A dominant-negative effect of cynomolgus monkey tripartite motif protein TRIM5α on anti-simian immunodeficiency virus SIVmac activity of an African green monkey orthologue

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Abstract

African green monkey (AGM) tripartite motif protein (TRIM) 5α can inhibit both human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus SIVmac, whereas cynomolgus monkey (CM) TRIM5α can inhibit HIV-1, but not SIVmac. We previously reported that the 17-amino-acid region and an adjacent 20-amino-acid duplication in the SPRY(B30.2) domain of AGM TRIM5α determined the species specificity. In the present study, we demonstrated that CM TRIM5α had a dominant-negative effect on the anti-SIVmac activity of AGM TRIM5α. In contrast, mutant TRIM5αs lacking the 20-amino-acid duplication did not have the dominant-negative effect, even though they failed to restrict SIVmac. These results indicated that oligomerization of the SPRY domain is required for anti-SIVmac activity and suggest that tight interaction between the viral capsid and all three molecules in one TRIM5α trimer may not be necessary for restriction activity. © 2006 Elsevier Inc. All rights reserved.

Keywords: TRIM5α; Human immunodeficiency virus; Simian immunodeficiency virus; SPRY domain; Dominant-negative; African green monkey; Cynomolgus monkey

Introduction

Human immunodeficiency virus type 1 (HIV-1) efficiently enters cells of Old World monkeys but encounters a block before reverse transcription (Himathongkham and Luciw, 1996; Hofmann et al., 1999; Shibata et al., 1995). This restriction is mediated by a dominant-repressive factor, tripartite motif protein (TRIM) 5α (Stremlau et al., 2004). Rhesus and cynomolgus monkey (CM) TRIM5α restricted HIV-1 infection (Nakayama et al., 2005), whereas human TRIM5α restricts N-tropic murine leukemia virus (N-MLV) infection (Hatziioannou et al., 2004; Keckesova et al., 2004; Perron et al., 2004; Yap et al., 2004). African green monkey (AGM) TRIM5α restricts simian immunodeficiency virus SIVmac, human immunodeficiency virus type 2 (HIV-2), and equine infectious anemia virus in addition to HIV-1 infection (Hatziioannou et al., 2004; Keckesova et al., 2004; Nakayama et al., 2005). TRIM5α shares a common amino-terminal TRIM motif, comprising RING, B-box, and coiled-coil domains, with other splicing variants, and encodes a unique SPRY (B30.2) domain (Reymond et al., 2001). Several recombinant studies between human and rhesus monkey TRIM5α revealed that the determinant of the species specificity lies in the SPRY (B30.2) domain (Reymond et al., 2001). Several recombinant studies between human and rhesus monkey TRIM5α revealed that the determinant of the species specificity lies in the SPRY (B30.2) domain of TRIM5α (Perez-Caballero et al., 2005; Sawyer et al., 2005; Stremlau et al., 2005; Yap et al., 2005). We also previously demonstrated that 17-amino-acid residues and the adjacent AGM-specific 20-amino-acid duplication in the SPRY domain determined species-specific restriction of SIVmac (Nakayama et al., 2005). Owl monkey TRIM5 is fused with cyclophilin A (cypA) within the SPRY domain and has strong anti-HIV-1 activity (Nisole et al., 2004; Sayah et al., 2004). Structural predictions suggested that the coiled-coil domain exhibits a propensity to form oligomers. It was first reported that rhesus TRIM5γ, a splice variant lacking SPRY (B30.2) domain, had a dominant-negative effect on HIV-1 restriction activity of rhesus TRIM5α (Stremlau et al., 2004).
Recently, Berthoux et al. reported that expression of rhesus monkey TRIM5α or owl monkey TRIM5-cypA in human cells interferes with the anti-N-MLV activity of endogenous human TRIM5α (Berthoux et al., 2005). Here, we show that CM TRIM5α lacking anti-SIVmac activity had a dominant-negative effect on the anti-SIVmac activity of AGM TRIM5α.

