JOURNAL OF VIROLOGY, Jan. 2011, p. 208–216 Vol. 85, No. 1
0022-538X/11/$12.00 doi:10.1128/JVI.01810-10

Association of TRIM22 with the Type 1 Interferon Response and Viral Control during Primary HIV-1 Infection▽

Ravesh Singh,[1] Gaurav Gaiha,[2] Lise Werner,[3] Kevin McKim,[2] Koleka Mlisana,[3] Jeremy Luban,[4] Bruce D. Walker,[1,2,5] Salim S. Abdool Karim,[3] Abraham L. Brass,[2] Thumbi Ndung'u,[1,2,3]* and the CAPRISA Acute Infection Study Team

HIV Pathogenesis Programme, Doris Duke Medical Research Institute, Nelson R. Mandela School of Medicine, University of KwaZulu-Natal, Durban, South Africa[1]; Ragon Institute of Massachusetts General Hospital, Massachusetts Institute of Technology and Harvard University, Boston, Massachusetts[2]; Centre for the AIDS Programme of Research in South Africa (CAPRISA), University of KwaZulu-Natal, Durban, South Africa[3]; Department of Microbiology, University of Geneva, Geneva, Switzerland[4]; and Howard Hughes Medical Institute, Chevy Chase, Maryland[5]

Received 26 August 2010/Accepted 14 October 2010

Type 1 interferons (IFNs) induce the expression of the tripartite interaction motif (TRIM) family of E3 ligases, but the contribution of these antiviral factors to HIV pathogenesis is not completely understood. We hypothesized that the increased expression of select type 1 IFN and TRIM isoforms is associated with a significantly lower likelihood of HIV-1 acquisition and viral control during primary HIV-1 infection. We measured IFN-α, IFN-β, myxovirus resistance protein A (MxA), human TRIM5α (huTRIM5α), and TRIM22 mRNA levels in peripheral blood mononuclear cells (PBMCs) of high-risk, HIV-1-uninfected participants and HIV-1-positive study participants. Samples were available for 32 uninfected subjects and 28 infected persons, all within 1 year of infection. HIV-1-positive participants had higher levels of IFN-β ($P = 0.0005$), MxA ($P = 0.007$), and TRIM22 ($P = 0.01$) and lower levels of huTRIM5α ($P < 0.001$) than did HIV-1-negative participants. TRIM22 but not huTRIM5α correlated positively with type 1 IFN (IFN-α, IFN-β, and MxA) (all $P < 0.0001$). In a multivariate model, increased MxA expression showed a significant positive association with viral load ($P = 0.0418$). Furthermore, TRIM22 but not huTRIM5α, IFN-α, IFN-β, or MxA showed a negative correlation with plasma viral load ($P = 0.0307$) and a positive correlation with $CD4^+$ T-cell counts ($P = 0.0281$). *In vitro* studies revealed that HIV infection induced TRIM22 expression in PBMCs obtained from HIV-negative donors. Stable TRIM22 knockdown resulted in increased HIV-1 particle release and replication in Jurkat reporter cells. Collectively, these data suggest concordance between type 1 IFN and TRIM22 but not huTRIM5α expression in PBMCs and that TRIM22 likely acts as an antiviral effector *in vivo*.

Tripartite interaction motif (TRIM) E3 ligases represent a recently described family of proteins with potent antiviral activity (39). There are approximately 70 TRIM family members, and they are characterized by the presence of a tripartite motif, which consists of a RING domain, one or two B-box motifs, and a coiled-coil region (21, 29, 39). The presence of the RING domain suggests that these proteins function as E3 ubiquitin ligases and mediate ubiquitylation events (6). The E3 ubiquitin ligase activity of the RING domain is important for the antiretroviral function of many TRIM proteins (29, 38).

The prototype member of this family, TRIM5α, is responsible for the complete block of HIV-1 replication in Old World monkey cells (33, 36). This effect is mediated through the interaction of rhesus monkey TRIM5α (TRIM5αrh) with the HIV-1 capsid (36). Further studies suggested that in addition to the effects of TRIM5αrh on HIV via binding to capsid, other mechanisms of viral inhibition are possible (27, 31). TRIM5α is responsible for the species-specific postentry restriction of retroviruses, such as N-tropic murine leukemia virus (N-MuLV) and HIV-1, in primate cells (36, 45). Other TRIM E3 ligases with antiviral activity have been described (45). TRIM family proteins affect specific steps in the HIV life cycle (13). TRIM proteins appear to mediate their antiviral activities via diverse mechanisms: interference with the uncoating of the viral preintegration complex was noted for TRIM5α (24), and an inhibition of viral budding has been described for TRIM22 (26).

Although the antiretroviral activity of TRIM E3 ligases is established, the contribution of this family of proteins to protection against HIV-1 infection or to the control of disease progression is largely unknown. Many *in vitro* studies have suggested that human TRIM5α (huTRIM5α) has little effect on HIV replication. However, some huTRIM5α genetic variants have been associated with reduced susceptibility to HIV infection (14, 35), suggesting that huTRIM5α may have a protective role in infection. Modest effects of huTRIM5α genetic polymorphisms on the rate of disease progression have also been reported (9, 41), and it was suggested previously that human TRIM5α may select for HIV-1 escape mutants after a prolonged duration of infection (17).

In a prospective cohort study of HIV-1-negative individuals at high risk for HIV-1 infection, we have recently shown that elevated levels of expression of huTRIM5α are associated with decreased susceptibility to HIV-1 infection (34). Furthermore,

* Corresponding author. Mailing address: Doris Duke Medical Research Institute, University of KwaZulu-Natal, Private Bag X7, Congella, Durban 4001, South Africa. Phone: 2731 260 4727. Fax: 2731 260 4623. E-mail: ndungu@ukzn.ac.za.

▽ Published ahead of print on 27 October 2010.

we found that huTRIM5α mRNA levels were neither actively downregulated nor upregulated among individuals in this cohort who eventually became HIV-1 infected. The latter finding was surprising, because previous studies demonstrated that type 1 interferons (IFNs) are dysregulated during HIV-1 infection (10, 15), and TRIM5α is a well-known type 1 IFN-inducible gene (1, 31). Therefore, we would have expected to observe trends for huTRIM5α similar to those reported for type 1 IFN. In addition to TRIM5α, several TRIM proteins with antiviral activity were recently described and were shown to be IFN inducible (2, 39). However, there are also notable exceptions to this IFN inducibility rule (4, 28). IFNs are themselves the main mediators of innate immunity to viral infection, and they play a significant role by upregulating the expression of many antiviral effectors within the cell (5, 30). Our understanding of the type 1 IFN regulation of TRIMs is complicated by the fact that IFN-α has 13 functional isoforms. It is unclear whether all these isoforms have the same effect on IFN-stimulated genes (23).

Here we investigated the expression of the type 1 IFNs IFN-α (IFN-α2b isoform), IFN-β, a surrogate interferon-inducible gene (myxovirus resistance protein A [MxA]), and TRIM22 in a longitudinal cohort of black African females at high risk for HIV-1 infection, for which we have previously demonstrated that enhanced TRIM5α mRNA expression is associated with reduced susceptibility to HIV infection (34). TRIM22 was selected for this analysis because in addition to TRIM5α, it is one of the relatively well-characterized TRIM E3 ligases, has been shown to be type 1 IFN inducible *in vitro*, and appears to possess anti-HIV-1 activity (2, 4, 25). Specifically, we tested the hypotheses that the increased expression of type 1 IFNs and TRIM22 is associated with a significantly lower likelihood of HIV-1 acquisition and lower viral loads or higher $CD4^+$ T-cell counts during primary HIV-1 infection. We tested whether there are differences in mRNA levels of select type 1 IFNs, huTRIM5α, and TRIM22 in peripheral blood mononuclear cells (PBMCs) from HIV-negative versus HIV-positive individuals. We used multivariate analysis models in order to better understand the kinetics and antiviral implications of the expression of these genes *in vivo*. Finally, we performed *in vitro* experiments to better understand the relationship between type 1 IFNs, TRIM22 expression, and antiviral activity.

