Published in final edited form as:
Virology. 2009 August 15; 391(1): 140–147. doi:10.1016/j.virol.2009.05.038.

A Rare Null Allele Potentially Encoding a Dominant-Negative TRIM5α Protein in Baka Pygmies

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Abstract

The global acquired immunodeficiency syndrome (AIDS) pandemic is thought to have arisen by the transmission of human immunodeficiency virus (HIV-1)-like viruses from chimpanzees in southeastern Cameroon to humans. TRIM5α is a restriction factor that can decrease the susceptibility of cells of particular mammalian species to retrovirus infection. A survey of *TRIM5* genes in 127 indigenous individuals from southeastern Cameroon revealed that approximately 4 percent of the Baka pygmies studied were heterozygous for a rare variant with a stop codon in exon 8. The predicted product of this allele, TRIM5 R332X, is truncated in the functionally important B30.2(SPRY) domain, does not restrict retrovirus infection, and acts as a dominant-negative inhibitor of wild-type human TRIM5α. Thus, some indigenous African forest dwellers potentially exhibit diminished

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TRIM5α function; such genetic factors, along with the high frequency of exposure to chimpanzee body fluids, may have predisposed to the initial cross-species transmission of HIV-1-like viruses.

Keywords

HIV-1; susceptibility; restriction factor; cross-species transmission; polymorphism; mutant; Africa

Introduction

The primate immunodeficiency viruses include the human immunodeficiency virus type 1 and type 2 (HIV-1 and HIV-2, respectively) and simian immunodeficiency virus (SIV). Simian immunodeficiency viruses infect many of the feral chimpanzee and monkey species indigenous to various parts of Africa. (Allan et al., 1991; Apetrei et al., 2005; Bibollet-Ruche et al., 2004; Jolly et al., 1996; Souquière et al., 2001; Tomonaga et al., 1993; Santiago et al., 2002, 2005). SIV_{sm} in sooty mangabeys in West Africa is closely related to HIV-2, supporting the hypothesis that HIV-2 arose by transmission of these viruses from monkeys to humans (Gao et al., 1992; Santiago et al., 2005; Chen et al., 1996, 1997; Lemey et al., 2003; Marx et al., 1991; Peeters et al., 1994). Although HIV-2 infection can result in acquired immunodeficiency syndrome (AIDS) in humans, the major cause of AIDS is HIV-1. Phylogenetically, HIV-1 is classified into a Major (M) Group, an Outlier (O) Group and the non M-non O (N) Group (Lemey et al., 2004; Ayouba et al., 2000, 2001; HIV Sequence Compendium, 2008). HIV-1 Group M variants, responsible for the vast majority of AIDS cases globally, have been further subdivided into phylogenetically distinct clades that differ in prevalence according to geographic region (HIV Sequence Compendium, 2008).

Several pieces of evidence support a west central African origin for HIV-1. Firstly, HIV-1-like viruses in feral chimpanzees (SIV_{cpz}), gorillas and cercopithecine monkeys have been identified in Cameroon (Van Heuverswyn and Peeters, 2007; Van Heuverswyn et al., 2005, 2006; Nerrienet et al., 2005; Courgnaud et al., 2003; Keele et al., 2006; Corbet et al., 2000, Gao et al., 1999). Secondly, significant HIV-1 genetic diversity exists among viruses isolated from humans in central Africa, indicating the presence of the virus for a long period of time relative to other regions (Peeters et al., 2003; Worobey et al., 2008; Vergne et al., 2003; Konings et al., 2006; Zhong et al., 2003). For example, individuals from rural villages in Cameroon are infected with HIV-1 from diverse clades and often harbor a number of circulating recombinant forms indicative of multiple infections (Vergne et al. 2003; Konings et al., 2006). Previous studies have documented multiple infections not only with HIV-1, but also with human T-cell leukemia viruses (HTLV-III, HTLV-IV), simian foamy virus, hepatitis C virus and GB virus C (GBV-C) in individuals in rural Cameroon (Konings et al, 2006; Switzer et al, 2006; Wolfe et al, 2004; Njouom et al, 2003; Kondo et al, 1997; Torimiro et al, unpublished data). Thirdly, rural populations in west central Africa are frequently exposed to the body fluids of non-human primates through hunting, butchering and keeping of pets (Hahn et al., 2000). The Baka pygmies are hunter-gatherers of southwestern Cameroon (Figure 1) whose semi-nomadic lifestyle has persisted, largely unchanged, for thousands of years. The repeated exposure of these forest-dwelling people to the body fluids of non-human primates may have created circumstances favorable to the initial transmission of HIV-1-like viruses from chimpanzees to a small number of humans. Once introduced into the human host, HIV-1 diverged from SIV_{cpz}, presumably allowing improved replication and spread in the human population.

A major barrier to cross-species transmission of retroviruses is mediated by the TRIM5α protein (Bieniasz, 2003; Hatziioannou et al., 2003; Hofmann et al., 1999; Stremlau et al., 2004a). Variants of TRIM5α in different primate species block the early, post-entry phase of infection of cells by particular retroviruses (Hatziioannou et al., 2004; Keckesova, Ylinen, and

Towers, 2004; Song et al., 2005b; Perron et al., 2004; Yap et al., 2004). For example, the TRIM5α protein of rhesus monkeys restricts HIV-1 infection; even when expressed at comparable levels, human TRIM5α (TRIM5α_{hu}) is less potent at suppressing HIV-1 or SIV$_{cpz}$ (Hatziioannou et al. 2004; Keckesova, Ylinen, and Towers, 2004; Stremlau et al. 2004a; Yap et al. 2004; Kratovac et al., 2008). On the other hand, TRIM5α_{hu} more potently restricts infection by the N-tropic murine leukemia virus (N-MLV) than TRIM5α_{rh} (Hatziioannou et al. 2004; Keckesova et al., 2004; Perron et al. 2004; Song et al., 2005b; Yap et al. 2004).

