 811. *J. Virol.* 70, 5751–5757.

Fan, L., and Peden, K. (1992). Cell-free transmission of Vif mutants of HIV-1. *Virology* 190, 19–29.

Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T., and Schmid, F. X. (1989). Cyclophilin and peptidyl-prolyl *cis–trans* isomerase are probably identical proteins. *Nature* 337, 476–478.

Fisher, A. G., Ensoli, B., Ivanoff, L., Chamberlain, M., Petteway, S., Ratner, L., Gallo, R. C., and Wong-Staal, F. (1987). The sor gene of HIV-1 is required for efficient virus transmission *in vitro*. *Science* 237, 888–893.

Franke, E. K., and Luban, J. (1996). Inhibition of HIV-1 replication by cyclosporine A or related compounds correlates with the ability to disrupt the Gag-cyclophilin A interaction. *Virology* 222, 279–282.

Franke, E. K., Yuan, H. E., and Luban, J. (1994). Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* 372, 359–362.

Gabuzda, D. H., Lawrence, K., Langhoff, E., Terwilliger, E., Dorfman, T., Haseltine, W. A., and Sodroski, J. (1992). Role of vif in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes. *J. Virol.* 66, 6489–6495.

Gamble, T. R., Vajdos, F. F., Yoo, S., Worthylake, D. K., Houseweart, M., Sundquist, W. I., and Hill, C. P. (1996). Crystal structure of human cyclophilin A bound to the amino-terminal domain of HIV-1 capsid. *Cell* 87, 1285–1294.

Ganser-Pornillos, B. K., Chandrasekaran, V., Pornillos, O., Sodroski, J. G., Sundquist, W. I., and Yeager, M. (2011). Hexagonal assembly of a restricting TRIM5alpha protein. *Proc. Natl. Acad. Sci. U.S.A.* 108, 534–539.

Gao, F., Bailes, E., Robertson, D. L., Chen, Y., Rodenburg, C. M., Michael, S. F., Cummins, L. B., Arthur, L. O., Peeters, M., Shaw, G. M., Sharp, P. M., and Hahn, B. H. (1999). Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* 397, 436–441.

Gautsch, J. W., Elder, J. H., Schindler, J., Jensen, F. C., and Lerner, R. A. (1978). Structural markers on core protein p30 of murine leukemia virus: functional correlation with Fv-1 tropism. *Proc. Natl. Acad. Sci. U.S.A.* 75, 4170–4174.

Goff, S. P. (2004). Genetic control of retrovirus susceptibility in mammalian cells. *Annu. Rev. Genet.* 38, 61–85.

Guo, F., Cen, S., Niu, M., Saadatmand, J., and Kleiman, L. (2006). Inhibition of formula-primed reverse transcription by human APOBEC3G during human immunodeficiency virus type 1 replication. *J. Virol.* 80, 11710–11722.

Guo, F., Cen, S., Niu, M., Yang, Y., Gorelick, R. J., and Kleiman, L. (2007). The interaction of APOBEC3G with human immunodeficiency virus type 1 nucleocapsid inhibits tRNA3Lys annealing to viral RNA. *J. Virol.* 81, 11322–11331.

Harris, R. S., Bishop, K. N., Sheehy, A. M., Craig, H. M., Petersen-Mahrt, S. K., Watt, I. N., Neuberger, M. S., and Malim, M. H. (2003). DNA deamination mediates innate immunity to retroviral infection. *Cell* 113, 803–809.

Harris, R. S., and Liddament, M. T. (2004). Retroviral restriction by APOBEC proteins. *Nat. Rev. Immunol.* 4, 868–877.

Hatziioannou, T., Cowan, S., Goff, S. P., Bieniasz, P. D., and Towers, G. J. (2003). Restriction of multiple divergent retroviruses by Lv1 and Ref1. *EMBO J.* 22, 385–394.

Hatziioannou, T., Cowan, S., von Schwedler, U. K., Sundquist, W. I., and Bieniasz, P. D. (2004a). Species-specific tropism determinants in the human immunodeficiency virus type 1 capsid. *J. Virol.* 78, 6005–6012.

Hatziioannou, T., Perez-Caballero, D., Yang, A., Cowan, S., and Bieniasz, P. D. (2004b). Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5alpha. *Proc. Natl. Acad. Sci. U.S.A.* 101, 10774–10779.

Hatziioannou, T., Perez-Caballero, D., Cowan, S., and Bieniasz, P. D. (2005). Cyclophilin interactions with incoming human immunodeficiency virus type 1 capsids with opposing effects on infectivity in human cells. *J. Virol.* 79, 176–183.