MATERIALS AND METHODS

Subjects. Study subjects were part of the CAPRISA 002 Acute Infection Study, which is an observational natural history study of HIV-1 subtype C infection established in Durban, South Africa, in 2004 (34, 40). The cohort consisted of 245 high-risk seronegative women who were monitored to identify acute or recent infections. Participants were enrolled into the acute-infection phase if they were antibody positive within 5 months of a previous antibody-negative test or if they had evidence of viral replication without HIV-1 antibodies, as assessed by rapid tests and PCR testing. Women from other seroincident cohorts in Durban were enrolled into CAPRISA 002 if they met the above-described criteria. Time of infection was defined as the midpoint between the last HIV antibody-negative test and the first HIV antibody-positive test or 14 days prior to the first positive HIV RNA PCR assay for those identified as being antibody negative but HIV RNA PCR positive. The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, and participants provided written informed consent.

PBMCs from a total of 32 HIV-1-uninfected and 28 recently HIV-1-infected individuals from the CAPRISA study cohort were available for use in this study.

Sample processing, viral load quantification, and CD4 cell enumeration. PBMCs were isolated by Ficoll-Histopaque (Sigma) density gradient centrifugation from blood within 6 h of phlebotomy and frozen in liquid nitrogen until use. Viral load was determined by using the automated COBAS Amplicor HIV-1 Monitor Test v1.5 (Roche). $CD4^+$ cells were enumerated by using the Multitest kit (CD4/CD3/CD8/CD45) on a four-parameter FACSCalibur flow cytometer (Becton Dickinson).

Lentivirus production. Lentiviruses containing short hairpin RNAs (shRNAs) expressed under the control of the U6 promoter in a lentiviral vector (pLKO.1) that also confers puromycin resistance were generated in 293T cells as previously described (22).

Plasmids pLKO.1, pLKO.1/scramble$_{shRNA}$ (Harvard Institute of Proteomics), and pLKO.1/TRIM22$_{shRNA}$ 3′UTR (5′-CCGGTATTGGTGTTCAAGACTATATCTCGAGATATAGTCTTGAACACCAATATTTTTG-3′; Sigma Aldrich) were used. 293T cells were transfected with a packaging plasmid (pCMVdR8.2 dvpr; Addgene), a pRSV-Rev (Addgene) envelope plasmid (VSV-G/pMD2.G; Addgene), and the corresponding pLKO.1 vector.

Viral infection. Peripheral blood mononuclear cells (1×10^6 cells) isolated from healthy HIV-negative donors were placed into a 12-well plate in the presence or absence of CD3.8 antibody (0.5 μg/ml) and incubated for 3 days at 37°C in 5% CO_2. Following stimulation, cells were washed with R10 medium and then infected with HIV-IIIB (NIH AIDS Reagent Repository) by spinoculation (2 h at 2,500 rpm at 37°C) at 2×10^5 cells/well in a 96-well plate. Virus was subsequently removed, and cells were washed once and then allowed to incubate for an additional 3 days before analysis of TRIM22 expression by Western blotting.

For Jurkat LTR-G cell experiments (JLTR-G; NIH AIDS Reagent Repository), cells were transduced by using spinoculation as described above, and the cells were puromycin selected 48 h later. Stably transduced JLTR-G cells in 96-well plates (2×10^5 cells/well) were incubated with IFN-α2a (1,000 U/ml; Pestka Biomedical Laboratories, NJ) for 24 h. Cells were then infected by spinoculation with HIV-IIIB. Cells were allowed to incubate for 2 h and then analyzed for HIV long terminal repeat (LTR)-dependent green fluorescent protein (GFP) expression by flow cytometry on days 2 and 7. Cell culture supernatants from day 7 samples were harvested and analyzed by a p24 enzyme-linked immunosorbent assay (ELISA) (Becton Dickinson).

Type 1 IFN stimulation of immune cell lines. CEM, Jurkat, and THP1 cells in 96-well plates (2×10^5 cells/well) were stimulated with IFN-α for 6 h, whereupon gene expression was assessed for TRIM5α and TRIM22.

RNA isolation and analysis. For all samples, RNA was extracted immediately after thawing and counting of PBMCs without *in vitro* stimulation. RNA was extracted from 2×10^6 PBMCs by using TRIzol LS reagent (Invitrogen). The total RNA concentration was quantified, and samples were used only if the optical density at 260 nm (OD_{260})/OD_{280} ratio was 1.90 or greater. All RNA samples were DNase treated. One microgram of total RNA from each sample was reversed transcribed by using the iScript cDNA synthesis kit (Bio-Rad).

RNA quantitation by real-time PCR. The PCR primers and cycling conditions used for IFN-α, IFN-β, MxA, huTRIM5α, and TRIM22 real-time quantitative PCR are provided in Table 1. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined to be the most suitable reference gene based on PCR efficiency. Each PCR mixture consisted of 3 mmol/μl $MgCl_2$, the respective primers (0.5 pmol/μl for MxA, huTRIM5α, and TRIM22 and 0.25 pmol/μl for IFN-α, IFN-β, and GAPDH), 1 μl Fast Start SYBR green I (Roche), 1 μg cDNA, and water (10-μl total volume). Reactions were run with a Roche LightCycler v1.5 instrument (1 cycle at 95°C for 10 min and then 45 cycles of denaturation, annealing, and extension [Table 1]). To confirm amplification specificity, the PCR products were subjected to a melting-curve analysis and agarose gel electrophoresis. Serial dilutions of cDNA from total RNA were performed for each target gene. These served as standard curves for quantitative analysis.

Western blotting. Antibodies used in this study were rabbit polyclonal anti-TRIM22 (Prestige, catalog number HPA003575; Sigma), mouse monoclonal anti-Ran (catalog number R4777; Sigma), or rabbit polyclonal anti-IFITM1 (catalog number ab70477; Abcam). Antibody-antigen complexes were detected by using enhanced chemiluminescence reagents (Invitrogen).

Statistical analysis. The generation of dot plots, nonparametric statistical analysis, and correlations (Pearson) were performed by using the statistical programs Instat Graphpad Prism V.5. and SAS. Values are expressed as medians. Differences between groups were evaluated by using a Student's *t* test. We correlated huTRIM5α and TRIM22 to type 1 IFN (MxA, IFN-α, and IFN-β) gene expression values and to each other. Pearson correlations were performed on log-transformed data. Univariate and multivariate generalized estimating equation (GEE) models were fitted to huTRIM5α, TRIM22, MxA, IFN-α, and IFN-β expressions; viral loads; and CD4 cell counts. Viral loads and expression

TABLE 1. Primers used in this study

Gene	GenBank accession no.	Sequence (5′–3′)[a]	Cycling conditions (denaturation, annealing, and extension)
MxA	NM_0024462	5′-AAGCTGATCCGCCTCCACTT-3′ (F) 5′-TGCAATGCACCCCTGTATACC-3′ (R)	95°C for 6 s, 60°C for 6 s, and 72°C for 10 s
IFN-α	NM_000069	5′-GAAACCACTGACTGTATATTGTGTGAAA-3′ (F) 5′-CAGCGTCACTAAAAACACTGCTTT-3′ (R)	95°C for 6 s, 60°C for 6 s, and 72°C for 10 s
IFN-β	L41942	5′-AGTCAGAGGGAATTGTTAAGAAGCA-3′ (F) 5′-TTTGGAATTAACTTGTCAATGATATAGGTG-3′ (R)	95°C for 6 s, 60°C for 6 s, and 72°C for 10 s
huTRIM5α	NM_033034	5′-AGGAGTTAAATGTAGTGCT-3′ (F) 5′-ATAGATGAGAAATCCATGGT-3′ (R)	95°C for 6 s, 60°C for 15 s, and 72°C for 6 s
TRIM22	NM_006074	5′-GGTTGAGGGGATCGTCAGTA-3′ (F) 5′-TTGGAAACAGATTTTGGCTTC-3′ (R)	95°C for 6 s, 60°C for 6 s, and 72°C for 10 s
GAPDH	NM_002046	5′-AAGGTCGGAGTCAACGGATT-3′ (F) 5′-CTCCTGGAAGATGGTGATGG-3′ (R)	95°C for 6 s, 65°C for 6 s, and 72°C for 6 s

[a] F, forward; R, reverse.

levels were log transformed, while square root transformation was applied to CD4 count data to ensure normality.