TRIM5 is a member of a family of proteins that contain a tripartite motif, hence the designation TRIM (Reymond et al., 2001). TRIM proteins have also been called RBCC proteins because the tripartite motif includes a RING domain, B-box 2 domain and coiled coil domain. TRIM proteins exhibit the propensity to assemble into cytoplasmic or nuclear bodies (Reymond et al., 2001). Many cytoplasmic TRIM proteins contain a C-terminal B30.2 or SPRY domain. Differential splicing of the TRIM5 primary transcript gives rise to the expression of several isoforms of the protein product. The TRIM5α isoform is the largest product (~493 amino acid residues in humans) and contains the B30.2(SPRY) domain. The B30.2(SPRY) domain of rhesus monkey TRIM5α is essential for recognition of the retroviral capsid and for anti-HIV-1 activity (Stremlau et al., 2004; Perez-Caballero et al., 2005; Kar et al., 2008; Langelier et al., 2008). Moreover, the difference in the anti-HIV-1 potency of rhesus and human TRIM5α proteins is determined by variable regions within the B30.2(SPRY) domain (Song et al., 2005a; Sawyer et al., 2005; Perez-Caballero et al., 2005; Stremlau et al., 2005; Yap, Nisole and Stoye, 2005). The decreased potency of human TRIM5α in restricting HIV-1, for example, is due to the presence of arginine 332 in the v1 variable loop of the B30.2(SPRY) domain (Li et al., 2006; Yap, Nisole and Stoye, 2005). This arginine residue decreases the affinity of human TRIM5α for the HIV-1 capsid (Li et al., 2006). TRIM5α binding to the retroviral capsid has been shown to lead to accelerated capsid uncoating, a process facilitated by the TRIM5α RING and B-box 2 domains (Stremlau et al., 2006; Perron et al., 2007; Diaz-Griffero et al., 2007a, b; Diaz-Griffero et al., 2008; Li and Sodroski, 2008).

Interspecies differences in primate TRIM5α proteins dictate the potency of restriction against particular retroviruses (Hatziioannou et al. 2004; Keckesova, Ylinen and Towers, 2004; Song et al., 2005b; Stremlau et al., 2004, 2005; Yap et al. 2004a; Kratovac et al., 2008). Considerable intra-species variation in the TRIM5α proteins of primates has also been documented (Liao et al., 2007; Newman et al., 2008; Brenner et al., 2008; Virgen et al., 2008; Wilson et al., 2008). In most reported studies, common polymorphisms in the coding exons of human *TRIM5* were not found to exert significant effects on the clinical progression of HIV-1 infection (Speelmon et al., 2006; Javanbakht et al., 2006; Goldschmidt et al., 2006; Sawyer et al., 2006; Nakayama et al., 2007; van Manen et al., 2008). One common nonsynonymous SNP (R136Q) exhibited an increased frequency among HIV-1-infected subjects relative to exposed seronegative persons, hinting that it may be linked to increased acquisition of infection (Speelmon et al., 2006). Moreover, some less common non-coding polymorphisms in African Americans have been associated with increases in susceptibility to HIV-1 infection (Javanbakht et al., 2006). The mechanism and importance of these potential regulatory polymorphisms require further investigation.

Here, we report the results of a survey of TRIM5 genotypes in indigenous Africans living in rural southeastern Cameroon, where HIV-1 infection in humans likely originated through contact with SIV$_{cpz}$-infected chimpanzees (Gao et al., 1999; Nerrienet et al., 2005; Van Heuverswyn et al., 2007; Van Heuverswyn and Peeters, 2007; Santiago et al., 2002; Corbet et al., 2000; Keele et al., 2006). In Baka pygmies, we identify a rare *TRIM5* allele that is predicted to encode a truncated TRIM5α protein defective for retrovirus restriction. The truncated TRIM5 variant exhibits dominant-negative effects on the wild-type TRIM5α protein. Thus,

some African forest dwellers, whose lifestyle results in frequent exposure to chimpanzee and other non-human primate body fluids, may possess lower-than-normal TRIM5-mediated retrovirus restriction activity.

Material and Methods

Study population

Administrative and ethical approval to carry out this project was obtained from the Cameroon Ministry of Public Health and all the collaborating institutions. From 2001 to 2002, adult volunteers living in southeastern Cameroon rainforest villages (Figure 1) participated in a study of retrovirus molecular epidemiology. For the human genetics component of the study, a purposive choice sampling technique was used to select 95 Baka pygmies (hunter-gatherers) and 32 non-pygmies.

TRIM5 resequencing

The complete exon 8 of human *TRIM5*, which encodes the B30.2(SPRY) domain, was resequenced using the following primers: 5′ TCCCTTAGCTGACCTGTTAATTT-3′ and 5′-GCTGTACAGAAGGGGCTGAG-3′. Samples were resequenced using protocols provided by Applied Biosystems, and analyzed on an ABI-3730XL instrument.

Cells

Cf2Th canine thymic epithelial cells and 293T cells were obtained from the American Type Culture Collection and propagated as recommended.

