Disruption of Human TRIM5α Antiviral Activity by Nonhuman Primate Orthologues

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TRIM5 is a determinant of species-specific differences in susceptibility to infection by retroviruses bearing particular capsids. Human immunodeficiency virus type 1 (HIV-1) infection is blocked by the alpha isoform of macaque TRIM5α (TRIM5αalpha) or by the product of the owl monkey TRIM5-cyclophilin A gene fusion (TRIMCyp). Human TRIM5α potently restricts specific strains of murine leukemia virus (N-MLV) but has only a modest effect on HIV-1. The amino termini of TRIM5 orthologues are highly conserved and possess a coiled-coil domain that promotes homomultimerization. Here we show that heterologous expression of TRIM5αalpha or TRIMCyp in human cells interferes with the anti-N-MLV activity of endogenous human TRIM5α (TRIM5αalpha). Deletion of the cyclophilin domain from TRIMCyp has no effect on heteromultimerization or colocalization with TRIM5αalpha but prevents interference with anti-N-MLV activity. These data demonstrate that TRIM5 orthologues form heteromultimers and indicate that C-terminal extensions alter virus recognition by multimers of these proteins.

Pools of transduced cells were selected in puromycin and then assessed for susceptibility to infection with HIV-1NL-GFP (2). For each transduced population we also tested the effect on HIV-1 infectivity of cyclosporine A (CsA), a drug that com-

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TRIM5 proteins inhibit the infectivity of a range of different retroviruses in a species-specific fashion (10). The capsid protein (CA) is the viral determinant for susceptibility to this restriction (3, 17). Rhesus macaque TRIM5α (TRIM5αalpha) restricts human immunodeficiency virus type 1 (HIV-1) replication (18). Human TRIM5α (TRIM5αalpha) restricts “N-tropic” strains of the murine leukemia virus (N-MLV) (8, 9, 13, 21). In owl monkey cells, HIV-1 is inhibited by TRIMCyp, the product of the TRIM5-cyclophilin (CypA) gene fusion (12, 15). The restriction activities of TRIM5αalpha and TRIMCyp are conferred to nonrestrictive cells upon transduction of the respective cDNAs (15, 18). CypA modulates the restriction of HIV-1 in human and owl monkey cells in opposite ways: HIV-1 CA binding to the CypA domain of TRIMCyp (4, 5) is necessary for inhibition of HIV-1 in owl monkey cells, while “free” CypA appears to protect HIV-1 from restriction in human cells (20).

At the C-terminus of TRIM5α is a variable SPRY domain that determines the species specificity of restriction (8, 9, 21). In owl monkeys, the SPRY domain was replaced by CypA via L1-mediated retrotransposition (12, 15). TRIM5α and TRIMCyp both contain a tripartite motif, composed of RING finger, B-Box, and coiled-coil domains (14, 15), that exhibits E3 ubiquitin ligase activity (6, 18). The coiled-coil domain promotes the formation of TRIM5 homomultimers (14). Here we asked whether TRIM5α or TRIMCyp associates with TRIM5αalpha and alters the antiviral activity of the human protein.

We transduced human rhabdomyosarcoma TE671 cells (19) with previously described LPCX vectors (15, 18) bearing cDNAs for TRIM5αalpha, TRIMCyp, or owl monkey CypA. Cells were also transduced with a vector expressing TRIMStop, a truncated version of TRIMCyp lacking the CypA domain (15).

FIG. 1. Inhibition of HIV-1 in human TE671 cells expressing owl monkey TRIMCyp or Rhesus macaque TRIM5α. LPCX-based retroviral vectors were used to transduce the indicated genes into human TE671 cells. Vect, Cyp, TS, TC and T5rh designate, respectively, Vector control, owl monkey CypA, TRIMStop, TRIMCyp, and TRIM5αalpha. These cells were then infected for 16 h with HIV-1NL-GFP (pseudotyped vesicular stomatitis virus G protein) in the presence (+) or absence (-) of CsA (5 μM). One twentieth of the cells was maintained in culture for another day and used to determine the percentage of GFP-positive cells by FACS (bottom of the figure). Total DNA was extracted from the remainder of the cells 16 h after infection, and 5 μg of each DNA sample was analyzed by Southern blotting. The positions of the linear, 1-LTR, and 2-LTR HIV-1 cDNA species are indicated on the left. “Total” DNA refers to a band specific to all HIV-1 cDNA forms, including the integrated DNA.
We monitored the percentage of infected (GFP-expressing) cells by fluorescence-activated cell sorting (FACS) and the synthesis of viral cDNA in the infected cells using a Southern blot designed to detect full-length, linear viral cDNA and circular viral cDNAs that form in the nucleus (1, 2, 22).