RESULTS

Relative expression levels of type 1 IFN (IFN-α and IFN-β), MxA, and huTRIM5α in PBMCs from HIV-1-uninfected versus HIV-1-infected subjects. We have previously shown that HIV-negative patients have higher levels of huTRIM5α than do HIV-1-positive patients (34). However, in matched pre- and postinfection samples, we did not see a significant dysregulation of TRIM5α. This result was surprising, as TRIM5α is a type 1 IFN-responsive gene, and type 1 IFN is dysregulated in primary HIV-1 infection (15). Furthermore, we found that women at high risk for HIV-1 infection who did not seroconvert following 2 years of follow-up had significantly higher TRIM5α mRNA levels in PBMCs than did seroconverters (34). A possible explanation for the latter finding is that high-risk nonseroconverter study participants have generally higher levels of innate antiviral defense mechanisms, perhaps mediated through type 1 IFN, thus providing an explanation for our observation of elevated huTRIM5α levels among nonseroconverters. We therefore sought to better understand the relationship between TRIM5α, IFN-α, IFN-β, and a type 1 IFN-inducible gene, MxA.

We compared mRNA levels of IFN-α, IFN-β, MxA, and TRIM5α in PBMCs from HIV-1-negative versus HIV-1-infected samples collected within the first 12 months postinfection. There were 32 individual HIV-1-negative samples available and 28 HIV-1-infected samples. Samples from HIV-1-positive individuals were available at multiple time points postinfection, and samples closest to the 12-month-postinfection time point were included in the analyses presented here. Only patients that remained HIV-1 negative upon 2 years of follow-up were used for this analysis ($n = 19$). The expression values were log transformed to ensure normality. Median expression levels between HIV-negative and HIV-positive samples were compared by using an unpaired Student's *t* test. There were no significant differences in IFN-α expression between HIV-1-negative and –positive participants (Fig. 1A). HIV-1-positive participants had significantly higher levels of IFN-β ($P = 0.0005$) and MxA ($P = 0.007$) (Fig. 1B and D). As we have previously reported, HIV-1-negative PBMCs had significantly higher levels of TRIM5α than did HIV-1-positive PBMCs ($P < 0.0001$) (Fig. 1C).

We next investigated the relationship between TRIM5α and type 1 IFN expression in HIV-1-negative and -positive samples. There was no correlation between TRIM5α and IFN-α or MxA (Fig. 1E and G) for both negative and positive time points. All HIV-1-negative ($n = 32$) and HIV-1-positive ($n = 75$) samples available at multiple time points were used for this analysis. We found a significant inverse correlation between IFN-β and TRIM5α in both HIV-1-negative ($r = -0.49$; $P = 0.004$) and HIV-positive ($r = -0.39$; $P = 0.0008$) samples (Fig. 1F). As expected, our data also indicated that MxA is a suitable surrogate for type 1 IFN induction, because MxA mRNA levels showed a significant positive correlation with IFN-α in both HIV-1-negative ($r = 0.8$; $P = 0.0001$) and HIV-1-positive ($r = 0.81$; $P = 0.0001$) PBMCs. MxA mRNA levels were also significantly correlated with IFN-β levels in both HIV-1-negative ($r = 0.7$; $P = 0.0001$) and HIV-1-positive ($r = 0.8$; $P = 0.0001$) samples (Fig. 1H). Thus, in this cohort of individuals at high risk for HIV-1 infection in a high-prevalence setting, the level of TRIM5α was higher in HIV-1-negative than in HIV-1-positive PBMCs, and surprisingly, there was an inverse correlation between TRIM5α and IFN-β.

Expression of TRIM22 in PBMCs from HIV-1-uninfected versus -infected subjects and association between type 1 IFN and TRIM22. We next wished to evaluate the expression of the TRIM22 gene, which is located downstream of TRIM5α on chromosome 11 (location 11p15) (32), because it was shown previously to be type 1 IFN inducible *in vitro* and has known antiviral activity (2). HIV-1-positive participants had higher mRNA levels of TRIM22 than did HIV-negative patients ($P = 0.01$) (Fig. 2A). IFN-α mRNA levels positively correlated with TRIM22 expression levels in both HIV-negative ($r = 0.91$; $P < 0.0001$) and HIV-1-positive ($r = 0.9$; $P < 0.0001$) subjects (Fig.

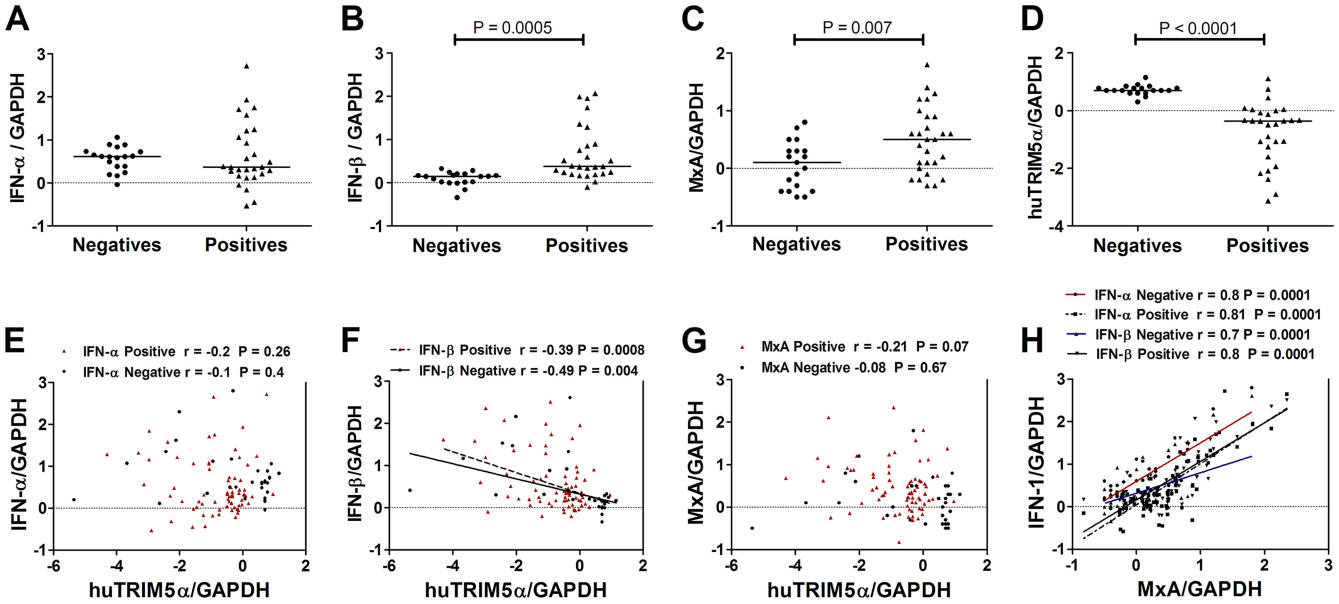

FIG. 1. Expression of type 1 IFN (IFN-α, IFN-β, and MxA) and huTRIM5α in PBMCs from HIV-1-uninfected versus HIV-1-infected subjects and association between type 1 IFN and huTRIM5α. The samples from infected participants were all collected within 12 months of infection. At least two time points were available postinfection for the primary infection samples. For the HIV-1-positive group we compared one time point, closest to the set point of 12 months postinfection ($n = 28$). Only patients that remained HIV-1 negative upon follow-up were used for this analysis ($n = 19$). Data are depicted as the normalized ratio of huTRIM5α, IFN-α, or IFN-β versus GAPDH. The expression values were log transformed to ensure normality. Median expression levels between HIV-negative and HIV-positive samples were compared. The differences between groups were evaluated by using an unpaired Student's t test. A P value of <0.05 was considered statistically significant. Pearson correlations were performed for huTRIM5α and IFN-α, IFN-β, or MxA for both negative and positive patients. Pearson correlations were also performed for MxA and IFN-α or IFN-β for both negative and positive patients.

