could also inhibit the replication of SIVmac239, while CV1-60tag, which contained the 182bp fragment of HSC-F TRIM5α in the background of CV1-TRIM5α–tag, could not. Conversely, the parental HSC-F-TRIM5α–tag did not inhibit the replication of SIVmac239 at all, while HSC-F+60tag, which contained the 242bp fragment of CV1-TRIM5α in the background of HSC-F-TRIM5α–tag, clearly inhibited SIVmac239 (Fig.4C). We obtained the same results as above when we used SeVs to express parental TRIM5α–tag or chimeras (Fig.4D). Those results unequivocally indicated that the determinant of the species-specific inhibition of SIVmac239 replication is located in the small region of 37 amino acid residues in the SPRY domain of CV1-TRIM5α.

HIV-2 GH123 is sensitive to cynomologus monkey TRIM5α as well as African green monkey TRIM5α.

HIV-2 is closely related to SIVmac (9). We tested whether or not the sensitivity of HIV-2 to various TRIM5αs was similar to that of SIVmac239. C143 cells expressing CV1, HSC-F and their chimeric TRIM5αs were infected with the VSV-G-pseudotyped HIV-2 strain GH123. Contrary to expectation, HSC-F-TRIM5α–tag inhibited HIV-2 replication as CV1-TRIM5α–tag did (Fig.5). Both chimeric TRIM5αs, CV1-60tag and HSC-F+60tag, also inhibited HIV-2 replication to a similar extent (Fig.5). These results indicated that HIV-2 strain GH123 was sensitive to cynomologus monkey TRIM5α, despite its high level of sequence homology to SIVmac239.

Levels of TRIM5α expression in various human cells.

TRIM5α is one of the splicing variants produced from the TRIM5 gene (27). We

established *real-time* PCR *methods to* specifically quantify TRIM5α mRNA as well as to quantify all the splicing variants transcribed from the TRIM5 gene. As shown in Fig.6, the levels of TRIM5 expression in un-stimulated PBMC, or CD14-positive monocytes were much lower than those in established cell lines, such as MT4. The TRIM5α transcript accounted for less than 20% of TRIM5 transcripts in all cell types studied. After 3 days stimulation with PHA and IL2, levels of TRIM5α and all the TRIM5 transcripts dramatically increased and were similar to those of established cell lines.

Discussion

In the present study, we showed that both cynomolgus and African green monkey TRIM5αs could inhibit HIV-1 infection. African green monkey TRIM5α could also inhibit SIVmac infection, whereas cynomolgus monkey TRIM5α could not. Experiments on chimeras of the cynomolgus and African green monkey TRIM5αs unequivocally demonstrated that a small region composed of 37 amino acid residues in the SPRY domain of African green monkey TRIM5α was responsible for restricting the SIVmac infection.

A previous study showed that rhesus monkey TRIM5γ, a splicing variant lacking the SPRY domain, did not suppress HIV-1 infection (33). In the case of TRIM7, the SPRY domain alone was sufficient for binding to its ligand glycogenin (36). Deletion of the entire SPRY domain from TRIM11 also abolished its ability to bind Humanin (21). Therefore, it is reasonable to assume that the variable N-terminal region of the SPRY domain of TRIM5α binds to HIV-1 or SIVmac CA protein. This assumption is consistent with the recent findings that in Owl monkey cells, HIV-1 infection was restricted by a TRIM5-cyclophilin A fusion protein, in which the SPRY domain was replaced with cyclophlin A, a well-known ligand of HIV-1 CA protein (22, 28).

Despite its close similarity to SIVmac, HIV-2 strain GH123 was restricted by cynomolgus monkey TRIM5α as well as by African green monkey TRIM5α. Although both HIV-2 and SIVmac was considered to come from SIVsm (9), it is possible that HIV-2 has been replicating in the human population in the absence of TRIM5α restriction for a certain period and has lost its ability to escape from cynomolgus monkey TRIM5α. However, it was also reported that there was a considerable degree of

variation in the ability to grow in monkey cells among HIV-2 strains (6, 8, 26). Therefore, it is necessary to examine various HIV-2 strains for their sensitivity to human and monkey TRIM5αs before drawing a definite conclusion. It would be also interesting to identify specific amino acid changes determining the sensitivity to cynomolgous monkey TRIM5α in viral CA proteins, since nearly 90% of amino acid residues in SIVmac239 CA protein are conserved in HIV-2GH123.