As expected, both TRIM5α<sub>rh</sub> and TRIMCyp inhibited HIV-1 infection of TE671 cells and inhibited HIV-1 cDNA synthesis (Fig. 1). CsA treatment rescued HIV-1 replication in TRIMCyp-expressing cells, as previously reported (15). CsA also enhanced HIV-1 infection of TE671-TRIM5α<sub>rh</sub>, indicating that CsA partially countered HIV-1 restriction when TRIM5α<sub>rh</sub> was expressed in human cells. This result was expected, as CsA also counteracts the restriction to HIV-1 in Old World monkey cells (1). At the high multiplicity of infection used here, CsA had little effect on HIV-1 infectivity; in experiments using a lower multiplicity of infection, CsA modestly decreased HIV-1 infection of control TE671 cells, as previously reported (data not shown and reference 20).

Next, we analyzed MLV replication in TE671-TRIM5α<sub>rh</sub>, TE671-TRIMCyp, and the control TE671-vector cells. We infected these cells with N- or B-tropic, GFP-expressing MLV vectors (2) that had identical titers in nonrestrictive Mus dunni tail fibroblasts and were normalized based on infection of these cells (19). In control cells, B-MLV was 200-fold more infectious than N-MLV (Fig. 2A). As<sub>2</sub>O<sub>3</sub>, a drug that counteracts restriction to N-MLV in TE671 cells (2, 9), specifically increased N-MLV infectivity by 10-fold or more (Fig. 2). In cells expressing TRIM5α<sub>rh</sub>, the N-MLV replication defect was reduced to about 20-fold (Fig. 2A); in cells expressing TRIMCyp, N-tropic restriction was fully abrogated (Fig. 2A). In either case, As<sub>2</sub>O<sub>3</sub> no longer enhanced infectivity, as would be expected for cells lacking the antiviral activity targeting N-MLV.

We also performed the reciprocal experiment, transducing owl monkey OMK cells with TRIM5α<sub>hu</sub> and challenging them...
with MLV or HIV-1. TRIM5α/H9251 had only a small (approximately twofold) restrictive effect on the replication of N-MLV and had no effect on B-MLV (Fig. 2B). Restriction of HIV-1 was similar in cells expressing TRIM5α/H9251 to that in the control cells (Fig. 2C), and HIV-1 replication was rescued by CsA in both cell lines, consistent with previous reports (15, 20). Altogether, the data in Fig. 2 suggest that TRIMCyp is dominant over TRIM5α/H9251.

Since TRIMCyp (but not TRIM5α/H9251rh) completely suppressed the antiviral activity of TRIM5α/H9251, we further investigated the effects of this orthologue. TRIMStop, a truncated version of TRIMCyp lacking the CypA domain (15), did not affect the capacity of TE671 cells to restrict N-MLV replication (Fig. 3A). To determine if this was because TRIMStop was expressed at lower levels than TRIMCyp, we performed reverse transcription-PCR (RT-PCR) using conventional methods (15). TRIMStop mRNA was expressed at least as well as TRIMCyp mRNA (Fig. 3B); TRIM5α/H9251 was expressed at similar levels in all three cell lines (Fig. 3B). By using Western blotting, we could not detect TRIM protein in the TE671 cell lines, but when 293T cells were transfected with the LPCX plasmids, TRIMStop was expressed at higher levels than TRIMCyp (Fig. 3C).

TRIM5 homomultimerization is promoted by the coiled-coil domain (14), which TRIMCyp also possesses. We hypothesized that the different TRIM5 orthologues heteromultimerize with each other and that TRIMStop did not interfere with TRIM5α/H9251 activity because these particular proteins are incapable of interacting. We used the yeast two-hybrid system to analyze interactions between TRIMStop, TRIMCyp, TRIM5α/H9251, TRIM5α/H11001, HIV-1 Gag, and huTRIMStop, a version of TRIM5α/H9251 which, like TRIMCyp, lacks the C-terminal SPRY domain. Fusions of these proteins with LexA and/or B42 were constructed, and the interactions between fusion proteins were analyzed using a previously described system (7) in which reporter gene β-galactosidase activity was assessed with a quantitative assay (16). TRIMCyp interacted equally well with TRIMCyp, TRIM5α/H9251, TRIM5α/H11001, HIV-1 Gag, and huTRIMStop, TRIMStop, huTRIMStop, and HIV-1 Gag (Fig. 4A). Though TRIMStop was not able to interact with Gag, presumably because the CypA domain was deleted, this protein was as competent as TRIMCyp in interactions with each of the TRIM5 orthologues.