Fig. 1. (A) Schematic representation of chimeric tripartite motif protein (TRIM) 5α and summary of the results. Alignment of amino-acid sequences of the highly variable region of the SPRY (B30.2) domain of CV1 and HSC-F TRIM5αs is shown on the top. Boxes in amino-acid sequence denote the CV1-TRIM5α-specific 20-amino-acid duplication. Filled and open bars denote CV1 and HSC-F sequences, respectively. "D" denotes the CV1-TRIM5α-specific 20-amino-acid duplication. + and − on the column of inhibition denote full and no suppression, respectively. + and − on the column of interference denote presence and absence of the dominant-negative effect on anti-SIVmac activity of CV1-TRIM5α, respectively. Anti-human immunodeficiency virus type 1 (HIV-1) activity of those TRIM5αs was reported previously (Nakayama et al., 2005). (B) MT4 cells infected with 10 plaque-forming units per cell of a recombinant Sendai virus (SeV) expressing hemagglutinin (HA)-tagged version of CV1-TRIM5α-tag (closed squares), HSC-F-TRIM5α-tag (open squares), CV1-60tag (open triangles), CV1-delete-tag (closed circles), HSC-F-delete-tag (open circles), or HSC-F +60tag (closed triangles) were challenged with simian immunodeficiency virus SIVmac239, and culture supernatants were assayed for levels of p27. The representative results of three independent experiments with similar results are shown. (C) MT4 cells co-infected with 5 plaque-forming unit per cell of an SeV expressing CV1-TRIMα-tag and 5 plaque-forming unit per cell of an SeV expressing HSC-F-TRIM5α-tag (open squares), CV1-60tag (open triangles), CV1-delete-tag (closed circles), HSC-F-delete-tag (open circles), or HSC-F +60tag (closed triangles) were challenged with simian immunodeficiency virus SIVmac239, and culture supernatants were assayed for levels of p27. The representative result of three independent experiments with similar results is shown. (D) Comparison of expression levels of HA-tagged TRIM5α proteins. MT4 cells were infected with SeVs expressing CV1-TRIMα-tag and 5 plaque-forming units per cell of each SeV were simultaneously infected, 5 plaque-forming units per cell of each SeV were inoculated (lanes 6 to 9). Twelve hours after SeV infection, cells were lysed and immunoprecipitated by an anti-HA antibody. A closed triangle indicates HA-tagged CV1-TRIM5αα, which contained the 20-amino-acid duplication. An open triangle indicates an HA-tagged HSC-F-TRIM5αα (lanes 2 and 6), CV1-60 (lanes 3 and 7), CV1-delete (lanes 4 and 8), and HSC-F-delete (lanes 5 and 9), lacking the 20-amino-acid duplication.
Results

Among Old World monkey cells, an AGM kidney cell line CV1 is resistant to not only HIV-1, but also simian immunodeficiency virus SIVmac. TRIM5α cDNA of CV1 and HSC-F cells, a T cell line from a CM, were cloned into Sendai virus vectors (SeVs). As described previously (Nakayama et al., 2005), both CV1- and HSC-F-TRIM5αs inhibited HIV-1 infection (Fig. 1A). CV1-TRIM5α could also inhibit SIVmac infection, whereas HSC-F-TRIM5α could not (Figs. 1A, B). A comparison of the CV1 and HSC-F TRIM5α sequences showed the presence of a highly variable region in the N-terminal portion of the SPRY domain. In this region, CV1-TRIM5α had a 20-amino-acid duplication, which was totally absent in HSC-F-TRIM5α (Fig. 1A). We constructed chimeric TRIM5αs from hemagglutinin (HA)-tagged version of HSC-F-TRIM5α and CV1-TRIM5α by using SpIHI and BamHI restriction enzyme digestion (Fig. 1A). HSC-F +60tag contained the 242 bp SpIHI-BamHI fragment of CV1-TRIM5α in the background of HA-tagged version of HSC-F-TRIM5α. The reciprocal chimera, CV1-60tag, contained a corresponding 182 bp fragment of HSC-F-TRIM5α in the background of HA-tagged version of CV1-TRIM5α. In this SpIHI-BamHI fragment, the differences between CV1 and HSC-F TRIM5α were located in a small region of 37-amino-acid residues including CV1-specific 20-amino-acid duplication and adjacent 17-amino-acid residues. In CV1-delete-tag, CV1-specific 20-amino-acid duplication was deleted by PCR mutagenesis. In HSC-F-delete-tag, the SpIHI-BamHI fragment of HSC-F-TRIM5α was replaced with the corresponding fragment of CV1-delete-tag. As shown in Fig. 1B, HSC-F +60tag, the chimeric TRIM5α containing 37-amino-acid residues from CV1-TRIM5α in the background of HSC-F-TRIM5α fully acquired the ability to inhibit SIVmac infection. Conversely, all the chimeric TRIM5αs lacking the CV1-specific 20-amino-acid duplication (CV1-60tag, CV1-delete-tag, and HSC-F-delete-tag) failed to restrict SIVmac infection (Figs. 1A, B). All the mutant TRIM5αs described above showed potent anti-HIV-1 activity as described previously (Fig. 1A) (Nakayama et al., 2005).

