

Human TRIM5 α Expression Levels and Reduced Susceptibility to HIV-1 Infection

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Background. Human TRIM5 α (TRIM5 α hu), a member of the tripartite motif protein family, displays some anti-human immunodeficiency virus type 1 (HIV-1) activity in vitro, although it is substantially less potent than its rhesus monkey counterpart (TRIM5 α rh). The effects of levels of TRIM5 α hu on prevention or control of HIV-1 infection in vivo are unknown.

Methods. We used a quantitative real-time polymerase chain reaction (PCR) assay to measure levels of TRIM5 α hu expression in peripheral blood mononuclear cells (PBMCs) obtained from a cohort of individuals at high risk for HIV-1 infection in Durban, South Africa. Samples were available from 38 infected subjects (with all these samples obtained within 1 year of infection) and from 57 uninfected persons. Matched preinfection and postinfection samples were available from 13 individuals.

Results. TRIM5 α hu messenger RNA levels were lower in the PBMCs of HIV-1-infected subjects than in those of uninfected subjects ($P < .001$). Seroconverters had lower preinfection levels of TRIM5 α hu than did nonseroconverters ($P < .001$). TRIM5 α hu levels did not change significantly after infection. There was no correlation between TRIM5 α hu levels and viral loads or CD4⁺ T cell counts.

Conclusions. High expression of TRIM5 α hu is associated with reduced susceptibility to HIV-1 infection. Furthermore, infection is not associated with dysregulation of TRIM5 α hu. TRIM5 α hu expression levels do not contribute to the control of primary HIV-1 viremia.

In Old World monkeys, a member of the tripartite motif protein family known as “rhesus TRIM5 α ” (TRIM5 α rh) possesses potent anti-human immunodeficiency virus

(HIV) type 1 (HIV-1) activity and restricts HIV-1 infection [1]. TRIM5 α rh inactivates HIV-1 by binding to the viral capsid, leading to its premature disassembly, and it may also block virus production [2–6]. Human TRIM5 α (TRIM5 α hu) has also been shown to have some in vitro anti-HIV activity, but it is substantially less potent than TRIM5 α rh [1, 7]. Genetic variants of TRIM5 α hu have been shown to influence relative susceptibility to HIV-1 infection, rates of clinical disease progression, and in vitro HIV-1 replication [8–10]. Furthermore, HIV-1 capsid escape mutants from TRIM5 α hu recognition have been identified, suggesting antiviral immune pressure by this protein [11]. In the present study, we tested the hypothesis that TRIM5 α hu messenger RNA (mRNA) levels are significantly higher in peripheral blood mononuclear cells (PBMCs) obtained from HIV-1-uninfected persons than in those obtained from HIV-1-infected persons. Furthermore, we characterized baseline (preinfection) and postinfection TRIM5 α hu expression in seroconverters, as well as TRIM5 α hu expression at baseline in nonseroconverters from a longitudinal cohort of individuals at high risk for

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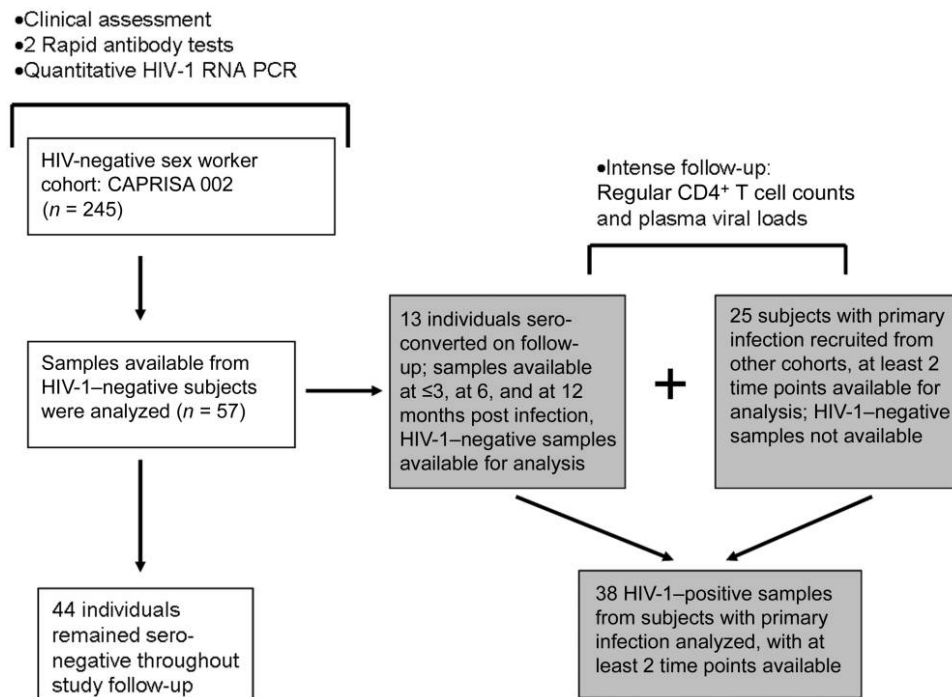


Figure 1. Study participants from a longitudinal cohort of individuals who had primary infection with human immunodeficiency virus type 1 (HIV-1) subtype C. A total of 57 HIV-uninfected study participants who were at high risk for infection were monitored for 36 months. HIV-1-negative samples obtained during sampling performed at study enrollment were available. Forty-four individuals remained seronegative throughout follow-up. Thirteen participants acquired HIV-1 infection and had samples collected at ≤ 3 months, at 6 months, and at 12 months after infection. In addition, 25 participants without matching preinfection samples were recruited and were monitored during primary HIV-1 infection through multiple sampling. CAPRISA, Centre for the AIDS Programme of Research in South Africa; PCR, polymerase chain reaction.