Plasmids

The wild-type TRIM5α_{hu} cDNA was described previously (Stremlau et al., 2004), and corresponds to the major allelic variant in European Americans and African Americans (Javanbakht et al., 2006). The R332X mutation was introduced into the wild-type *TRIM5* cDNA by PCR-directed mutagenesis. The TRIM5α_{hu} proteins possess C-terminal epitope tags derived from either the influenza virus hemagglutinin (HA) or the P and V proteins of simian virus 5 (V5).

Creation of cells stably expressing TRIM5 variants

A retroviral vector encoding the wild-type TRIM5α_{hu}-HA protein was created using the pLPCX plasmid (Stratagene) (Stremlau et al., 2004). The pLPCX plasmid contains only the amino acid-coding sequence and not the untranslated region of the TRIM5α cDNA. Recombinant viruses were produced in 293T cells by cotransfecting the pLPCX plasmids with the pVPack-GP and pVPack-VSV-G packaging plasmids (Stratagene). The pVPack-VSV-G plasmid encodes the vesicular stomatitis virus (VSV) G envelope glycoprotein, which allows efficient entry into a wide range of vertebrate cells (Yee et al., 1994). Cf2Th cells stably expressing the wild-type TRIM5α_{hu}-HA proteins were established by incubation of ~ 1×10^5 cells with recombinant virus in the presence of 5 μg/ml polybrene. Cells were selected in 5 μg/ml puromycin.

The R332X human TRIM5 protein with a V5 epitope tag was expressed using the Viral Power system (Invitrogen) (Diaz-Griffero et al., 2006). Recombinant lentiviruses were produced according to the manufacturer's protocol. The resulting virus particles were used to transduce ~ 1×10^5 Cf2Th cells (or Cf2Th cells expressing wild-type TRIM5α_{hu}-HA) in the presence of 5 μg/ml polybrene. Cells were selected in either 5 μg/ml blasticidin for cells expressing R332X TRIM5α_{hu}-V5, or 5 μg/ml puromycin and 5 μg/ml blasticidin for cells expressing both wild-type and R332X TRIM5α_{hu} proteins.

TRIM5 protein analysis

Cellular proteins were extracted with radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris, pH 7.4, 100 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% Nonidet P-40, 1 mg/ml aprotinin, 2 mg/ml leupeptin, 1 mg/ml pepstatin A, 100 mg/ml phenylmethylsulfonyl fluoride). The cell lysates were analyzed by SDS-PAGE (10% acrylamide), followed by blotting onto nitrocellulose membranes (Amersham Biosciences). Detection of protein by Western blotting utilized monoclonal antibodies that are specifically reactive with the HA (Roche Applied Science) or V5 (Invitrogen) epitope tags. Detection of proteins was performed by enhanced chemiluminescence (PerkinElmer Life Sciences), using the following secondary antibodies obtained from Amersham Biosciences: anti-mouse (for V5) and anti-rat (for HA).

Co-localization experiments

Co-localization was studied as previously described (Javanbakht et al., 2005). Briefly, cells were grown overnight on 12-mm-diameter coverslips and fixed in 3.9% paraformaldehyde (Sigma) in phosphate-buffered saline (PBS, Cellgro) for 30 minutes. Cells were washed in PBS, incubated in 0.1 M glycine (Sigma) for 10 minutes, washed in PBS, and permeabilized with 0.05% saponin (Sigma) for 30 minutes. Samples were blocked with 10% donkey serum (Dako, Carpentaria, CA) for 30 minutes and incubated for 1 hour with antibodies. The anti-HA fluorescein isothiocyanate-conjugated 3F10 antibody (Roche Applied Sciences) and anti-V5 Cy3-conjugated antibody (Sigma) were used to stain HA- or V5-tagged TRIM5α proteins, respectively. Subsequently, samples were mounted for fluorescence microscopy by using the ProLong Antifade Kit (Molecular Probes, Eugene, OR). Images were obtained with a Bio-Rad Radiance 2000 laser scanning confocal microscope with Nikon 60X numerical aperture 1.4 optics.

Infection with viruses expressing green fluorescent protein

Recombinant viruses (HIV-1-GFP, N-MLV-GFP and B-MLV-GFP) expressing the humanized Renilla reniformis green fluorescent protein (GFP) were prepared as previously described (Perron et al., 2004; Stremlau et al., 2004). The viral stocks were quantified by measuring reverse transcriptase activity, as described previously (Rho et al., 1981). Cf2Th cells expressing the wild-type (wt) and R332X mutant TRIM5 proteins, or control Cf2Th cells transduced with the empty pLPCX vector, were incubated with various amounts of the recombinant viruses, as described (Perron et al., 2004; Stremlau et al., 2004). For infections, 3×10^4 Cf2Th cells seeded in 24-well plates were incubated with virus for 24 hours. Cells were washed and returned to culture for 48 hours, and then subjected to FACS analysis with a FACScan (Becton Dickinson).

Results

A human *TRIM5* allele encoding a truncated protein

To examine the diversity of *TRIM5* within a population of individuals in central Africa, genomic DNA was prepared from the PBMC of 127 subjects from Cameroon. This cohort included 95 Baka pygmies and 32 non-pygmies. The complete *TRIM5* exon 8, which encodes the B30.2(SPRY) domain of TRIM5α, was resequenced. A rare mutation that specifies a premature stop codon (TGA) replacing the CGA codon for arginine 332 of TRIM5α was observed in four heterozygotes, all Baka pygmies from the same study site. Thus, 4.2 percent of the sampled Baka pygmies are heterozygous for this allele, with an allele frequency of 2.1 percent in this group. The allele is predicted to encode a truncated TRIM5α protein (R332X) missing 83% of the B30.2(SPRY) domain (Figure 2); the other TRIM5 isoforms, TRIM5γ and TRIM5δ, should not be affected by this mutation.