In the present study, we examined whether or not simultaneous expression of HSC-F-TRIM5α or TRIM5α SPRY domain mutants that failed to inhibit SIVmac infection altered the anti-SIVmac activity of CV1-TRIM5α. Human T cell line MT4 cells were first infected with an SeV expressing CV1-TRIM5α-tag and an SeV expressing HSC-F-TRIM5α-tag, CV1-60tag, CV1-delete-tag, HSC-F-delete-tag, or HSC-F +60tag, incubated at 37 °C for 9 h, and then challenged with SIVmac239. As shown in Fig. 1C, HSC-F-TRIM5α and CV1-60tag strongly interfered with the anti-SIVmac activity of CV1-TRIM5α-tag. The HSC-F-TRIM5α and CV1-60tag shared the 17-amino-acid region of the HSC-F-TRIM5α SPRY domain and lacked the CV1-specific 20-amino-acid duplication.

Contrary to our expectation, however, the CV1-delete-tag and HSC-F-delete-tag, which failed to restrict SIVmac infection, also failed to show the dominant-negative effect on anti-SIVmac activity of CV1-TRIM5α-tag. Both of these mutant TRIM5αs lacked the CV1-specific 20-amino-acid duplication but shared the 17-amino-acid region of the SPRY domain with the parental CV1-TRIM5α. These results indicated that the ability of TRIM5α to interfere with the anti-SIVmac activity of CV1-TRIM5α was not only determined by its lack of restriction activity against SIVmac. On the other hand, the HSC-F +60tag that inhibited SIVmac infection (Fig. 1B) did not interfere with the restricting activity of CV1-TRIM5α-tag (Fig. 1C).

The levels of expression of those TRIM5αs were comparable to each other (Fig. 1D lanes 1 to 5). Co-expression of CV1-TRIM5α-tag did not alter the levels of expression of those TRIM5αs (Fig. 1D, lanes 6 to 9). Conversely, the expression levels of co-expressed CV1-TRIM5α-tag were also comparable to each other (Fig. 1D, lanes 6 to 9).

A single round replication assay further confirmed the above findings since co-expression of HSC-F-TRIM5α-tag or CV1-60tag with CV1-TRIM5α-tag strongly interfered with anti-SIVmac activity of CV1-TRIM5α-tag in CD4 negative HeLa cells infected with vesicular stomatitis virus G-protein-pseudotyped SIVmac. Similarly, co-expression of CV1-delete-tag or HSC-F-delete-tag with CV1-TRIM5α-tag did not interfere with anti-SIVmac activity of CV1-TRIM5α-tag in a single round replication assay (Fig. 2).

To assess whether or not these parental and chimeric TRIM5αs really form hetero-oligomer, we performed immunoprecipitation followed by Western blot analysis. We used hamster kidney cell line TK-ts13 since it lacks primate TRIM5α proteins. Cells were transfected with plasmid expressing various HA-tagged version of chimeric TRIM5αs with a plasmid expressing myc-tagged version of CV1-TRIM5α. Sixty hours after transfection, cells were lysed and TRIM5α proteins were immunoprecipitated with an antibody against HA. Resultant immunoprecipitants were subjected to Western blot analysis using...
anti-myc or anti-HA antibodies as probe. As shown in Fig. 3B, myc-tagged CV1-TRIM5αs were clearly co-immunoprecipitated with HA-tagged version of CV1-, CV1-60, CV1-delete, HSC-F, and HSC-F-delete TRIM5α proteins. These results suggested that not only homologous but also heterologous oligomers of TRIM5αs examined above were formed in cells. A heat shock protein inhibitor geldanamycin was reported to inhibit cytoplasmic body formation (Song et al., 2005). Addition of 10 μM of geldanamycin in the culture media did not affect the homo- or hetero-oligomerization of TRIM5αs (data not shown), excluding the possibility that co-immunoprecipitation of CV1-TRIM5α with various TRIM5αs was caused by non-specific aggregation of TRIM5α proteins in cytoplasmic bodies. Therefore, the lack of the dominant-negative effect of chimeric TRIM5α on anti-SIVmac activity of CV-1-TRIM5α was not due to a lack of oligomer formation between CV1 and chimeric TRIM5αs.