2B). IFN-β also showed a significant positive correlation with TRIM22 in both HIV-negative ($r = 0.93$; $P < 0.0001$) and HIV-1-positive ($r = 0.87$; $P < 0.0001$) subjects (Fig. 2C). Likewise, MxA mRNA levels correlated positively with TRIM22 mRNA levels in both HIV-1-negative ($r = 0.81$; $P < 0.0001$) and HIV-1-positive ($r = 0.92$; $P < 0.0001$) subjects (Fig. 2D). Thus, TRIM22 positively correlates with type 1 IFN expression in both HIV-1-negative and HIV-1-positive PBMCs *in vivo*.

Expression of type 1 IFN (IFN-α and IFN-β), MxA, and TRIM22 mRNA in PBMCs at baseline (study enrollment) from nonseroconverters versus seroconverters. We next addressed whether preinfection samples from seroconverters differed from those from nonseroconverters in IFN-α, IFN-β, MxA, and TRIM22 expression levels. Although seroconverters showed generally higher mRNA levels of IFN-α and MxA than nonseroconverters, the differences between the groups did not

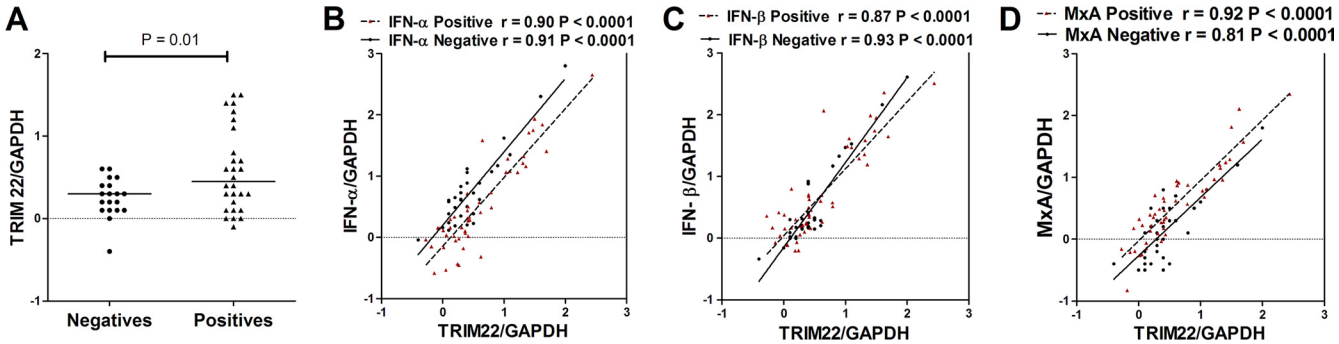

FIG. 2. Expression of TRIM22 in PBMCs from HIV-1-uninfected versus HIV-1-infected subjects and association between type 1 IFN and TRIM22. The samples from infected participants were all collected within 12 months of infection (primary infection phase). At least two time points were available postinfection for the primary infection samples. For the HIV-1-positive group we compared one time point, closest to the set point of 12 months postinfection ($n = 28$). Only patients that remained HIV-1 negative upon follow-up were used for this analysis ($n = 19$). Data are depicted as the normalized ratio of TRIM22 versus GAPDH. The expression values were log transformed to ensure normality. Median expression levels between HIV-negative and HIV-positive samples were compared. The differences between groups were evaluated by using an unpaired Student's t test. A P value of <0.05 was considered statistically significant. Pearson correlations were performed for TRIM22 and IFN-α, IFN-β, or MxA for both negative and positive patients.

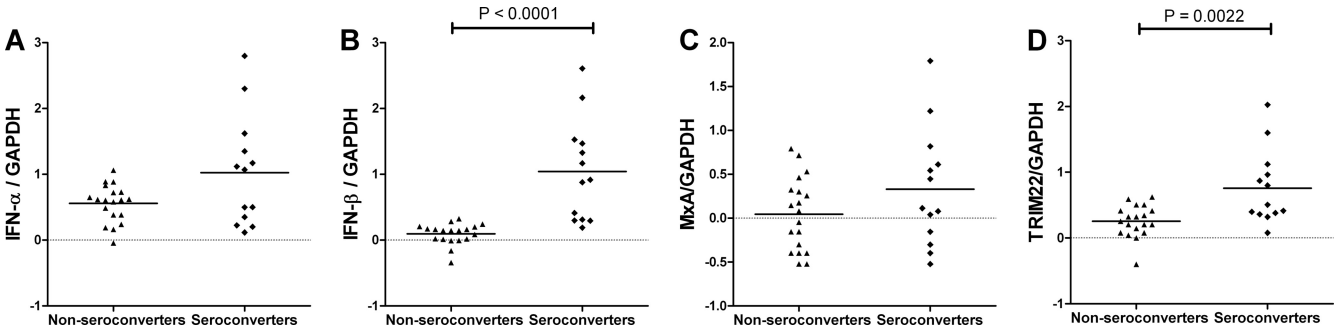

FIG. 3. Expression of type 1 IFN (MxA, IFN-α, and IFN-β) and TRIM22 mRNA in PBMCs at baseline (study enrollment) for nonseroconverters versus seroconverters. Participants included in this analysis were all enrolled as high-risk HIV-1-uninfected individuals and were longitudinally monitored for at least 36 months each at the time of analysis. Data are depicted as the normalized ratio of IFN-α, IFN-β, MxA, and TRIM22 versus GAPDH. The expression values were log transformed to ensure normality. The horizontal line represents the median. The differences between groups were evaluated by using an unpaired Student's *t* test. A *P* value of <0.05 was considered statistically significant.

reach statistical significance (Fig. 3A and C). Individuals who became HIV-1 positive ($n = 13$) during the 2-year study follow-up period had significantly higher IFN-β ($P < 0.0001$) and TRIM22 ($P = 0.0022$) mRNA levels preinfection than did those who remained HIV-1 negative ($n = 19$) ($P < 0.0001$; $P = 0.0022$) (Fig. 3B and D).

Association between antiviral gene expression, viral load, and CD4 T-cell counts. To determine if IFN-α, IFN-β, MxA, huTRIM5α, and TRIM22 gene expressions had functional implications for viral control during primary infection, we used a generalized estimating equation (GEE) model to evaluate viral load or $CD4^+$ T-cell counts, adjusting for repeated measurements for the same individual. In the univariate models, MxA displayed a statistically significant association with HIV-1 plasma viral load. For every log increase in MxA mRNA levels, the viral load increased by 0.29 log copies/ml ($P = 0.0444$). IFN-β also showed a positive association with viral load; however, this was not statistically significant ($P = 0.0995$).

Following adjustment for the other antiviral factors included in this study, MxA and TRIM22 maintained a statistically significant association with viral load. The association between MxA and HIV-1 viral load increased after adjusting for the other antiviral factor expression variables, with every log increase in MxA increasing the viral load by 0.85 log copies/ml ($P = 0.0418$). On the other hand, for every log increase in TRIM22, the viral load decreased by 0.98 log copies/ml ($P = 0.0307$) (Table 2).

$CD4^+$ T-cell counts are an important correlate of disease progression rate and outcome in HIV-1 infection. We therefore investigated whether IFN-α, IFN-β, MxA, huTRIM5α, and TRIM22 had any association with $CD4^+$ T-cell counts during primary HIV-1 infection. A GEE model was fitted to $CD4^+$ T-cell counts, adjusting for repeated measurements for the same individual. In the univariate models, MxA, TRIM22, IFN-α, and IFN-β all had significant a negative association with $CD4^+$ T-cell counts; thus, as the expression increased with 1 log, $CD4^+$ T-cell counts decreased by 2.12, 1.79, 1.75, and 2.04 square root $CD4^+$ T cells/μl for these factors, respectively. However, in the multivariate model, only TRIM22 remained statistically significant ($P = 0.0281$), showing a positive association with $CD4^+$ T-cell counts (Table 3).