Anti-retroviral activity of TRIM5$_{hu}$ R332X

The TRIM5α B30.2(SPRY) domain is essential for retrovirus restriction (Stremlau et al., 2004; Perez-Caballero et al., 2005). Furthermore, arginine 332 in the v1 variable region is a determinant of anti-HIV-1 potency (Li et al., 2006; Yap, Nisole and Stoye, 2005). These observations suggest that the truncated TRIM5$_{hu}$ R332X protein is probably defective for retrovirus inhibitory activity. To test this, Cf2Th canine thymic epithelial cells were established that express wild-type TRIM5α$_{hu}$–HA, TRIM5$_{hu}$ R332X-V5, or both wild-type TRIM5α$_{hu}$–HA and TRIM5$_{hu}$ R332X-V5. These proteins have C-terminal HA or V5 epitope tags. Two independent cell lines expressing both TRIM5 variants were established. Cf2Th cells were used for these experiments because dogs do not express a functional TRIM5 protein (Sawyer et al., 2007), thus allowing an assessment of the human TRIM5 phenotypes in a clean background. The wild-type TRIM5α$_{hu}$ protein was expressed at comparable levels in the control cells and in each of the independent clones in which TRIM5$_{hu}$ R332X-V5 was also expressed (Figure 3). The TRIM5$_{hu}$ R332X-V5 protein was expressed at higher levels in the control LPCX cells than in the cells expressing the wild-type TRIM5α$_{hu}$–HA protein. The expression of the wild-type TRIM5α$_{hu}$–HA protein may have decreased the efficiency of transduction of the cells by the lentivirus vector expressing TRIM5$_{hu}$ R332X-V5.

The susceptibility of the Cf2Th cells expressing the TRIM5$_{hu}$ variants to retrovirus infection was assessed. The cells were exposed to increasing concentrations of recombinant HIV-1, B-MLV and N-MLV expressing GFP. Forty-eight hours later, the level of infection achieved was assessed by FACS analysis (Figure 4). Relative to the control cells transduced with the empty LPCX vector, the cells expressing only wild-type TRIM5α$_{hu}$ were less infectible by HIV-1. This result is consistent with previous reports demonstrating that TRIM5α$_{hu}$ partially restricts HIV-1 infection (Stremlau et al., 2004,2005;Perez-Caballero et al., 2005;Sawyer et al., 2005). By contrast, HIV-1 infection was not inhibited in cells expressing TRIM5$_{hu}$ R332X. Neither was HIV-1 infection affected in the cells expressing both wild-type TRIM5α$_{hu}$ and TRIM5$_{hu}$ R332X proteins. Apparently, the coexpression of TRIM5$_{hu}$ R332X diminishes the partial HIV-1-restricting ability of the wild-type TRIM5α$_{hu}$ protein.

As expected (Perron et al., 2004; Hatziioannou et al., 2004; Keckesova et al., 2004; Yap et all, 2004), the cells expressing the wild-type TRIM5α$_{hu}$ protein were very resistant to N-MLV infection. No inhibition of N-MLV infection was observed for the TRIM5$_{hu}$ R332X protein. Compared with cells expressing only the wild-type TRIM5α$_{hu}$ protein, cells expressing both wild-type TRIM5α$_{hu}$ and TRIM5$_{hu}$ R332X proteins exhibited less restriction of N-MLV infection. Thus, for both N-MLV and HIV-1 infections, wild-type TRIM5α$_{hu}$ is a less efficient restriction factor when TRIM5$_{hu}$ R332X is co-expressed.

None of the TRIM5 variants tested here affected the efficiency of B-MLV infection. This indicates the specificity of the inhibitory effects observed for HIV-1 and N-MLV.

Co-localization and association of wild-type TRIM5 α$_{hu}$ and TRIM5$_{hu}$ R332X

The above results suggest that TRIM5$_{hu}$ R332X can exert some dominant-negative effects on retroviral restriction by wild-type TRIM5α$_{hu.}$ As both proteins retain all of the TRIM5 regions shown to be sufficient for dimerization (Javanbakht et al., 2006), we examined the colocalization and association of these TRIM5 variants. Wild-type TRIM5α$_{hu}$ and TRIM5$_{hu}$ R332X colocalized in the cytoplasm of cells coexpressing these proteins; the TRIM5 proteins exhibited a speckled pattern superimposed on a diffuse staining of the cytoplasm (Figure 5A). The wild-type TRIM5$_{hu}$ protein was coprecipitated with the TRIM5$_{hu}$ R332X-V5 protein in coexpressing cells (Figure 5B). Thus, the wild-type TRIM5α$_{hu}$ and TRIM5$_{hu}$ R332X proteins colocalize and associate, consistent with the observed dominant-negative effects.