Hatziioannou, T., Princiotta, M., Piatak, M. Jr., Yuan, F., Zhang, F., Lifson, J. D., and Bieniasz, P. D. (2006). Generation of simian-tropic HIV-1 by restriction factor evasion. *Science* 314, 95.

Himathongkham, S., and Luciw, P. A. (1996). Restriction of HIV-1 (subtype B) replication at the entry step in rhesus macaque cells. *Virology* 219, 485–488.

Hirsch, V. M., Olmsted, R. A., Murphey-Corb, M., Purcell, R. H., and Johnson, P. R. (1989). An African primate lentivirus (SIVsm) closely related to HIV-2. *Nature* 339, 389–392.

Hofmann, W., Schubert, D., Labonte, J., Munson, L., Gibson, S., Scammell, J., Ferrigno, P., and Sodroski, J. (1999). Species-specific, postentry barriers to primate immunodeficiency virus infection. *J. Virol.* 73, 10020–10028.

Hrecka, K., Hao, C., Gierszewska, M., Swanson, S. K., Kesik-Brodacka, M., Srivastava, S., Florens, L., Washburn, M. P., and Skowronski, J. (2011). Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. *Nature* 474, 658–661.

Ikeda, Y., Ylinen, L. M., Kahar-Bador, M., and Towers, G. J. (2004). Influence of gag on human immunodeficiency virus type 1 species-specific tropism. *J. Virol.* 78, 11816–11822.

Javanbakht, H., Diaz-Griffero, F., Yuan, W., Yeung, D. F., Li, X., Song, B., and Sodroski, J. (2007). The ability of multimerized cyclophilin A to restrict retrovirus infection. *Virology* 367, 19–29.

Kamada, K., Igarashi, T., Martin, M. A., Khamsri, B., Hatcho, K., Yamashita, T., Fujita, M., Uchiyama, T., and Adachi, A. (2006). Generation of HIV-1 derivatives that productively infect macaque monkey lymphoid cells. *Proc. Natl. Acad. Sci. U.S.A.* 103, 16959–16964.

Kar, A. K., Diaz-Griffero, F., Li, Y., Li, X., and Sodroski, J. (2008). Biochemical and biophysical characterization of a chimeric TRIM21-TRIM5alpha protein. *J. Virol.* 82, 11669–11681.

Keckesova, Z., Ylinen, L. M., and Towers, G. J. (2004). The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proc. Natl. Acad. Sci. U.S.A.* 101, 10780–10785.

Kootstra, N. A., Munk, C., Tonnu, N., Landau, N. R., and Verma, I. M. (2003). Abrogation of postentry restriction of HIV-1-based lentiviral vector transduction in simian cells. *Proc. Natl. Acad. Sci. U.S.A.* 100, 1298–1303.

Kozak, C. A., and Chakraborti, A. (1996). Single amino acid changes in the murine leukemia virus capsid protein gene define the target of Fv1 resistance. *Virology* 225, 300–305.

Laguette, N., Sobhian, B., Casartelli, N., Ringeard, M., Chable-Bessia, C., Segeral, E., Yatim, A., Emiliani, S., Schwartz, O., and Benkirane, M. (2011). SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 474, 654–657.

Lecossier, D., Bouchonnet, F., Clavel, F., and Hance, A. J. (2003). Hypermutation of HIV-1 DNA in the absence of the Vif protein. *Science* 300, 1112.

Le Tortorec, A., and Neil, S. J. (2009). Antagonism to and intracellular sequestration of human tetherin by the human immunodeficiency virus type 2 envelope glycoprotein. *J. Virol.* 83, 11966–11978.

Li, X., and Sodroski, J. (2008). The TRIM5alpha B-box 2 domain promotes cooperative binding to the retroviral capsid by mediating higher-order self-association. *J. Virol.* 82, 11495–11502.

Li, X. Y., Guo, F., Zhang, L., Kleiman, L., and Cen, S. (2007). APOBEC3G inhibits DNA strand transfer during HIV-1 reverse transcription. *J. Biol. Chem.* 282, 32065–32074.

Liddament, M. T., Brown, W. L., Schumacher, A. J., and Harris, R. S. (2004). APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 *in vivo*. *Curr. Biol.* 14, 1385–1391.

Luban, J., Bossolt, K. L., Franke, E. K., Kalpana, G. V., and Goff, S. P. (1993). Human immunodeficiency virus type 1 Gag protein binds to cyclophilins A and B. *Cell* 73, 1067–1078.

Luo, K., Wang, T., Liu, B., Tian, C., Xiao, Z., Kappes, J., and Yu, X. F. (2007). Cytidine deaminases APOBEC3G and APOBEC3F interact with human immunodeficiency virus type 1 integrase and inhibit proviral DNA formation. *J. Virol.* 81, 7238–7248.