By using a real time PCR method, we found that levels of TRIM5 gene expression were almost identical among actively dividing human cells, while quiescent human cells showed very low levels of expression. When we analyzed CV1 cells, a level of TRIM5 gene expression was approximately 3×10^6 copies/μg total RNA, being similar to that in other human cell lines examined. Although HIV-1 infection was suppressed in hamster TK-ts13 or human C143 cells expressing CV1-TRIN5α, relatively high levels of TRIM5α, nearly 5×10^7 copies/μg total RNA, appeared to be required for a similar level of suppression to that observed in CV1 cells (data not shown). One possible explanation for this discrepancy is that certain molecules co-operating with TRIM5α also showed species-specificity, and CV1-TRIM5α was not fully supported in hamster and human cells. Because TRIM5 gene products are suspected to be an E3 ubiquitin ligase (34), it is important to identify the E2 ubiquitin-conjugating enzyme interacting with TRIM5α. Alternatively, restriction factors other than TRIM5α may exist in CV1 cells, or certain molecules required for efficient lentivirus infection may be absent in CV1 cells.

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Legends for figures

Figure 1. (A) Alignment of amino acid sequences of African green monkey (AGM: CV1-TRIM5α–type 1), cynomolgus monkey (CM: HSC-F-TRIM5α) and human TRIM5α (Hu: MT4-TRIM5α) predicted from the sequences of the cDNAs used in this study with key domains indicated. (B) Alignment of amino acids sequences of the highly variable region within the SPRY (B30.2) domain of TRIM5α. The rhesus monkey TRIM5α (Rh) sequence published by Stremlau et al. (33) was added. A box indicates the 20-amino acid duplication within African green monkey TRIM5α. (C) A phylogenetic tree of various TRIM5α sequences produced by the UPGMA method.

Figure 2. (A) TK-ts13 *cell clones expressing* MT4-TRIM5α (open squares), CV1-TRIM5α-type 1 (closed circles), CV1- TRIM5α-type 2 (closed squares), HSC-F-TRIM5α (closed triangles), or empty vector (open circles) were exposed to the indicated green fluorescence protein (GFP)-expressing HIV-1 vector. GFP-positive cells were counted with a flowcytometer. Data typical of at least three independent clones for each TRIM5α is shown. (B) Lysates from MT4 cells infected with recombinant Sendai virus (SeV) expressing CV1-TRIM5α-tag, HSC-F-TRIM5α-tag, or the parental Z strain were immunoprecipitated by anti-HA antibody. Resultant immunoprecipitates were visualized by western blotting with an antibody against HA. (C) MT4 cells were mock-infected (open circle), or infected with SeV expressing

CV1-TRIM5α-tag (closed square), HSC-F-TRIM5α-tag (open triangle) or the parental Z strain (closed circle). Nine hours after infection, cells were inoculated with an HIV-1 strain, NL43. Data points are means for triplicate samples with SD.

Figure 3. (A) MT4 (open circles), HSC-F (open triangles) or CV1 (closed square) cells were infected with VSV-pseudotyped NL43 or VSV-pseudotyped SIVmac239. Data points are means for triplicate samples with SD. (B) MT4 cells were mock-infected (open circle), or infected with SeV expressing CV1-TRIM5α-tag (closed square) or HSC-F-TRIM5α-tag (open triangle). Nine hours after infection, cells were inoculated with SIVmac239. Data points are means for triplicate samples with SD.

Figure 4. (A) Schematic representation of chimeric TRIM5α and summary of the results. Filled and open bars denote CV1 and HSC-F sequences, respectively. The length of the fragment from the Sph I site to BamHI site is shown in the box. + denotes suppression of virus by TRIM5α and – denotes no suppression. (B) Lysates from C143 cells expressing CV1-TRIM5α-tag (lane 1), HSC-F-TRIM5α-tag (lane 2), HSC+60tag (lane 3), CV1-60tag (lane 4), or empty vector (lane 5) were immunoprecipitated by anti-HA antibody. Resultant immunoprecipitates were visualized by western blotting with an antibody against HA. (C) C143 cells expressing CV1-TRIM5α-tag (closed squares), HSC-F-TRIM5α-tag (open squares), HSC+60tag (closed

triangles), CV1-60tag (open triangles), or empty vector (open circles) were infected with VSV-pseudotyped NL43 or SIVmac239. Data points are means for triplicate samples with SD. (D) MT4 cells infected with SeV expressing CV1-TRIM5α-tag (closed squares), HSC-F-TRIM5α-tag (open squares), HSC+60tag (closed triangles), CV1-60tag (open triangles), or empty vector (open circles) were infected with NL43 or SIVmac239. Data points are means for triplicate samples with SD.