TRIM22 expression is induced in HIV-negative PBMCs by infection with HIV-1. We next sought to determine whether infection of PBMCs isolated from HIV-negative donors could induce TRIM22 expression *in vitro*. Infection of PBMCs in the presence or absence of stimulating bispecific CD3.8 antibody (11) resulted in the upregulation of the TRIM22 protein in comparison to uninfected controls (Fig. 4A and B). The stim-

TABLE 2. Association between gene expression and viral load[a]

Gene[b]	Unadjusted model		Adjusted model	
	Effect estimate (SE)	*P* value	Effect estimate (SE)	*P* value
MxA	0.2887 (0.1436)	**0.0444**	0.8539 (0.4195)	**0.0418**
IFN-α	0.2163 (0.1363)	0.1126	0.2512 (0.6476)	0.6981
IFN-β	0.2216 (0.1345)	0.0995	0.1693 (0.4987)	0.7343
huTRIM5α	0.0368 (0.1308)	0.7785	0.1539 (0.1439)	0.2851
TRIM22	0.2154 (0.1532)	0.1597	−0.9807 (0.4539)	**0.0307**

[a] A GEE model was fitted to viral load, adjusting for repeated measurements for the same individual. Unadjusted models were fitted for each expression level in order to determine the effect on viral load. An adjusted model was fitted, including all expression variables in the model to determine whether they have an effect while adjusting for other expression levels. Viral load and expression level were log transformed to ensure normality. Boldface type indicates significance.

[b] Versus GAPDH.

TABLE 3. Association between gene expression and $CD4^+$ T-cell counts after HIV infection[a]

Gene[b]	Unadjusted model		Adjusted model	
	Effect estimate (SE)	*P* value	Effect estimate (SE)	*P* value
MxA	−2.1227 (0.8552)	0.0131	−4.1024 (2.3833)	0.0852
IFN-α	−1.7526 (0.7378)	0.0175	−2.9797 (2.4699)	0.2277
IFN-β	−2.0405 (0.6065)	0.0008	−0.8828 (2.3954)	0.7125
huTRIM5α	−2.0405 (0.6065)	0.0008	−0.3175 (0.5683)	0.5764
TRIM22	−1.7936 (0.8945)	0.0450	6.0974 (2.7761)	**0.0281**

[a] A GEE model was fitted to $CD4^+$ T-cell counts, adjusting for repeated measurements for the same individual. Unadjusted models were fitted for each expression level in order to determine the effect on $CD4^+$ T-cell counts. An adjusted model was fitted, including all expression variables in the model to determine whether they have an effect while adjusting for other expression levels. A square root transformation was applied to $CD4^+$ T-cell counts to ensure normality. The expression level was log transformed. Boldface type indicates significance.

[b] Versus GAPDH.

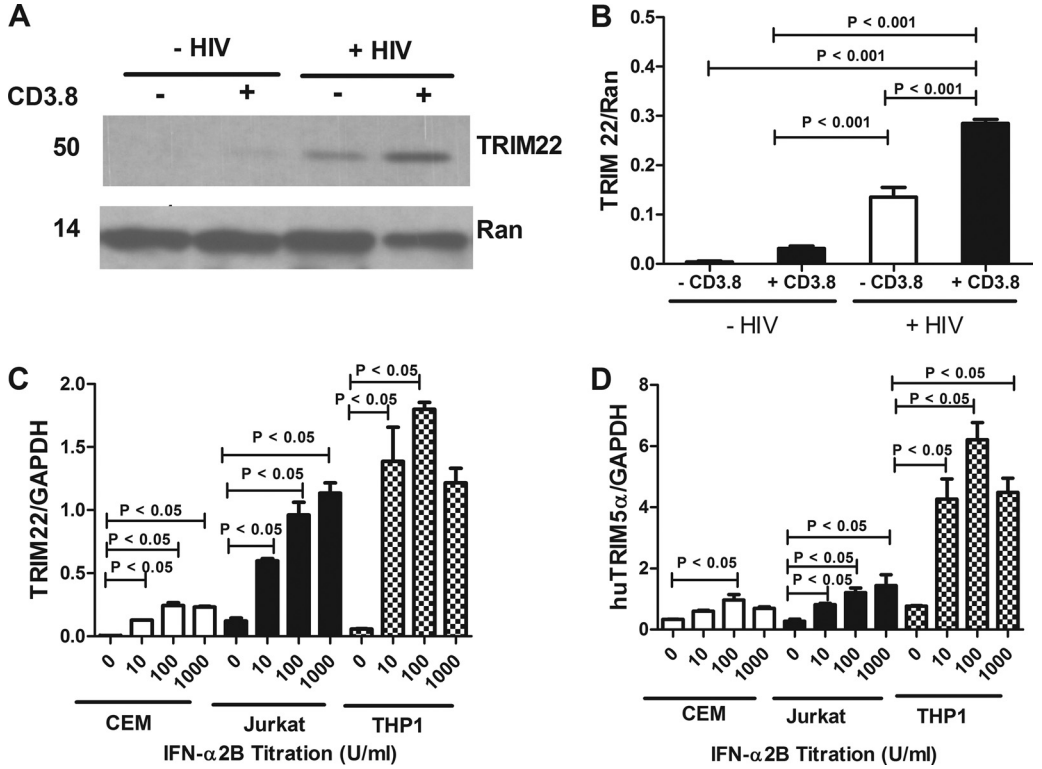

FIG. 4. Induction of TRIM22 expression by HIV and IFN-α. Shown is a representative example of data for TRIM22 induction in PBMCs from a healthy HIV-negative donor following infection with HIV-IIIB in the presence or absence of stimulating CD3.8 antibody. (A) Expression of TRIM22 following HIV infection was assessed by Western blotting. Ran levels are shown as a loading control. (B) Relative fold induction of TRIM22 by HIV averaged over three HIV-negative donors as determined by densitometric analysis (NIH Image). Values were normalized to TRIM22 expression following CD3.8 stimulation and HIV infection in each donor. (C and D) Dose-dependent increases in TRIM22 (C) and huTRIM5α (D) in various immune cell lines as determined by RT-PCR.

ulation of PBMCs with CD3.8 antibody with no infection also resulted in a slight increase in the TRIM22 expression level, indicating that activation alone was enough to alter TRIM22 expression.

To validate the role of IFN-α in TRIM22 induction, we stimulated a number of cell lines used in HIV infection assays (CEM, Jurkat, and THP-1) with increasing amounts of IFN-α and examined TRIM22 and huTRIM5α expression by reverse transcription (RT)-PCR. IFN-α significantly upregulated TRIM22 and huTRIM5α expression in a dose-dependent manner in all three cell lines tested (Fig. 4C and D).

Silencing of TRIM22 increases HIV infection and virus release in the presence of IFN-α. To determine its functional role in HIV infection, we tested the role of TRIM22 in HIV infection. JLTR-G reporter cells were stably transduced with empty pLKO vector, control scrambled shRNA (control), or an shRNA directed against the 3′ untranslated region (3′UTR) of TRIM22 and then challenged with HIV-IIIB (a fully infectious laboratory strain), with our without stimulation by IFN-α. At day 7, the percentage of HIV-infected cells (GFP-positive staining) in the 3′UTR cells treated with type 1 IFN exhibited a significantly higher percentage of infected cells (51.2%) than did vector-treated (22%) and control (24.5%) cells in the presence of IFN-α (Fig. 5A). Furthermore, the percentage of infected TRIM22-depleted cells was nearly equivalent regardless of whether cells had been treated with IFN-α or not, strongly demonstrating a significant functional role for TRIM22 in the anti-HIV IFN-α response. Data for culture supernatants collected on day 7 were consistent with these observations when assessed for p24 levels by ELISA (Fig. 5B). The knockdown of TRIM22 by the 3′UTR shRNA in the presence of IFN-α was validated by both RT-PCR and Western blotting (Fig. 5C and D).

DISCUSSION

For species other than humans, it has been demonstrated that restriction factors can completely block or partially restrict retroviral infection (7, 8, 12, 24, 36). In contrast, little is known about the *in vivo* regulation of restriction factors or their possible role in protecting or controlling retroviral infections in humans. In this study, we used a well-characterized clinical cohort of high-risk seronegative and acute or primary infection samples to investigate the association of the expression of select type 1 IFN isoforms, two well-characterized TRIM E3 ligases (TRIM5α and TRIM22), and the impact on HIV-1 susceptibility and viral control during primary HIV-1 infection.