Mangeat, B., Turelli, P., Caron, G., Friedli, M., Perrin, L., and Trono, D. (2003). Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. *Nature* 424, 99–103.

Mangeat, B., Turelli, P., Liao, S., and Trono, D. (2004). A single amino acid determinant governs the species-specific sensitivity of APOBEC3G to Vif action. *J. Biol. Chem.* 279, 14481–14483.

Mariani, R., Chen, D., Schrofelbauer, B., Navarro, F., Konig, R., Bollman, B., Munk, C., Nymark-Mcmahon, H., and Landau, N. R. (2003). Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. *Cell* 114, 21–31.

Mbisa, J. L., Barr, R., Thomas, J. A., Vandegraaff, N., Dorweiler, I. J., Svarovskaia, E. S., Brown, W. L., Mansky, L. M., Gorelick, R. J., Harris, R. S., Engelman, A., and Pathak, V. K. (2007). Human immunodeficiency virus type 1 cDNAs produced in the presence of APOBEC3G exhibit defects in plus-strand DNA transfer and integration. *J. Virol.* 81, 7099–7110.

Mische, C. C., Javanbakht, H., Song, B., Diaz-Griffero, F., Stremlau, M., Strack, B., Si, Z., and Sodroski, J. (2005). Retroviral restriction factor TRIM5alpha is a trimer. *J. Virol.* 79, 14446–14450.

Munk, C., Brandt, S. M., Lucero, G., and Landau, N. R. (2002). A dominant block to HIV-1 replication at reverse transcription in simian cells. *Proc. Natl. Acad. Sci. U.S.A.* 99, 13843–13848.

Neil, S. J., Zang, T., and Bieniasz, P. D. (2008). Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. *Nature* 451, 425–430.

Newman, R. M., Hall, L., Connole, M., Chen, G. L., Sato, S., Yuste, E., Diehl, W., Hunter, E., Kaur, A., Miller, G. M., and Johnson, W. E. (2006). Balancing selection and the evolution of functional polymorphism in Old World monkey TRIM5alpha. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19134–19139.

Nisole, S., Stoye, J. P., and Saib, A. (2005). TRIM family proteins: retroviral restriction and antiviral defence. *Nat. Rev. Microbiol.* 3, 799–808.

Ott, D. E., Coren, L. V., Johnson, D. G., Sowder, R. C. II, Arthur, L. O.,

and Henderson, L. E. (1995). Analysis and localization of cyclophilin A found in the virions of human immunodeficiency virus type 1 MN strain. *AIDS Res. Hum. Retroviruses* 11, 1003–1006.

Owens, C. M., Song, B., Perron, M. J., Yang, P. C., Stremlau, M., and Sodroski, J. (2004). Binding and susceptibility to postentry restriction factors in monkey cells are specified by distinct regions of the human immunodeficiency virus type 1 capsid. *J. Virol.* 78, 5423–5437.

Owens, C. M., Yang, P. C., Gottlinger, H., and Sodroski, J. (2003). Human and simian immunodeficiency virus capsid proteins are major viral determinants of early, postentry replication blocks in simian cells. *J. Virol.* 77, 726–731.

Pacheco, B., Finzi, A., Mcgee-Estrada, K., and Sodroski, J. (2010). Species-specific inhibition of foamy viruses from South American monkeys by New World Monkey TRIM5{alpha} proteins. *J. Virol.* 84, 4095–4099.

Perron, M. J., Stremlau, M., Song, B., Ulm, W., Mulligan, R. C., and Sodroski, J. (2004). TRIM5alpha mediates the postentry block to N-tropic murine leukemia viruses in human cells. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11827–11832.

Rein, A., Kashmiri, S. V., Bassin, R. H., Gerwin, B. L., and Duran-Troise, G. (1976). Phenotypic mixing between N- and B-tropic murine leukemia viruses: infectious particles with dual sensitivity to Fv-1 restriction. *Cell* 7, 373–379.

Reymond, A., Meroni, G., Fantozzi, A., Merla, G., Cairo, S., Luzi, L., Riganelli, D., Zanaria, E., Messali, S., Cainarca, S., Guffanti, A., Minucci, S., Pelicci, P. G., and Ballabio, A. (2001). The tripartite motif family identifies cell compartments. *EMBO J.* 20, 2140–2151.

Rose, K. M., Marin, M., Kozak, S. L., and Kabat, D. (2005). Regulated production and anti-HIV type 1 activities of cytidine deaminases APOBEC3B, 3F, and 3G. *AIDS Res. Hum. Retroviruses* 21, 611–619.