To investigate TRIM5:TRIMCyp heterodimerization in mammalian cells, we expressed TRIM5α/H9251 in fusion with glutathione-S-transferase (GST) and transfected 293T cells with either GST or GST-TRIM5α/H9251. These cells were cotransfected with the LPCX plasmid constructs described above that express TRIMCyp or TRIMStop. GST pull down on glutathione-Sepharose beads (Sigma) followed by Western blotting with anti-TRIM antibody showed that both TRIMCyp and TRIMStop associated with TRIM5α/H9251 (Fig. 4B). The blot was also probed with an anti-cyclophilin A antibody, confirming the identity of TRIMCyp (Fig. 4B).

Finally, to examine the distribution of the tagged proteins in cells by immunofluorescence, we cloned TRIMCyp and
FIG. 4. TRIMCyp and TRIMStop both bind TRIM5α<sub>hu</sub>. (A) Yeast two-hybrid system. TRIM5α<sub>hu</sub>, TRIM5α<sub>rh</sub>, TRIMCyp, TRIMStop, huTRIMStop, and HIV-1 Gag were fused to lexA. TRIMCyp and TRIMStop were expressed in fusion with the B42 activation domain. Pairs of fused LexA and B42 expression plasmids were transformed into <i>Saccharomyces cerevisiae</i> strain EGY48. For each transformant, β-galactosidase activity for three colonies is reported in Miller units with the standard deviation. (B) Binding in mammalian cells. TRIM5α<sub>hu</sub> was fused to GST. 293T cells were transfected with GST or GST-TRIM5α<sub>hu</sub> (GST-T5<sub>α<sub>hu</sub></sub>) and cotransfected with LPCX (C), LPCX-TRIMCyp (TC), or LPCX-TRIMStop (TS). Thirty-six hours later, the cells were lysed in 50 mM Tris-Cl (pH 8.0), 150 mM NaCl, 1% NP40, 0.1% SDS, and GST was pulled down using glutathione-coated Sepharose beads (Pharmacia). One percent of the pre-pull-down lysate and 25% of the bound proteins were analyzed by Western blotting, using polyclonal antibodies directed against TRIM5, cyclophilin A, or GST.
TRIMStop into p3xFLAG.CMV (SIGMA), encoding N-terminal FLAG-tagged versions of the two proteins. These constructs were each transfected into TE671 cells and detected with anti-FLAG antibody. Both TRIMCyp and TRIMStop were diffusely distributed in the cytoplasm and concentrated around the nucleus in about half of the cells. TRIM5α<sub>hu</sub>, expressed in fusion with GST and detected with anti-GST antibody, was cytoplasmic as well and was partly localized to cytoplasmic bodies as previously reported (14). When TRIMCyp or TRIMStop were coexpressed in the same cells with TRIM5α<sub>hu</sub>, both TRIMCyp and TRIMStop showed partial localization to TRIM5α<sub>hu</sub> cytoplasmic bodies, suggesting that TRIMCyp and TRIMStop are both capable of heterodimerizing with TRIM5α<sub>hu</sub> in cells. Altogether, the results shown in Fig. 4 and 5 show that TRIM5 orthologues from different primate species heteromultimerize with each other and that failure of TRIMStop to block restriction activity of TRIM5α<sub>hu</sub> is not due to failure to multimerize.

In this work we show that TRIM5 proteins from different primate species interact with each other and can interfere with each other’s function. The CypA domain of TRIMCyp is necessary for interference with endogenous TRIM5α (Fig. 3). CsA did not affect interference by TRIMCyp (data not shown), demonstrating that CypA peptidyl-isomerase activity was not relevant for the effect. A likely possibility is that when TRIM5α<sub>hu</sub>/TRIMCyp heterodimers are formed, the cyclophilin A domain of TRIMCyp interferes with TRIM5α<sub>hu</sub>’s ability to recognize the N-MLV target. Perhaps TRIM5α and TRIMCyp function as multimers, with the TRIM5 C-terminus facing the viral target (consistent with the role of CypA in binding HIV-1 CA). Similar to the results reported here with different TRIM5 orthologues, the gamma isoform of macaque TRIM5 was found to inhibit TRIM5α<sub>rh</sub> anti-HIV restriction activity (18).

We thank Joseph Sodroski and Matthew Stremlau for reagents.

This work was funded by National Institutes of Health grant RO1 AI36199 and used core facilities of the Columbia-Rockefeller Center for AIDS Research. L.B. was supported by a fellowship (106524-35-RFHF) from the American Foundation for AIDS Research (AmFAR). D.M.S. was supported by the Columbia University College of Physicians and Surgeons Medical Scientist Training Program.

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