Our earlier study revealed that lower huTRIM5α mRNA expression levels were associated with increased susceptibility to HIV-1 infection in a cohort of high-risk black African females (34). In addition, we found that in matched samples of

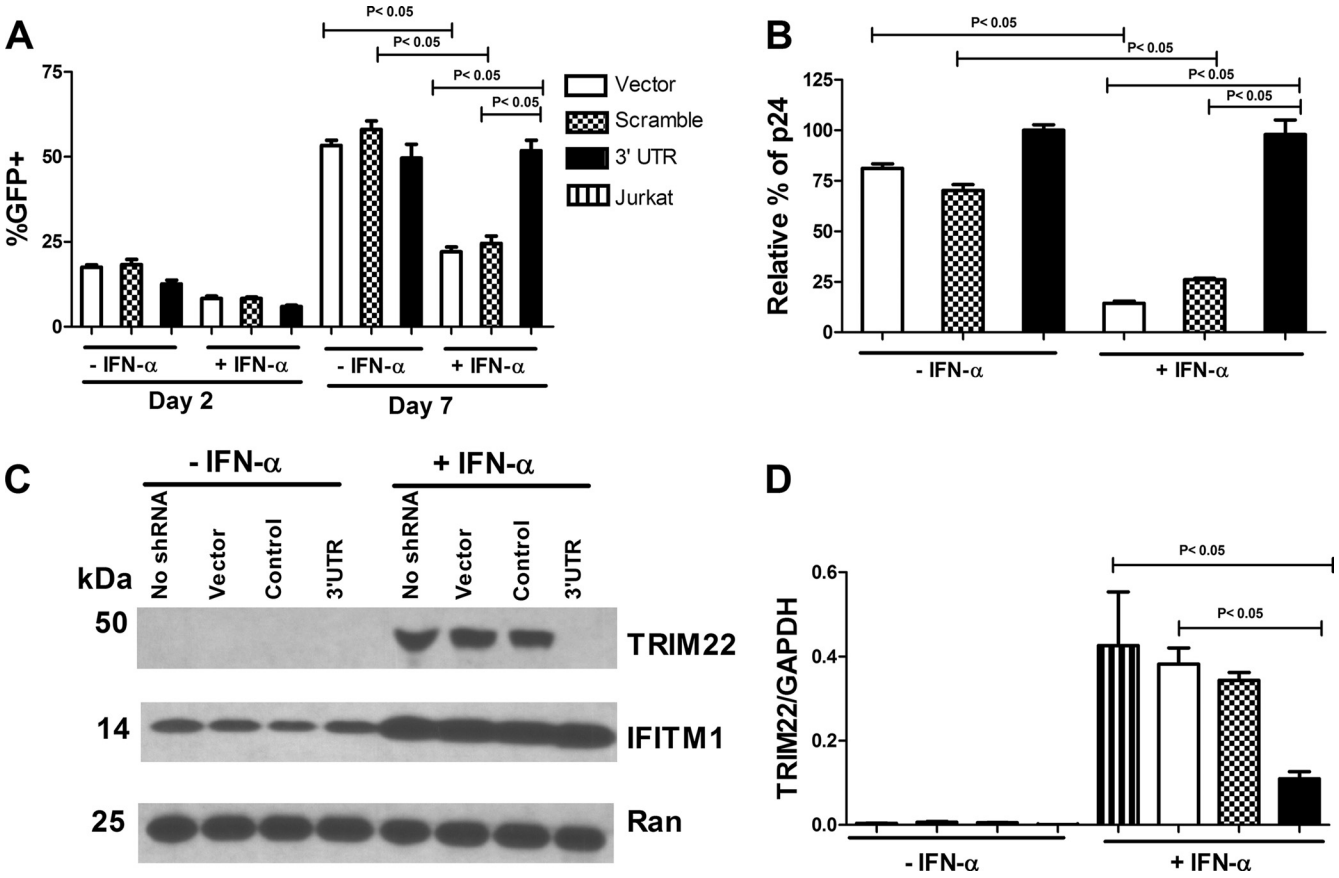

FIG. 5. TRIM22 silencing increases HIV infection and virus accumulation. (A) Jurkat reporter cells (LTR-G) transduced with the empty pLKO vector, control shRNA, or an anti-TRIM22 shRNA targeting the 3′UTR were infected with HIV-IIIB following a 1-day stimulation with IFN-α. Infection of JLTR-G cells (on day 7 postinfection) was assessed by GFP expression using flow cytometry. (B) TRIM22 silencing enhances accumulation of HIV particles in culture supernatants as determined by p24 ELISA on day 7 postinfection. (C and D) JLTR-G cells transduced with the indicated lentivirus were assessed for TRIM22 expression by RT-PCR and Western blotting. IFITM1 was used as a control (3a) for IFN induction, while Ran was used as a loading control.

HIV-1-negative individuals who later became HIV-1 positive, huTRIM5α levels were not dysregulated following infection.

Here we sought to understand the relationship between huTRIM5α and type 1 IFN in HIV-1-negative and -positive donor PBMCs, in part because huTRIM5α is type 1 IFN inducible (4, 31). Similarly, we also assessed the expression and activity of the related TRIM22 protein, focusing on this protein because, like TRIM5α, it is IFN inducible and has been demonstrated to have anti-HIV-1 activity (2, 4, 25). We found that PBMCs from HIV-1-positive study subjects had higher levels of IFN-β and MxA, suggesting that these antiviral proteins are actively upregulated following HIV-1 infection. This result is consistent with findings from other groups (42, 44). We did not see significant differences in IFN-α expression between HIV-1-negative and -positive PBMCs, suggesting that there may be differences in the mobilization of the varied type 1 IFN isoforms (10, 23) following HIV-1 infection. However, overall, in both HIV-1-negative and -positive PBMCs, we found a strong positive correlation between the two type 1 IFN isoforms tested and the IFN-inducible gene MxA, as previously described (18). Surprisingly, huTRIM5α showed a significant inverse correlation with IFN-β and had no association with IFN-α or MxA, even though huTRIM5α was shown previously to be an IFN-α-inducible gene *in vitro* (31). These results could reflect the limitations of our methodology here in analyzing global expression in PBMCs rather than in specific constituent cellular compartments or using frozen samples instead of fresh samples. Alternatively, we can speculate that distinct type 1 IFN isoforms may differentially regulate huTRIM5α expression, or huTRIM5α expression *in vivo* may involve more-complex pathways in addition to type 1 IFN. Further studies are needed to comprehensively investigate how different type 1 IFN isoforms and other cellular proteins may function in the regulation of huTRIM5α in different cellular environments and varied cohorts.

Unlike huTRIM5α, TRIM22 mRNA levels were higher in HIV-1-positive participants than in HIV-1-negative ones. We also found that TRIM22 correlated with IFN-α, IFN-β, and MxA in both HIV-1-negative and -positive samples. These results show an association between TRIM22 and type 1 IFN expression *in vivo*, suggesting that TRIM22 is a type 1 IFN-responsive gene *in vivo*, as was shown previously in *in vitro* experiments (2, 3). Higher levels of IFN-α, IFN-β, MxA, and TRIM22 were detected in seroconverters than in nonseroconverters at baseline, with differences reaching significance for IFN-β and TRIM22. These results suggest that there is im-

mune activation or another dysfunction before these persons become HIV-1 positive, and this may have contributed to the increased susceptibility to infection for these study subjects. It was previously demonstrated for a cohort of individuals at high risk of HIV-1 acquisition that participants who remained seronegative had lower levels of $CD4^+$ T-cell activation at baseline (when both groups where HIV-1 negative) (16). Similarly, levels of type 1 IFNs are elevated during immune activation, which in turn has been associated with increased HIV-1/AIDS pathology (19, 20). Further analysis of the dynamics of the expression of these antiviral factors in matched pre- and postinfection samples, especially from longitudinal cohorts of low- and high-risk study subjects with frequent sampling, may further help to better understand how intrinsic immunity is mobilized in acute HIV-1 infection and its possible contribution to antiviral control, especially in the critical acute phase of infection. It may also be useful to investigate the impact of HIV-1 infection on the type 1 IFN pathway response genes in mucosal tissues.