Figure 5. C143 cells expressing CV1-TRIM5α-tag (closed squares), HSC-F-TRIM5α-tag (open squares), HSC+60tag (closed triangles), CV1-60tag (open triangles), or empty vector (open circles) were infected with VSV-pseudotyped GH123. Data points are means for triplicate samples with SD.

Figure 6. Levels of TRIM5α expression in various human cells. The total amount of TRIM5 gene transcripts was measured by *TaqMan* PCR using a primer-probe set which detects the region common for all splicing variants (open bars). Alpha-isoforms were specifically measured by using a primer-probe set targeting the α-isoform-specific exon boundary (closed bars). Peripheral blood mononuclear cells (PBMC) were stimulated with PHA for 3days (PHA blast) and compared with un-stimulated PBMC (PBMC) or un-stimulated CD14-positive monocytes (mono). A TK-ts13-derived cell clone expressing MT4-TRIM5α-tag was used as a

control.

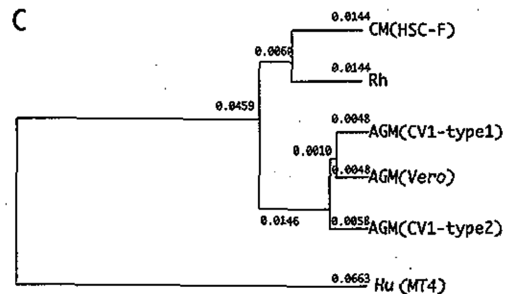
A

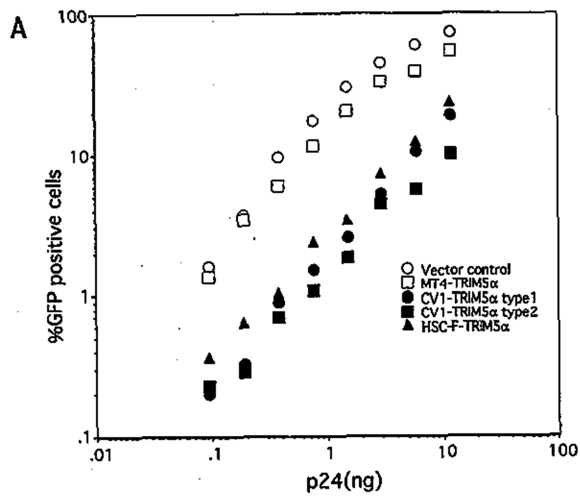
```
                         RING domain                                                      B-box2 domain
AGM    1:  MASGILLNVKEEVTCPICLELLTEPLSLPCGHSFCQACITANHKESMLYKEEERSCPVCRISYQPENIQPNRHVANIVEKLREVKLSPEEGQKVDHCARHGEKLLLFCQEDSKVICWLCE  120
CM     1:  ---------------------------H--------------K------G----------------------------------------------------------------------  120
Hu     1:  ------V----------------Q----D---------L-----K---D- G-S-------------R------------------ -----------------------G--------  118

                                                                                                      Coiled-coil domain
AGM  121:  RSQEHRGHHTFLMEEVAQEYHVKLQTALEMLRQKQQEAEKLEADIREEKASWKIQIDYDKTNVSADFEQLREILDWEESNELQNLEKEEEDILKSLTKSETEMVQQTQYMRELISDLEHR  240
CM   121:  -----------------------------------------------------H-----L-----------R------------K------------K-------V---------  240
Hu   119:  ------------T----R--Q----A-------------E-------------T--Q------L-------D------------------------N----------SL---------  238

                                                                                              SPRY (B30.2) domain
AGM  241:  LQGSMMELLQGVDGIIKRIENMTLKKPKTFHKNQRRVFRAPDLKGMLDMFRELTDVRRYWVDVTLAPNNISHAVIAEDKRQVSYQNPQIMYQAPGSLFGSLTNFNYCTGVRGSQSITSGK  360
CM   241:  ---------------------------------------------------------A--------------------------SR----V--S--T--Q-                  340
Hu   239:  ----V-----D---V---T--V-----E--P----------------EV---------------V------C---S--------SPK---I-G-R-TRYQT                    338

AGM  361:  LTNFNYCTGVLGSQSITSGKHYWEVDVSKKSAWILGVCAGFQPDATYNIEQNENYQPKYGYWVIGLQEGDKYSVFQDGSSHTPFAPFIVPLSVIICPDRVGVFVDYEACTVSFFNITNHG  480
CM   341:  ----------------------------------------S--MC---------------------V---------L--------------------------------------  460
Hu   339:  FV-------I-------------------T--------------MC---K---------------E--V-C-A---S-F---SV------------------L---------------  458

AGM  481:  FLIYKFSQCSFSKPVFPYLNPRKCTVPMTLCSPSS  515
CM   461:  -----------------------------------  495
Hu   459:  -------H----Q-----------G----------  493
```