Discussion

The existence of SIV_{cpz} phylogenetically related to HIV-1 Group M in chimpanzees in Cameroon (Santiago et al., 2002; Gao et al., 1999; Keele et al., 2006; Nerrienet et al., 2005; Van Heuverswyn et al., 2007) supports the assertion that immunodeficiency viruses causing the global AIDS pandemic entered the human population in west central Africa. There, indigenous forest dwellers hunt and butcher non-human primates, including chimpanzees, and would thus be potentially exposed to retroviruses and other infectious agents in these animals. Here we report that approximately 4% of Baka pygmies are heterozygous for a rare allele (*R332X*) of *TRIM5*, which encodes one of the host barriers to cross-species transmission of retroviruses (Stremlau et al., 2004a; Hatziioannou et al., 2004; Keckesova et al., 2004; Perron et al., 2004; Yap et al., 2004; Song et al., 2005b). The presence of a stop codon in the *TRIM5 R332X* allele is predicted to result in the production of a TRIM5α variant that is truncated in the B30.2(SPRY) domain. This truncated TRIM5 protein lacks detectable antiretroviral activity and can exert a dominant-negative effect on the moderate HIV-1-restricting ability of the wild-type TRIM5α protein. The *TRIM5 R332X* allele has not been identified in previous surveys of African Americans or South Africans (Javanbakht et al., 2006), indicating that the distribution of this allele is limited. Null mutations in human *TRIM5* are apparently rare, whereas a TRIM5 variant (H43Y) exhibiting modestly decreased antiretroviral activity is quite frequent in modern humans (Sawyer et al., 2006; Javanbakht et al., 2006). Species-wide disruption of *TRIM5* has occurred in some mammals, such as dogs (Sawyer et al., 2007).

The observed phenotypes of $TRIM5_{hu}$ R332X are consistent with expectations based on numerous structure-function studies of TRIM5α proteins. $TRIM5_{hu}$ R332X is truncated within the v1 variable region of the B30.2(SPRY) domain, and therefore lacks most of the beta strands that contribute to the fold of this domain (Seto et al., 1999; Woo et al., 2006a, b; Yao et al., 2006; Grütter et al., 2006; James et al., 2007). The remnant of the B30.2(SPRY) domain in $TRIM_{hu}$ R332X is presumably folded into some non-native structure. $TRIM5_{hu}$ R332X thus lacks an intact capsid-binding domain, explaining its defectiveness in retroviral restriction (Stremlau et al., 2004; Perez-Caballero et al., 2005; Kar et al., 2008; Langelier et al., 2008).

TRIM5α forms dimers, allowing bivalent binding to the retroviral capsid (Javanbakht et al., 2006; Kar et al., 2008; Langelier et al., 2008). Because $TRIM5_{hu}$ R332X retains the coiled coil and L2 linker needed for oligomerization (Javanbakht et al., 2006), $TRIM5_{hu}$ R332X can form hetero-oligomers with wild-type TRIM5α. As these heterodimers have only one intact capsid-binding domain, the avidity for capsid is diminished compared with that of the wild-type TRIM5α dimer. Thus, as has been previously seen for TRIM5γ and other TRIM5 variants with deleted B30.2(SPRY) domains (Stremlau et al., 2004; Perez-Caballero et al., 2005), $TRIM5_{hu}$ R332X exhibits dominant-negative effects on retroviral restriction when coexpressed with wild-type $TRIM5\alpha_{hu}$.

Additional investigation will be required to assess the potential biological consequences of *TRIM5 R332X* heterozygosity. For example, the relative levels of expression of the wild-type $TRIM5\alpha_{hu}$ and $TRIM5_{hu}$ R332X proteins in natural target cells would be expected to modulate the degree to which dominant-negative inactivation of wild-type TRIM5α function occurs. Although a previous study (Javanbakht et al., 2006) hinted that some regulatory changes in *TRIM5* might affect HIV-1 susceptibility, whether functional decreases in $TRIM5\alpha_{hu}$ antiretroviral activity resulting from $TRIM5_{hu}$ R332X expression could predispose to a higher risk of HIV-1 or SIV_{cpz} infection is unknown. Because of the rarity of the *TRIM5 R332X* allele, it is not feasible to perform a case-control study to evaluate the effect of this polymorphism on susceptibility to HIV-1 infection. In addition to facilitating the cross-species transmission of SIV_{cpz} from chimpanzees to humans, decreased TRIM5 function might also allow reinfection with HIV-1-like viruses, which promotes the generation of virus diversity through

recombination. Indeed, diverse viruses from multiple clades and circulating recombinant forms of HIV-1 have been documented in the inhabitants of rural villages in the equatorial rain forest and grass field regions in Cameroon (Zhong et al., 2003; Konings et al., 2006). Future studies can explore the intriguing possibility that host genetic factors particular to the forest-dwelling peoples of west central Africa contributed to the initiation and evolution of HIV-1 infection in humans.

Acknowledgments

We thank Ms. Yvette McLaughlin and Ms. Elizabeth Carpelan for preparation of the manuscript. This work has been supported, in part, by a Fellowship/Grant from the Fogarty International Center/USNIH: Grant # 2 D 43 TW000010-16 – AITRP; by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research; by the National Institutes of Health (AI063987, AI076094, AI067854 and a Center for AIDS Research Award AI060354); by the International AIDS Vaccine Initiative; by the Global Viral Forecasting Initiative; and by the late William F. McCarty-Cooper. This project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U. S. Government.