We also investigated the association of antiviral gene expression with viral load and CD4 T-cell counts, two commonly used markers of disease progression. We found that MxA mRNA levels showed a positive association with plasma viral load. For every log increase in MxA, the viral load increased by 0.85 log copies/ml. This is consistent with data from previous studies that demonstrated that levels of type 1 IFN increase as the viral load increases (18). Interestingly, we also observed that TRIM22 has a negative association with plasma viral load and a positive correlation with $CD4^+$ T-cell counts. We found that for every log increase in TRIM22 mRNA levels, there is an associated viral load decrease of 0.98 log copies/ml ($P = 0.0307$). TRIM22 also showed a positive association with $CD4^+$ T-cell counts, as every log increase in TRIM22 expression was associated with a 6.09 square root increase in $CD4^+$ cells/μl ($P = 0.0281$).

The new findings of a paradoxical elevation of TRIM22 levels in HIV-positive versus HIV-negative PBMCs and the favorable association of TRIM22 expression with markers of disease outcome prompted us to investigate whether TRIM22 could be induced by HIV-1 infection of HIV-negative donor PBMCs. Our experiments confirmed that TRIM22 was induced by HIV (Fig. 5A and B) and provided an *in vitro* corroboration of results demonstrating that HIV-1-positive subjects have higher levels of TRIM22 than do HIV-negative donors. This was similar to data from the work of Wang et al. (43), who showed that pseudotyped HIV-1 infection could induce APOBEC3G expression. Since PBMC populations are composed of a number of immune cell types (T cells and monocytes, etc.), we also demonstrated that IFN-α exerts an enhancing effect on TRIM22 expression in a dose-dependent manner in CEM and Jurkat T-cell lines and the monocyte cell line THP1 (Fig. 4C).

In addition, we found that the silencing of TRIM22 in a T-cell line nearly completely abrogated the IFN-mediated restriction of HIV-1 (Fig. 5A). TRIM22 silencing also resulted in an increased accumulation of HIV particles in culture supernatants (Fig. 5B), suggesting a role for TRIM22 in late viral replication activities, such as viral release or budding. Overall, therefore, our results are in agreement with data from several studies that have suggested that TRIM22 is induced by type 1 IFN and that TRIM22 can potently inhibit HIV replication and release (2, 3, 37).

Together, these data are suggestive of both *in vivo* and *in vitro* anti-HIV roles for TRIM22, although it is difficult to prove a cause-effect relationship between TRIM22 expression levels and viral load or CD4 T-cell count variables. Based on our findings, we speculate that the targeted enhancement of the expression of TRIM22 in HIV-1-infected individuals may be beneficial in reducing the viral load and could be employed as a novel antiviral strategy.

In conclusion, we have demonstrated with a cohort of HIV-1-uninfected and -infected individuals in a high-prevalence setting that HIV-1 infection is associated with an increased expression of the antiviral factor genes IFN-β and MxA, key components of the type 1 IFN pathway. However, we did not find a correlation between IFN-α or MxA and huTRIM5α, a previously described type 1 IFN-responsive host restriction factor. Indeed, we found a significant negative correlation between IFN-β and huTRIM5α. In contrast, we found that TRIM22 levels strongly correlated with IFN-α, IFN-β, and MxA expression in both HIV-1-negative and -positive PBMCs and were upregulated in HIV-1-positive study subjects. Remarkably, TRIM22 was associated with lower plasma HIV viral loads and higher CD4 T-cell counts in multivariate models adjusted for multiple antiviral factors analyzed, suggesting that TRIM22 could have antiviral effects *in vivo*. We show *in vitro* that TRIM22 is induced by type 1 IFN and HIV-1 infection. Furthermore, we demonstrate that TRIM22 plays a critical role in type 1 IFN-induced anti-HIV-1 activity in tissue cultures. This is the first study to provide evidence suggesting an *in vivo* antiviral activity of TRIM22. Further studies will be needed to address what specific cell types in the PBMC milieu express TRIM22 and the other members of the TRIM family, to better define how the expression of these proteins is regulated and to address whether these proteins can be harnessed as antiviral therapies or prophylactics.

ACKNOWLEDGMENTS

The CAPRISA 002 study was supported by the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), U.S. Department of Health and Human Services (grant U19 AI 51794). This study was funded by grants from the Switzerland South African Joint Research Programme (SSAJRP) (J.L. and T.N.), the Hasso Plattner Foundation and the South African DST/NRF Research Chair in Systems Biology of HIV/AIDS (T.N.), the NIAID (grant R01AI059159), and grant 3100AO-128655 from the SNF (J.L.). R.S. was a recipient of a KwaZulu-Natal Research Institute for TB and HIV (K-RITH) travel award.

We thank the study participants and the CAPRISA clinical and laboratory staff for providing specimens. We give special acknowledgments to the following members of the CAPRISA Acute Infection Study team: Carolyn Williamson, Lynn Morris, Clive Gray, Winston Hide, and Francois van Loggerenberg.

REFERENCES

1. **Asaoka, K., K. Ikeda, T. Hishinuma, K. Horie-Inoue, S. Takeda, and S. Inoue.** 2005. A retrovirus restriction factor TRIM5alpha is transcriptionally regulated by interferons. Biochem. Biophys. Res. Commun. **338:**1950–1956.
2. **Barr, S. D., J. R. Smiley, and F. D. Bushman.** 2008. The interferon response inhibits HIV particle production by induction of TRIM22. PLoS Pathog. **4:**e1000007.
3. **Bouazzaoui, A., M. Kreutz, V. Eisert, N. Dinauer, A. Heinzelmann, S. Hallenberger, J. Strayle, R. Walker, H. Rubsamen-Waigmann, R. Andreesen, and H. von Briesen.** 2006. Stimulated trans-acting factor of 50 kDa (Staf50) inhibits HIV-1 replication in human monocyte-derived macrophages. Virology **356:**79–94.

3a. **Brass, A. L., I. C. Huang, Y. Benita, S. P. John, M. N. Krishnan, E. M. Feeley, B. J. Ryan, J. L. Weyer, L. van der Weyden, E. Fikrig, D. J. Adams, R. J. Xavier, M. Farzan, and S. J. Elledge.** 2009. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus. West Nile virus, and dengue virus. Cell **139:**1243–1254.

4. **Carthagena, L., A. Bergamaschi, J. M. Luna, A. David, P. D. Uchil, F. Margottin-Goguet, W. Mothes, U. Hazan, C. Transy, G. Pancino, and S. Nisole.** 2009. Human TRIM gene expression in response to interferons. PLoS One **4:**e4894.

5. **Fensterl, V., and G. C. Sen.** 2009. Interferons and viral infections. Biofactors **35:**14–20.

6. **Gack, M. U., Y. C. Shin, C. H. Joo, T. Urano, C. Liang, L. Sun, O. Takeuchi, S. Akira, Z. Chen, S. Inoue, and J. U. Jung.** 2007. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. Nature **446:**916–920.

7. **Gao, G., X. Guo, and S. P. Goff.** 2002. Inhibition of retroviral RNA production by ZAP, a CCCH-type zinc finger protein. Science **297:**1703–1706.

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

9. **Goldschmidt, V., G. Bleiber, M. May, R. Martinez, M. Ortiz, and A. Telenti.** 2006. Role of common human TRIM5alpha variants in HIV-1 disease progression. Retrovirology **3:**54.

10. **Hardy, G. A., S. F. Sieg, B. Rodriguez, W. Jiang, R. Asaad, M. M. Lederman, and C. V. Harding.** 2009. Desensitization to type I interferon in HIV-1 infection correlates with markers of immune activation and disease progression. Blood **113:**5497–5505.

11. **Harrer, T., E. Harrer, S. A. Kalams, T. Elbeik, S. I. Staprans, M. B. Feinberg, Y. Cao, D. D. Ho, T. Yilma, A. M. Caliendo, R. P. Johnson, S. P. Buchbinder, and B. D. Walker.** 1996. Strong cytotoxic T cell and weak neutralizing antibody responses in a subset of persons with stable nonprogressing HIV type 1 infection. AIDS Res. Hum. Retroviruses **12:**585–592.