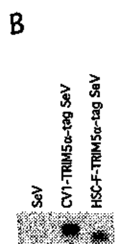
B

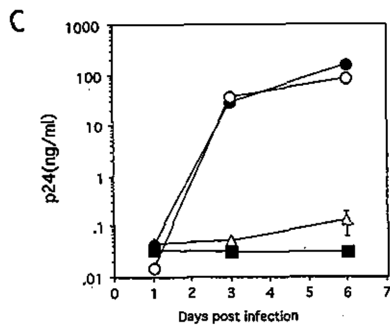
```
                         Sph I
                         ▼
AGM(CV1-type1) 312:  HAVIAEDKRQVSYQNPQIMYQAPGSLFGS|LTNFNYCTGVRGSQSITSGK|
AGM(CV1-type2) 312:  --------------------------S--|------------L--------|
AGM (Vero)     312:  -------------R-------S-------|----S-----P----------|
CM(HSC-F)      312:  ------------SR----V--S--T--Q-|
Rh             312:  ------------SR----------T--TRPS
Hu (MT4)       310:  ---S--------SPK---I-G-R-TRYQT|
                                                                               Bam HI
                                                                                 ▼
                                                |LTNFNYCTGVLGSQSITSGK|HYWEVDVSKKSAWILGV:397
                                                |--------------------|-----------------:397
                                                |--------------------|-----------------:397
                                                |--------------------|-----------------:377
                                                |--------------------|-----------------:379
                                                |FV-------I----------|-----------T-----:375
```

C

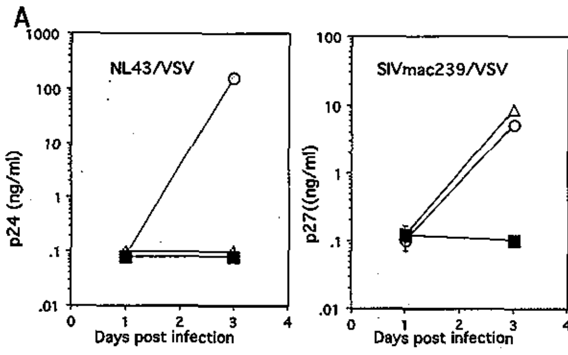



A
%GFP positive cells
p24(ng)
Vector control
MT4-TRIM5α
CV1-TRIM5α type1
CV1-TRIM5α type2
HSC-F-TRIM5α