Sakai, H., Shibata, R., Sakuragi, J., Sakuragi, S., Kawamura, M., and Adachi, A. (1993). Cell-dependent requirement of human immunodeficiency virus type 1 Vif protein for maturation of virus particles. *J. Virol.* 67, 1663–1666.

Sakuma, R., Noser, J. A., Ohmine, S., and Ikeda, Y. (2007). Rhesus monkey TRIM5alpha restricts HIV-1 production through rapid degradation of viral Gag polyproteins. *Nat. Med.* 13, 631–635.

Sayah, D. M., Sokolskaja, E., Berthoux, L., and Luban, J. (2004). Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* 430, 569–573.

Schrofelbauer, B., Chen, D., and Landau, N. R. (2004). A single amino acid of APOBEC3G controls its species-specific interaction with virion infectivity factor (Vif). *Proc. Natl. Acad. Sci. U.S.A.* 101, 3927–3932.

Shibata, R., Kawamura, M., Sakai, H., Hayami, M., Ishimoto, A., and Adachi, A. (1991). Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells. *J. Virol.* 65, 3514–3520.

Shibata, R., Sakai, H., Kawamura, M., Tokunaga, K., and Adachi, A. (1995). Early replication block of human immunodeficiency virus type 1 in monkey cells. *J. Gen. Virol.* 76(Pt 11), 2723–2730.

Si, Z., Vandegraaff, N., O'huigin, C., Song, B., Yuan, W., Xu, C., Perron, M., Li, X., Marasco, W. A., Engelman, A., Dean, M., and Sodroski, J. (2006). Evolution of a cytoplasmic tripartite motif (TRIM) protein in cows that restricts retroviral infection. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7454–7459.

Simon, J. H., Miller, D. L., Fouchier, R. A., Soares, M. A., Peden, K. W., and Malim, M. H. (1998). The regulation of primate immunodeficiency virus infectivity by Vif is cell species restricted: a role for Vif in determining virus host range and cross-species transmission. *EMBO J.* 17, 1259–1267.

Sokolskaja, E., Sayah, D. M., and Luban, J. (2004). Target cell cyclophilin A modulates human immunodeficiency virus type 1 infectivity. *J. Virol.* 78, 12800–12808.

Song, B., Javanbakht, H., Perron, M., Park, D. H., Stremlau, M., and Sodroski, J. (2005). Retrovirus restriction by TRIM5alpha variants from Old World and New World primates. *J. Virol.* 79, 3930–3937.

Strebel, K., Daugherty, D., Clouse, K., Cohen, D., Folks, T., and Martin, M. A. (1987). The HIV 'A' (sor) gene product is essential for virus infectivity. *Nature* 328, 728–730.

Stremlau, M., Owens, C. M., Perron, M. J., Kiessling, M., Autissier, P., and Sodroski, J. (2004). The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 427, 848–853.

Stremlau, M., Perron, M., Lee, M., Li, Y., Song, B., Javanbakht, H., Diaz-Griffero, F., Anderson, D. J., Sundquist, W. I., and Sodroski, J. (2006). Specific recognition and accelerated uncoating of retroviral capsids by the TRIM5alpha restriction factor. *Proc. Natl. Acad. Sci. U.S.A.* 103, 5514–5519.

Takahashi, N., Hayano, T., and Suzuki, M. (1989). Peptidyl-prolyl *cis–trans* isomerase is the cyclosporin A-binding protein cyclophilin. *Nature* 337, 473–475.

Takeuchi, H., Buckler-White, A., Goila-Gaur, R., Miyagi, E., Khan, M. A., Opi, S., Kao, S., Sokolskaja, E., Pertel, T., Luban, J., and Strebel, K. (2007). Vif counteracts a cyclophilin A-imposed inhibition of simian immunodeficiency viruses in human cells. *J. Virol.* 81, 8080–8090.

Takeuchi, H., Ishii, H., Kuwano, T., Inagaki, N., Akari, H., and Matano, T. (2012). Host cell species-specific effect of cyclosporine A on simian immunodeficiency virus replication. *Retrovirology* 9, 3.

Takeuchi, H., Kao, S., Miyagi, E., Khan, M. A., Buckler-White, A., Plishka, R., and Strebel, K. (2005). Production of infectious SIVagm from human cells requires functional inactivation but not viral exclusion of human APOBEC3G. *J. Biol. Chem.* 280, 375–382.

Thali, M., Bukovsky, A., Kondo, E., Rosenwirth, B., Walsh, C. T., Sodroski, J., and Gottlinger, H. G. (1994). Functional association of cyclophilin A with HIV-1 virions. *Nature* 372, 363–365.