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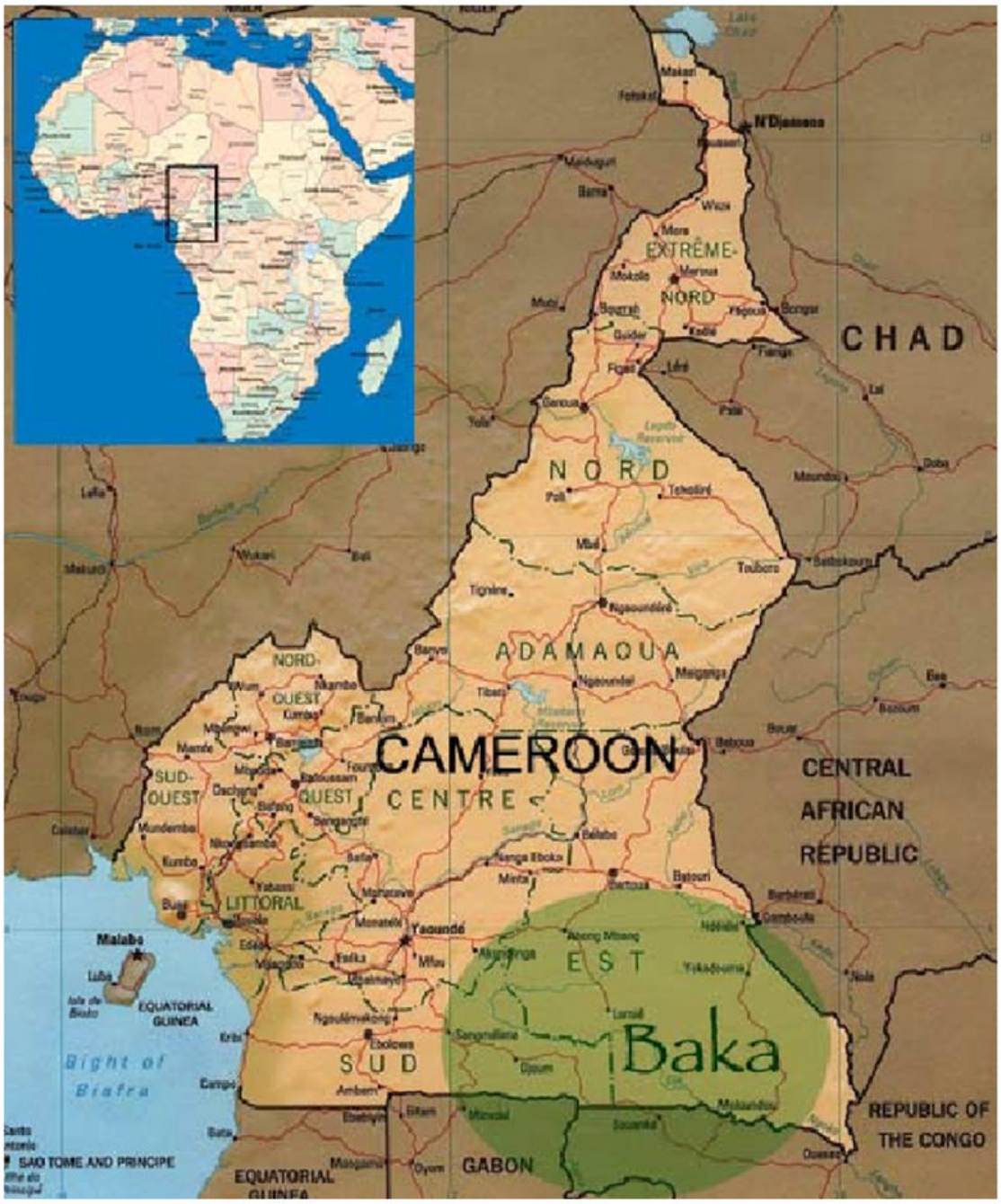

Figure 1. Geographic location of the Baka forest people

The region of Africa highlighted in the box in the inset is shown, with the range of the Baka pygmy population shaded green. The Baka people constitute an ethnic group inhabiting the rain forest of southeastern Cameroon, northern Republic of Congo, northern Gabon and southwestern Central African Republic. The Baka pygmies number from 5,000 to 28,000 individuals.

Figure 2. The TRIM5$_{hu}$ R332X protein

The human TRIM5α protein is shown, with the domains and interdomain linkers (L1 and L2) labeled (B2 = B-box 2; CC = coiled coil). The variable (v1–v4) regions of the B30.2(SPRY) domain are shown. The predicted sequence of the carboxyl terminus of the TRIM5$_{hu}$ R332X protein is shown beneath the wild-type TRIM5α$_{hu}$ sequence.