HIV-1 infection. We also tested whether TRIM5 α mRNA levels correlated with plasma viremia or CD4⁺ T cell counts during primary HIV-1 infection.

SUBJECTS, MATERIALS, AND METHODS

Subjects. Study subjects were part of the Centre for the AIDS Programme of Research in South Africa (CAPRISA) 002 Acute Infection Study, which is an observational natural history study of infection with HIV-1 subtype C that was established in Durban, South Africa, in 2004 [12]. Briefly, the cohort consisted of 245 high-risk seronegative women who were monitored to identify acute or recent infections. Two HIV-1 rapid tests, Capillus (Trinity Biotech) and Determine (Abbott Laboratories), were performed on a monthly basis to identify recent seroconversions. Antibody-negative samples were pooled for polymerase chain reaction (PCR) testing performed using Ampliscreen (version 1.5; Roche Diagnostics). Participants were enrolled in the acute infection phase of the study if they were found to be antibody positive within 5 months of receiving a previous antibody negative test result 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 in the CAPRISA 002 Acute Infection Study if they

met the aforementioned criteria. The time of infection was defined as (1) the midpoint between the last HIV antibody-negative test result and the first HIV antibody-positive test result or (2) 14 days before the first positive HIV RNA PCR assay result for those identified as antibody negative but HIV RNA PCR positive. The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu Natal, Durban, South Africa.

PBMCs obtained from a total of 57 HIV-1-uninfected and 38 recently infected individuals from the CAPRISA study cohort were available for use in the present study (figure 1). Samples from 10 HIV-1-negative individuals were available at 3-month intervals; this sampling interval allowed for longitudinal analysis. Samples were available from 13 study subjects before and after HIV-1 infection. Samples were available from at least 2 study time points for each of the individuals in the primary HIV-1 infection phase, and a total of 142 separate samples were analyzed for this group.

Sample processing, viral load quantification, and CD4⁺ T cell enumeration. PBMCs were isolated by Ficoll-Histopaque (Sigma) density gradient centrifugation from blood within 6 h of blood sample collection and were frozen until use. Viral loads were determined using the automated Cobas Amplicor HIV-1 Monitor Test (version 1.5; Roche). CD4⁺ T cells were enumer-

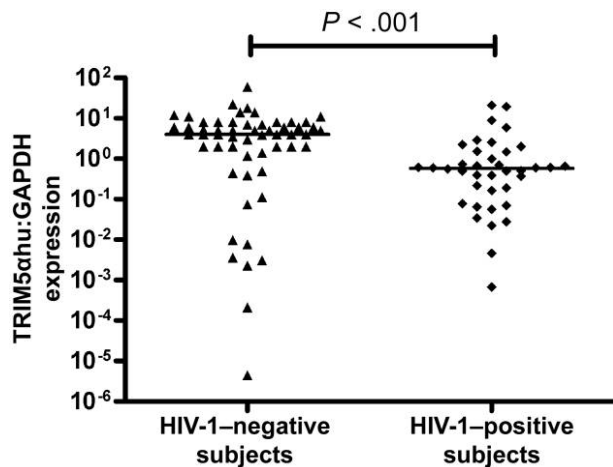


Figure 2. Expression of human TRIM5 α (TRIM5 α hu) in peripheral blood mononuclear cells (PBMCs) obtained from human immunodeficiency virus type 1 (HIV-1)-uninfected subjects and -infected subjects. The samples obtained from infected participants were all collected within 12 months of infection (i.e., during the primary infection phase). The samples obtained during primary infection were available from at least 2 time points after infection, and the average values of these TRIM5 α hu levels were determined and depicted as single data points. Data are depicted as the normalized ratio of TRIM5 α hu to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The horizontal line denotes the median value. The differences between groups were evaluated using the Mann-Whitney *U* test. $P < .05$ denoted statistical significance.

ated using the Multitest kit (CD4/CD3/CD8/CD45) on a 4-parameter FACSCalibur flow cytometer (Becton Dickinson).

RNA isolation and analysis. RNA was extracted from all samples immediately after PBMCs were thawed and counted without in vitro stimulation. RNA was extracted from 2×10^6 PBMCs by use of the TRIzol LS reagent (Invitrogen). RNA integrity was confirmed using 3-(*N*-morpholino)propanesulfonic acid (MOPS) gels. The total RNA concentration was quantified, and samples were used only if the ratio of the optical density measured at 260 nm to the optical density measured at 280 nm was ≥ 1.90 . All RNA samples were treated with DNase. One microgram of total RNA from each sample was reversed transcribed using the iScript complementary DNA (cDNA) synthesis kit (Bio-Rad).