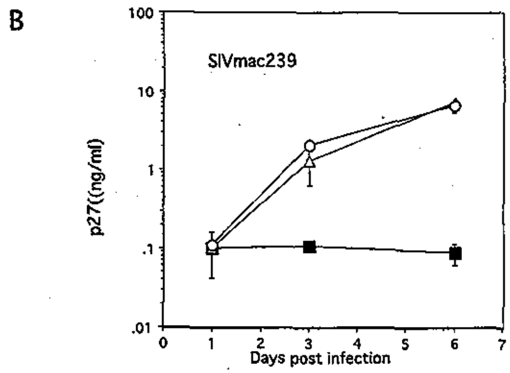

B
SeV
CV1-TRIM5α-tag SeV
HSC-F-TRIM5α-tag SeV

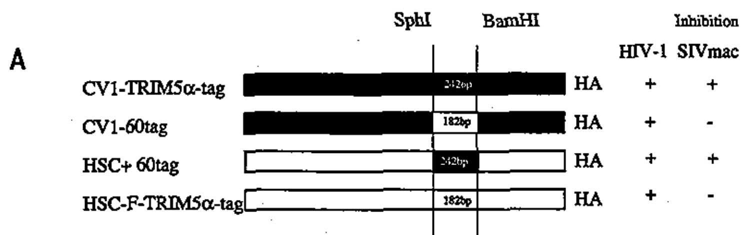

C
p24(ng/ml)
Days post infection

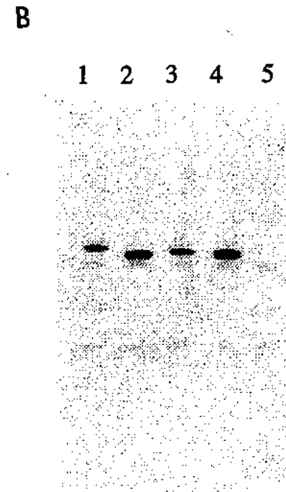
A

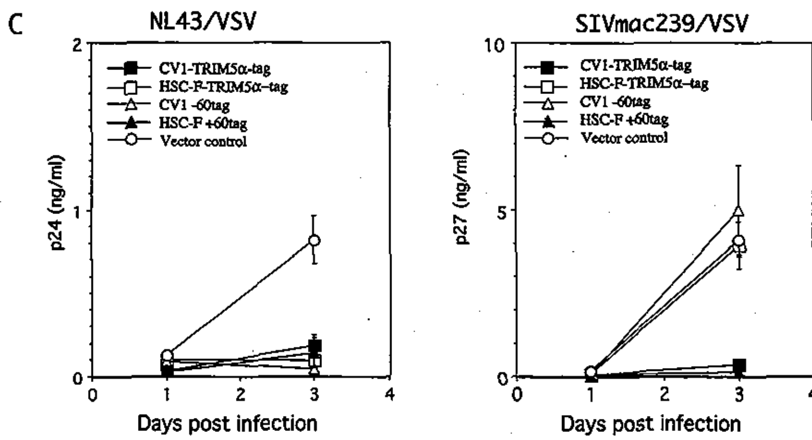


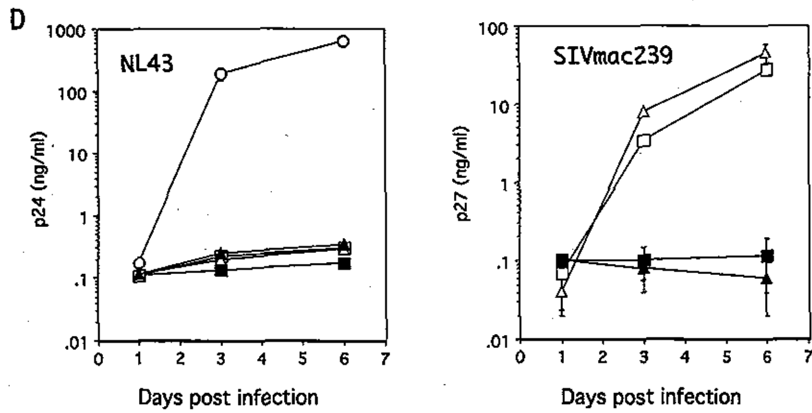
B

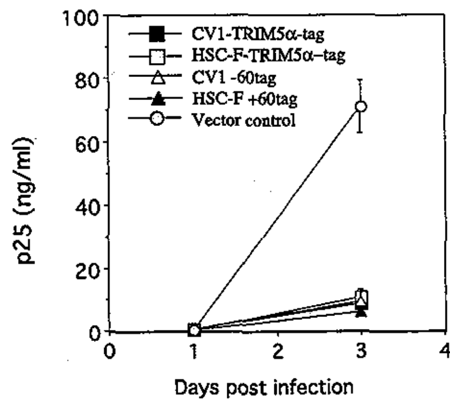



A
SphI
BamHI
Inhibition
HIV-1 SIVmac
CV1-TRIM5α-tag
HA + +
CV1-60tag
182bp
HA + -
HSC+ 60tag
HA + +
HSC-F-TRIM5α-tag
182bp
HA + -


B
1 2 3 4 5


C
NL43/VSV
CV1-TRIM5α-tag
HSC-F-TRIM5α-tag
CV1 -60tag
HSC-F +60tag
Vector control
p24 (ng/ml)
Days post infection
SIVmac239/VSV
p27 (ng/ml)
Days post infection


D
NL43
p24 (ng/ml)
Days post infection
SIVmac239
p27 (ng/ml)
Days post infection



100
80
60
40
20
0
p25 (ng/ml)
0
1
2
3
4
Days post infection
CV1-TRIM5α-tag
HSC-F-TRIM5α–tag
CV1 -60tag
HSC-F +60tag
Vector control