Towers, G., Bock, M., Martin, S., Takeuchi, Y., Stoye, J. P., and Danos, O. (2000). A conserved mechanism of retrovirus restriction in mammals. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12295–12299.

Towers, G. J., Hatziioannou, T., Cowan, S., Goff, S. P., Luban, J., and Bieniasz, P. D. (2003). Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. *Nat. Med.* 9, 1138–1143.

van Manen, D., Rits, M. A., Beugeling, C., Van Dort, K., Schuitemaker, H., and Kootstra, N. A. (2008). The effect of Trim5 polymorphisms on the clinical course of HIV-1 infection. *PLoS Pathog.* 4, e18. doi: 10.1371/journal.ppat.0040018

von Schwedler, U., Song, J., Aiken, C., and Trono, D. (1993). Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. *J. Virol.* 67, 4945–4955.

Wiegand, H. L., Doehle, B. P., Bogerd, H. P., and Cullen, B. R. (2004). A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. *EMBO J.* 23, 2451–2458.

Wilson, S. J., Webb, B. L., Ylinen, L. M., Verschoor, E., Heeney, J. L., and Towers, G. J. (2008). Independent evolution of an antiviral TRIMCyp in rhesus macaques. *Proc. Natl. Acad. Sci. U.S.A.* 105, 3557–3562.

Wu, X., Anderson, J. L., Campbell, E. M., Joseph, A. M., and Hope, T. J. (2006). Proteasome inhibitors uncouple rhesus TRIM5alpha restriction of HIV-1 reverse transcription and infection. *Proc. Natl. Acad. Sci. U.S.A.* 103, 7465–7470.

Xu, H., Svarovskaia, E. S., Barr, R., Zhang, Y., Khan, M. A., Strebel, K., and Pathak, V. K. (2004). A single amino acid substitution in human APOBEC3G antiretroviral enzyme confers resistance to HIV-1 virion infectivity factor-induced depletion. *Proc. Natl. Acad. Sci. U.S.A.* 101, 5652–5657.

Yap, M. W., Nisole, S., Lynch, C., and Stoye, J. P. (2004). TRIM5alpha protein restricts both HIV-1 and murine leukemia virus. *Proc. Natl. Acad. Sci. U.S.A.* 101, 10786–10791.

Ylinen, L. M., Keckesova, Z., Wilson, S. J., Ranasinghe, S., and Towers, G. J. (2005). Differential restriction of human immunodeficiency virus type 2 and simian immunodeficiency virus SIVmac by TRIM5alpha alleles. *J. Virol.* 79, 11580–11587.

Yu, Q., Chen, D., Konig, R., Mariani, R., Unutmaz, D., and Landau, N. R. (2004a). APOBEC3B and APOBEC3C are potent inhibitors of simian immunodeficiency virus replication. *J. Biol. Chem.* 279, 53379–53386.

Yu, Q., Konig, R., Pillai, S., Chiles, K., Kearney, M., Palmer, S., Richman, D., Coffin, J. M., and Landau, N. R. (2004b). Single-strand specificity of APOBEC3G accounts for minus-strand deamination of the HIV genome. *Nat. Struct. Mol. Biol.* 11, 435–442.

Yu, X., Yu, Y., Liu, B., Luo, K., Kong, W., Mao, P., and Yu, X. F. (2003). Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. *Science* 302, 1056–1060.

Zhang, F., Perez-Caballero, D., Hatziioannou, T., and Bieniasz, P. D. (2008). No effect of endogenous TRIM5alpha on HIV-1 production. *Nat. Med.* 14, 235–236; author reply 236–238.

Zhang, F., Wilson, S. J., Landford, W. C., Virgen, B., Gregory, D., Johnson, M. C., Munch, J., Kirchhoff, F., Bieniasz, P. D., and Hatziioannou, T. (2009). Nef proteins from simian immunodeficiency viruses are tetherin antagonists. *Cell Host Microbe* 6, 54–67.

Zhang, H., Yang, B., Pomerantz, R. J., Zhang, C., Arunachalam, S. C., and Gao, L. (2003). The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. *Nature* 424, 94–98.

Zhao, Y., Chen, Y., Schutkowski, M., Fischer, G., and Ke, H. (1997). Cyclophilin A complexed with a fragment of HIV-1 gag protein: insights into HIV-1 infectious activity. *Structure* 5, 139–146.

Zheng, Y. H., Irwin, D., Kurosu, T., Tokunaga, K., Sata, T., and Peterlin, B. M. (2004). Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication. *J. Virol.* 78, 6073–6076.

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