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

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

14. **Javanbakht, H., W. Yuan, D. F. Yeung, B. Song, F. Diaz-Griffero, Y. Li, X. Li, M. Stremlau, and J. Sodroski.** 2006. Characterization of TRIM5alpha trimerization and its contribution to human immunodeficiency virus capsid binding. Virology **353:**234–246.

15. **Kamga, I., S. Kahi, L. Develioglu, M. Lichtner, C. Maranon, C. Deveau, L. Meyer, C. Goujard, P. Lebon, M. Sinet, and A. Hosmalin.** 2005. Type I interferon production is profoundly and transiently impaired in primary HIV-1 infection. J. Infect. Dis. **192:**303–310.

16. **Koning, F. A., S. A. Otto, M. D. Hazenberg, L. Dekker, M. Prins, F. Miedema, and H. Schuitemaker.** 2005. Low-level CD4+ T cell activation is associated with low susceptibility to HIV-1 infection. J. Immunol. **175:**6117–6122.

17. **Kootstra, N. A., M. Navis, C. Beugeling, K. A. van Dort, and H. Schuitemaker.** 2007. The presence of the Trim5alpha escape mutation H87Q in the capsid of late stage HIV-1 variants is preceded by a prolonged asymptomatic infection phase. AIDS **21:**2015–2023.

18. **Lehmann, C., J. M. Harper, D. Taubert, P. Hartmann, G. Fatkenheuer, N. Jung, J. van Lunzen, H. J. Stellbrink, R. C. Gallo, and F. Romerio.** 2008. Increased interferon alpha expression in circulating plasmacytoid dendritic cells of HIV-1-infected patients. J. Acquir. Immune Defic. Syndr. **48:**522–530.

19. **Lehmann, C., D. Taubert, N. Jung, G. Fatkenheuer, J. van Lunzen, P. Hartmann, and F. Romerio.** 2009. Preferential upregulation of interferon-alpha subtype 2 expression in HIV-1 patients. AIDS Res. Hum. Retroviruses **25:**577–581.

20. **Mandl, J. N., A. P. Barry, T. H. Vanderford, N. Kozyr, R. Chavan, S. Klucking, F. J. Barrat, R. L. Coffman, S. I. Staprans, and M. B. Feinberg.** 2008. Divergent TLR7 and TLR9 signaling and type I interferon production distinguish pathogenic and nonpathogenic AIDS virus infections. Nat. Med. **14:**1077–1087.

21. **Meroni, G., and G. Diez-Roux.** 2005. TRIM/RBCC, a novel class of 'single protein RING finger' E3 ubiquitin ligases. Bioessays **27:**1147–1157.

22. **Moffat, J., D. A. Grueneberg, X. Yang, S. Y. Kim, A. M. Kloepfer, G. Hinkle, B. Piqani, T. M. Eisenhaure, B. Luo, J. K. Grenier, A. E. Carpenter, S. Y. Foo, S. A. Stewart, B. R. Stockwell, N. Hacohen, W. C. Hahn, E. S. Lander, D. M. Sabatini, and D. E. Root.** 2006. A lentiviral RNAi library for human and mouse genes applied to an arrayed viral high-content screen. Cell **124:**1283–1298.

23. **Mogensen, K. E., M. Lewerenz, J. Reboul, G. Lutfalla, and G. Uze.** 1999. The type I interferon receptor: structure, function, and evolution of a family business. J. Interferon Cytokine Res. **19:**1069–1098.

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

25. **Obad, S., T. Olofsson, N. Mechti, U. Gullberg, and K. Drott.** 2007. Regulation of the interferon-inducible p53 target gene TRIM22 (Staf50) in human T lymphocyte activation. J. Interferon Cytokine Res. **27:**857–864.

26. **Ozato, K., D. M. Shin, T. H. Chang, and H. C. Morse III.** 2008. TRIM family proteins and their emerging roles in innate immunity. Nat. Rev. Immunol. **8:**849–860.

27. **Perron, M. J., M. Stremlau, B. Song, W. Ulm, R. C. Mulligan, and J. Sodroski.** 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.

28. **Rajsbaum, R., J. P. Stoye, and A. O'Garra.** 2008. Type I interferon-dependent and -independent expression of tripartite motif proteins in immune cells. Eur. J. Immunol. **38:**619–630.

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

30. **Sadler, A. J., and B. R. Williams.** 2008. Interferon-inducible antiviral effectors. Nat. Rev. Immunol. **8:**559–568.

31. **Sakuma, R., A. A. Mael, and Y. Ikeda.** 2007. Alpha interferon enhances TRIM5alpha-mediated antiviral activities in human and rhesus monkey cells. J. Virol. **81:**10201–10206.

32. **Sawyer, S. L., L. I. Wu, M. Emerman, and H. S. Malik.** 2005. Positive selection of primate TRIM5alpha identifies a critical species-specific retroviral restriction domain. Proc. Natl. Acad. Sci. U. S. A. **102:**2832–2837.

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

34. **Sewram, S., R. Singh, E. Kormuth, L. Werner, K. Mlisana, S. S. Karim, and T. Ndung'u.** 2009. Human TRIM5alpha expression levels and reduced susceptibility to HIV-1 infection. J. Infect. Dis. **199:**1657–1663.

35. **Speelmon, E. C., D. Livingston-Rosanoff, S. S. Li, Q. Vu, J. Bui, D. E. Geraghty, L. P. Zhao, and M. J. McElrath.** 2006. Genetic association of the antiviral restriction factor TRIM5alpha with human immunodeficiency virus type 1 infection. J. Virol. **80:**2463–2471.

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

37. **Tissot, C., S. A. Taviaux, S. Diriong, and N. Mechti.** 1996. Localization of Staf50, a member of the Ring finger family, to 11p15 by fluorescence in situ hybridization. Genomics **34:**151–153.

38. **Towers, G. J.** 2005. Control of viral infectivity by tripartite motif proteins. Hum. Gene Ther. **16:**1125–1132.

39. **Uchil, P. D., B. D. Quinlan, W. T. Chan, J. M. Luna, and W. Mothes.** 2008. TRIM E3 ligases interfere with early and late stages of the retroviral life cycle. PLoS Pathog. **4:**e16.

40. **van Loggerenberg, F., K. Mlisana, C. Williamson, S. C. Auld, L. Morris, C. M. Gray, Q. A. Karim, A. Grobler, N. Barnabas, I. Iriogbe, and S. S. A. Karim.** 2008. Establishing a cohort at high risk of HIV infection in South Africa: challenges and experiences of the CAPRISA 002 acute infection study. PLoS One **3:**e1954.

41. **van Manen, D., M. A. Rits, C. Beugeling, K. van Dort, H. Schuitemaker, and N. A. Kootstra.** 2008. The effect of Trim5 polymorphisms on the clinical course of HIV-1 infection. PLoS Pathog. **4:**e18.

42. **von Wussow, P., D. Jakschies, H. K. Hochkeppel, C. Fibich, L. Penner, and H. Deicher.** 1990. The human intracellular Mx-homologous protein is specifically induced by type I interferons. Eur. J. Immunol. **20:**2015–2019.

43. **Wang, Y. J., X. Wang, H. Zhang, L. Zhou, S. Liu, D. L. Kolson, L. Song, L. Ye, and W. Z. Ho.** 2009. Expression and regulation of antiviral protein APOBEC3G in human neuronal cells. J. Neuroimmunol. **206:**14–21.

44. **Woelk, C. H., F. Ottones, C. R. Plotkin, P. Du, C. D. Royer, S. E. Rought, J. Lozach, R. Sasik, R. S. Kornbluth, D. D. Richman, and J. Corbeil.** 2004. Interferon gene expression following HIV type 1 infection of monocyte-derived macrophages. AIDS Res. Hum. Retroviruses **20:**1210–1222.

45. **Yap, M. W., S. Nisole, C. Lynch, and J. P. Stoye.** 2004. Trim5alpha protein restricts both HIV-1 and murine leukemia virus. Proc. Natl. Acad. Sci. U. S. A. **101:**10786–10791.