**Discussion**

It was first reported that rhesus monkey TRIM5γ, a splice variant lacking SPRY (B30.2) domain, had a dominant-negative effect on HIV-1 restriction activity of rhesus monkey TRIM5α (Stremlau et al., 2004). Subsequently, Berthoux et al. reported that expression of rhesus monkey TRIM5α or owl monkey TRIM5-cypA in human cells interferes with the anti-N-MLV activity of endogenous human TRIM5α (Berthoux et al., 2005). Perez-Caballero et al. reported that truncated human TRIM5α proteins lacking the B30.2/SPRY domain form heteromers with full-length human TRIM5α and are dominant inhibitors of its N-MLV restriction (Perez-Caballero et al., 2005). Furthermore, Mishe et al. clearly showed that the TRIM5α protein oligomerizes into trimers and proposed that trimerization potentially allows SPRY domain to interact with threefold pseudosymmetrical structures on retroviral capsids (Mishe et al., 2005).

In the present study, we demonstrated that the HSC-F-TRIM5α had a dominant-negative effect on the anti-SIVmac activity of CV1-TRIM5α. A chimeric TRIM5α carrying the HSC-F-derived 17-amino-acid residues without the CV1-specific 20-amino-acid duplication (CV1-60tag) also showed a dominant-negative effect. These results emphasized the importance of the 17-amino-acid residue as well as the 20-amino-acid duplication of CV1-TRIM5α in the restriction of SIVmac infection and further suggested that the oligomeric structure of SPRY domain is required for the restriction of SIVmac infection by CV1-TRIM5α. Nevertheless, two mutant TRIM5αs carrying the CV1-derived 17-amino-acid region without the 20-amino-acid duplication (HSC-F-delete-tag and CV1-delete-tag) did not show the dominant-negative effect, even though all the chimeric and mutant TRIM5αs mentioned above failed to restrict SIVmac as the parental HSC-F-TRIM5α-tag and CV1-60tag. Immunoprecipitation and Western blot analysis of cells co-transfected with myc-tagged version of CV1-TRIM5α and HA-tagged versions of those chimeric and mutant TRIM5αs confirmed their heterologous oligomers formation regardless of the presence or absence of a dominant-negative effect on anti-SIVmac activity of CV1-TRIM5α. These results indicated that it is not necessary for all the TRIM5α molecules in one trimer to carry the 20-amino-acid duplication for anti-SIVmac activity. It is possible that tight interaction between the viral capsid and all three TRIM5α molecules in one trimer may not be necessary for restriction activity. Alternatively, a certain capsid modification, which is not observed on all the capsid molecules in the virions, may mimic the CV1-specific 20-amino-acid duplication.

It is expected that four types of trimer form in heterologous TRIM5α expressing cells. In cells expressing equal amount of CV1-TRIM5α and HSC-F-TRIM5α, (1) one out of eight trimers would be a homo-trimer of CV1-TRIM5α molecules, (2) three out of eight trimers would be hetero-trimers composed of two molecules of CV1-TRIM5α and one molecule of HSC-F-TRIM5α, (3) three out of eight trimers would be hetero-trimers composed of one molecules of CV1-TRIM5α and two molecules of HSC-F-TRIM5α, and (4) one out of eight trimers would be a homo-trimer of HSC-F-TRIM5α molecules. The CV1-TRIM5α homo-trimer would completely inhibit SIVmac infection, whereas HSC-F-TRIM5α homo-trimer would fail to inhibit SIVmac. We showed that cells expressing CV1-TRIM5α-tag and HSC-F-TRIM5α-tag produced approximately two third of p27 antigen of the cells expressing HSC-F-TRIM5α-tag alone (Fig. 2). The slight reduction of p27 antigen production is most likely caused by the presence of at least one out of eight TRIM5α trimers possessing anti-SIVmac activity. Conversely, cells co-expressing CV1-delete-tag or HSC-F-delete-tag with CV1-TRIM5α-tag showed slight but significant increase in p27 production upon SIVmac infection compared with those expressing CV1-TRIM5α-tag alone. This is again explained by the presence of one out of eight TRIM5α trimers lacking anti-SIVmac activity. Therefore, determination of the exact numbers of molecules carrying the 20-amino-acid duplication within an
expressing myc-tagged version of CV1-TRIM5α cloned into pcDNA3.1(μTK-ts13) cells were co-transfected with 10 μg of plasmid expressing HA-tagged TRIM5α by using calcium phosphate method. Sixty hours after transfection, cells were harvested and lysed in lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P40, 0.5% sodium deoxycholate). TRIM5α proteins in the lysates were precipitated with anti-HA High Affinity rat monoclonal antibody (Roche) using a Protein G Immunoprecipitation Kit (Roche). Precipitated materials were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins in the gel were then electrophoretically transferred to a membrane (Immobilon, Millipore). Blots were blocked and probed with anti-myc mouse monoclonal antibody (9B11, Cell Signaling) or anti-HA High Affinity rat monoclonal antibody (Roche) overnight at 4 °C. Blots were then incubated with peroxidase-conjugated anti-mouse IgG (Kirkegaard and Perry Laboratories) or anti-rat IgG (American Qualex), and bound antibodies were visualized with a Chemilumi-one chemiluminescent kit (Nacalai tesque, Kyoto, Japan).