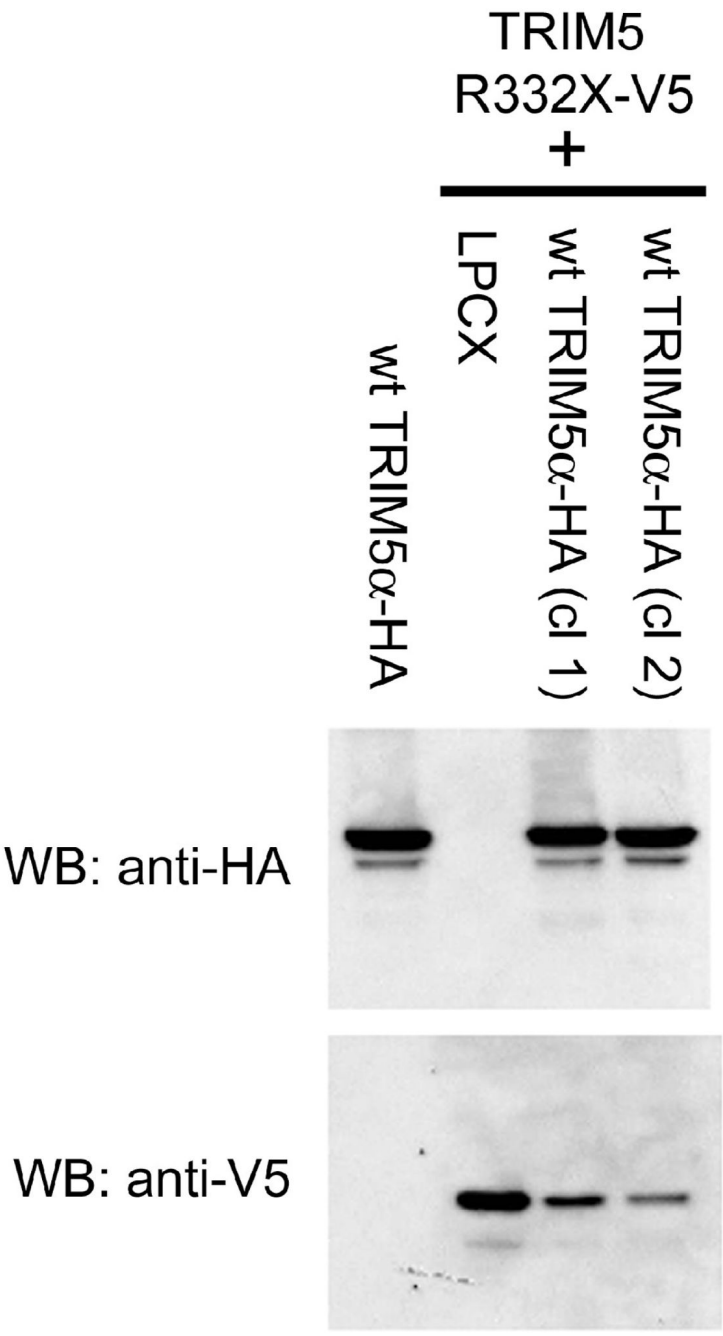

Figure 3. Expression of the human TRIM5 variants in canine epithelial cells

Cf2Th cells were transduced with either the empty LPCX vector or the LPCX vector expressing wild-type (wt) TRIM5α_{hu}-HA. Some of the cells were subsequently transduced with the gene encoding TRIM5$_{hu}$ R332X-V5 using the Viral Power system (Invitrogen). The results with two independent cultures (cl 1 and cl 2) of the Cf2Th cells coexpressing wt TRIM5α_{hu}-HA and TRIM5$_{hu}$ R332X-V5 proteins are shown. Cell lysates were Western blotted and probed with antibodies directed against either the HA epitope tag (top panel) or the V5 tag (lower panel).

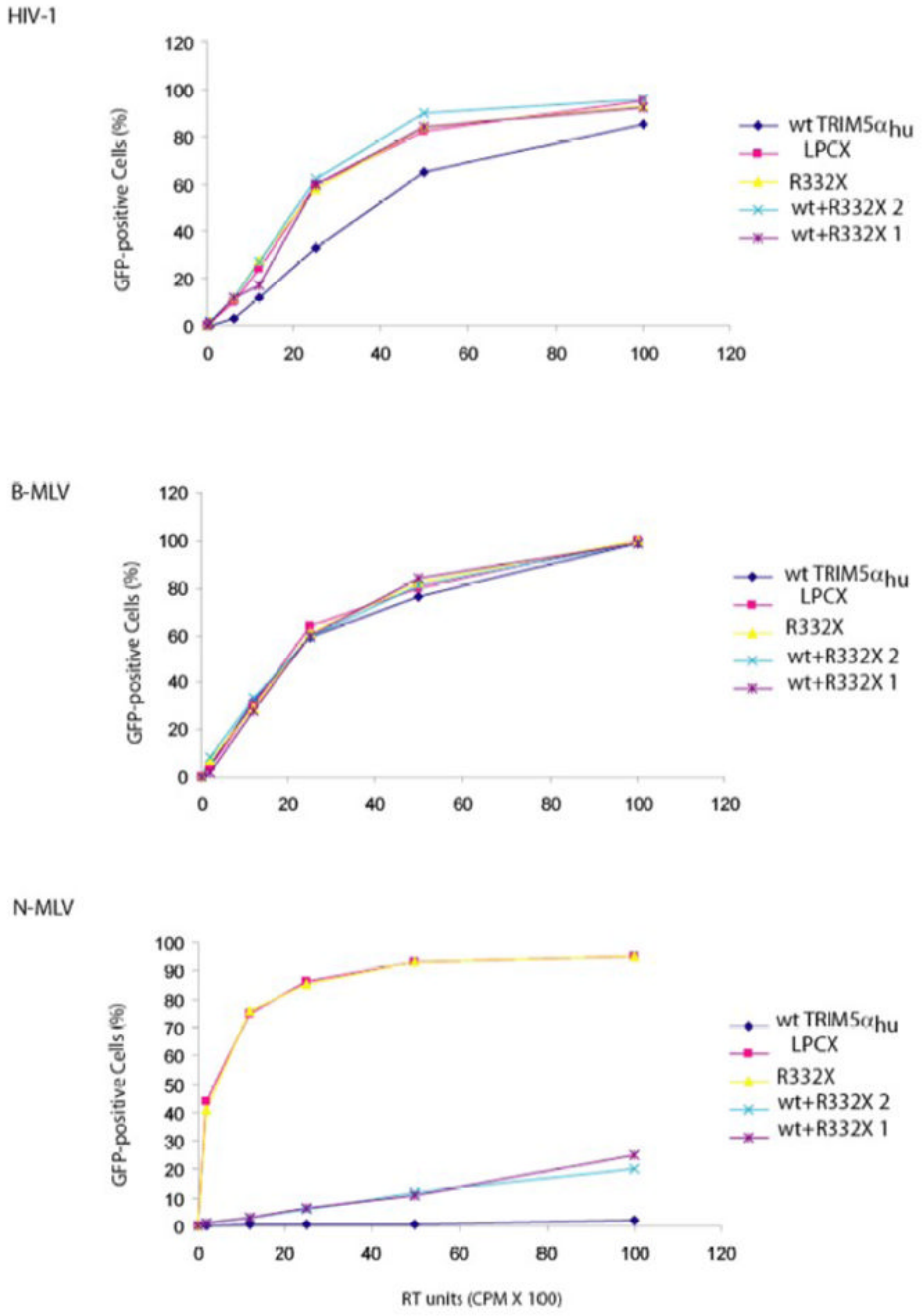

Figure 4. Susceptibility of cells expressing human TRIM5 variants to retrovirus infection