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References


Materials and methods

Construction of chimeric TRIM5α expressing plasmid

Construction of parental and chimeric TRIM5αs carrying an HA tag (YPYDVPDYA) at their C-termini was described previously (Nakayama et al., 2005). Briefly, cloned CV1-TRIM5α and HSC-F-TRIM5α cDNAs in pcDNA3.1 were used as templates for PCR amplification with a primer containing a nucleotide sequence corresponding to the HA-tag fused with the C-terminal portion of TRIM5α. The C-terminal portion of TRIM5α fused with the HA-tag (BamHI to NotI) and the N-terminal portion of TRIM5α (NotI to BamHI) were assembled in a pCEP4 vector (Invitrogen). To generate chimeric TRIM5α HSC-F +60tag, the 182 bp Spbl–BamHI fragment of HSC-F-TRIM5α-tag was replaced with the corresponding 242 bp Spbl–BamHI fragment of CV1-TRIM5α in the background of HSC-F-TRIM5α-tag. Conversely, the 242 bp Spbl–BamHI fragment of CV1-TRIM5α was replaced with the 182 bp Spbl–BamHI fragment of HSC-F-TRIM5α-tag in the background of CV1-TRIM5α-tag to generate CV1-60tag. CV1-delete-tag, which possessed the 3′ proximal 98 bp of the Spbl–BamHI fragment of HSC-F-TRIM5α in the background of CV1-TRIM5α-tag, was generated by a PCR-based mutagenesis of CV1-TRIM5α-tag. The Spbl–BamHI fragment of HSC-F-TRIM5α was replaced with the corresponding fragment of CV1-delete-tag to generate HSC-F-delete-tag.

SIVmac infection

Construction of recombinant SeVs expressing various TRIM5αs was described previously (Nakayama et al., 2005). For CD4-dependent infection assays, 2.5 × 10^5 MT4 cells were infected with SeV expressing CV1-TRIM5α-tag, HSC-F-TRIM5α-tag, CV1-60tag, HSC-F-delete-tag, CV1-delete-tag, or HSC-F +60tag at a multiplicity of infection of five plaque-forming units per cell for each SeV and incubated at 37 °C for 9 h. Cells were then super-infected with 30 ng of p27 of SIVmac239. The culture supernatants were collected periodically, and the level of p27 was measured with a RETROtek antigen ELISA kit (ZepTeMtrix). For a single round replication assay, CD4-negative HeLa cells were infected with SeV expressing various TRIM5αs at a multiplicity of infection of 5 plaque-forming unit per cell. Cells were then super-infected with 30 ng of p27 of vesicular stomatitis virus G-protein-pseudotyped SIVmac239.

Immunoprecipitation and Western blot analysis

For co-immunoprecipitation analysis, we constructed CV1-TRIM5α carrying myc-tag (EQKLISEEDL) at its C-termini and cloned into pcDNA3.1(−) vector (Invitrogen). Hamster kidney TK-ts13 cells were co-transfected with 10 μg of a plasmid expressing myc-tagged version of CV1-TRIM5α and 10 μg of a

active TRIM5α trimer against SIVmac would be important to help understand the precise molecular mechanisms of anti-viral activity of TRIM5α.


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