Cf2Th cells transduced with the empty LPCX vector or expressing the indicated $TRIM5_{hu}$ variants were incubated with various amounts of recombinant HIV-1, B-MLV or N-MLV expressing GFP. The results with two independent cultures (1 and 2) of the cells co-expressing the wt and R332X variants of TRIM5 are shown. Infected, GFP-positive cells were counted by FACS. The results of a typical experiment are shown. Similar results were obtained in three independent experiments.

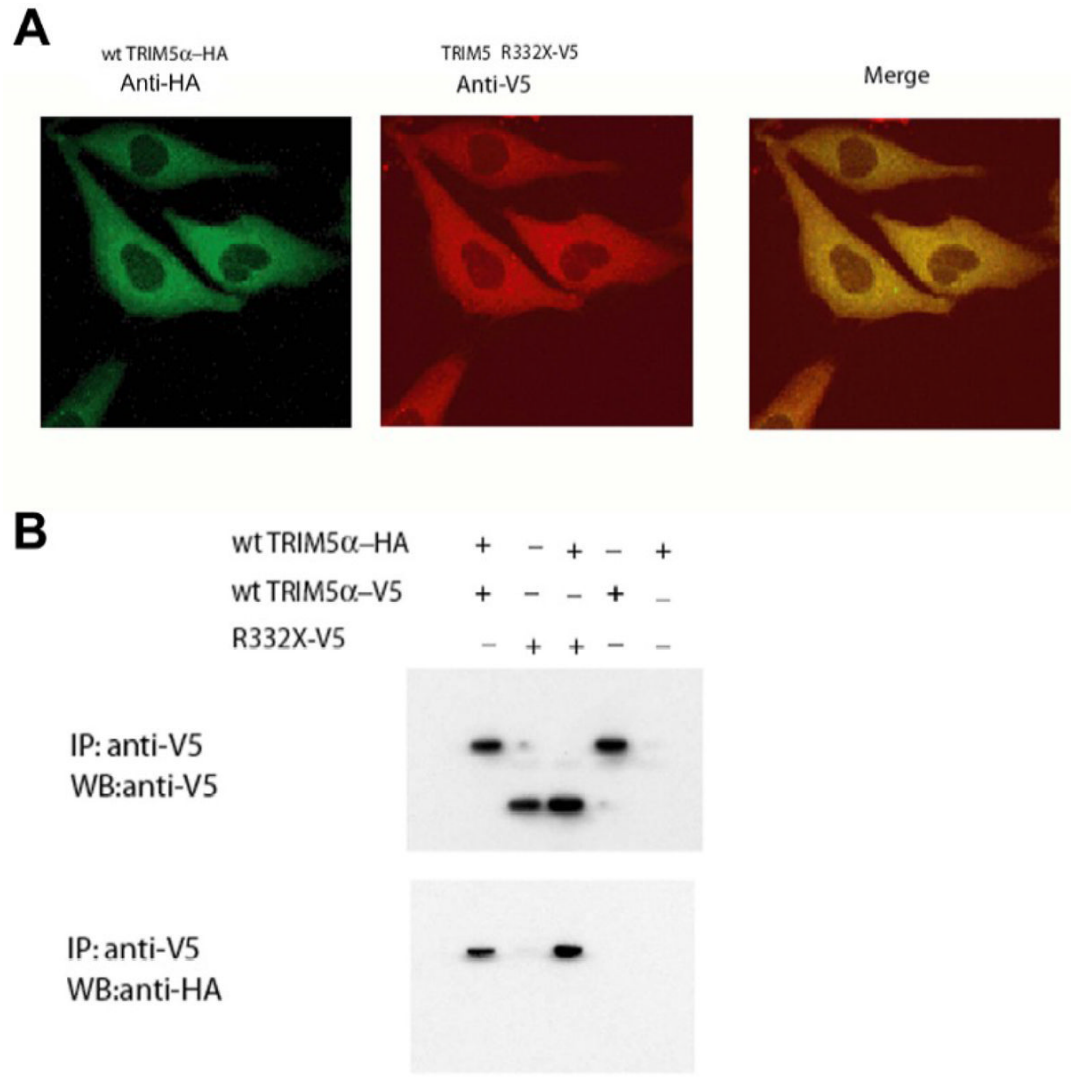

Figure 5. Association of TRIM5$_{hu}$ R332X and wild-type TRIM5α$_{hu}$

A. Cf2Th cells stably expressing wild-type TRIM5α $_{hu}$-HA and TRIM5$_{hu}$ R332X-V5 were fixed and stained with a FITC-conjugated anti-HA antibody or a Cy3-conjugated anti-V5 antibody. Representative confocal microscopic images of the Cf2Th cells are shown. The merged image is shown in the right panel. **B.** 293T cells were transfected with plasmids expressing the indicated TRIM5 variants containing either an HA or a V5 epitope tag. Cell lysates were precipitated (IP) with an anti-V5 antibody. The precipitates were then Western blotted (WB) with an anti-V5 antibody (upper panel) or an anti-HA antibody (lower panel).