Real-time PCR quantitation. The TRIM5 α hu gene was amplified using primers designed to uniquely amplify the α -isoform-specific SPRY domain of TRIM5 α hu (GenBank accession no. NM_033034). The primers used were 5'-AGGAGTTA-AATGTAGTGCT-3' (forward) and 5'-ACCATGGATTCTCA-TCTAT-3' (reverse). Glyceraldehyde 3-diphosphate dehydrogenase (GAPDH) was validated from among 5 genes as the most suitable reference gene, on the basis of its PCR efficiency. The GAPDH primers used were 5'-AAGGTCGGAGTCAACGG-ATT-3' (forward) and 5'-CTCCTGGAAGATGGTGATGG-3' (reverse). Each PCR comprised 3 mmol/ μ L MgCl₂, 0.5 pmol/ μ L each primer, 1 μ L of Fast Start SYBR Green I (Roche), 1 μ g of

cDNA, and water to 10 μ L. Reactions were run in duplicate on a Roche Lightcycler (version 1.5) as follows: 1 cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 6 s, at 60°C for 15 s, and at 72°C for 6 s. Relative mRNA levels were calculated by generating standard curves with the use of serial dilutions of a known concentration of cDNA sample for TRIM5 α hu and GAPDH. Individual samples were then analyzed against the standard curve and represented as levels of mRNA per microgram of cDNA. Results are depicted as a ratio of TRIM5 α hu to GAPDH per microgram of cDNA.

Statistical analysis. Dot plot generation, nonparametric statistical analysis, and correlations (Pearson) were performed using the statistical program InStat GraphPad Prism (version 3; GraphPad). Values are expressed as median values. Differences between >2 groups were evaluated using Dunn's multiple-comparison test, whereas the Mann-Whitney *U* test was used for any 2-group comparisons. $P < .05$ was considered to denote statistical significance.

RESULTS

To investigate whether there were differences in TRIM5 α hu gene expression between HIV-1-negative and HIV-1-positive subjects, we compared mRNA levels in PBMCs obtained from HIV-1-negative versus HIV-1-infected samples collected during the first 12 months after infection. There were 57 individual HIV-1-negative samples and 142 HIV-1-infected samples obtained from 38 individuals available for this comparison. Because multiple samples were available from HIV-1-infected per-

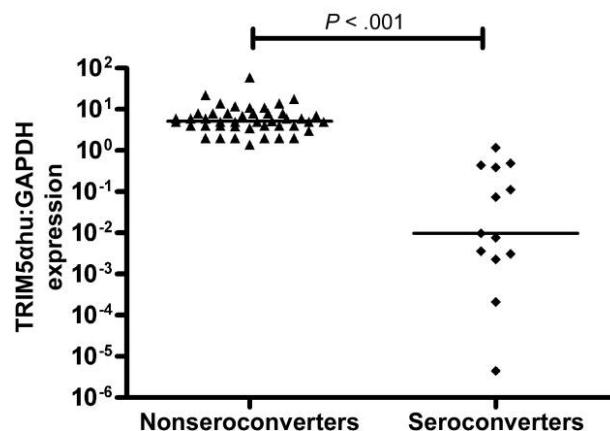


Figure 3. Expression of human TRIM5 α (TRIM5 α hu) messenger RNA in peripheral blood mononuclear cells (PBMCs) obtained from nonseroconverters vs. seroconverters at baseline (i.e., study enrollment). The participants included in this analysis were all enrolled as high-risk HIV-1-uninfected individuals and were longitudinally monitored for at least 36 months each at the time of analysis. Data are depicted as the normalized ratio of TRIM5 α hu to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The horizontal line denotes the median value. The differences between groups were evaluated using the Mann-Whitney *U* test. $P < .05$ denoted statistical significance.

sons, an average value for each individual was computed for this analysis. Levels of TRIM5 α hu mRNA expression in samples obtained from HIV-1–negative persons were significantly higher than the average levels noted in samples obtained from HIV-1–infected persons ($P < .001$) (figure 2). Median TRIM5 α hu mRNA levels were ~ 8 times higher in the uninfected group. There are 2 possible explanations for these results: (1) individuals who express low levels of TRIM5 α hu are more likely to become HIV-1 infected than are those who express high levels or (2) HIV-1 infection is associated with dysregulation of TRIM5 α hu mRNA expression.

We next addressed whether TRIM5 α hu expression levels differed in preinfection samples obtained from seroconverters versus samples obtained from nonseroconverters. Individuals who became HIV-1 positive ($n = 13$) during study follow-up had significantly lower TRIM5 α hu mRNA levels before infection, compared with individuals who remained HIV-1 negative

($n = 44$) ($P < .001$) (figure 3). Therefore, in this cohort, individuals with lower TRIM5 α hu levels were more likely to acquire viral infection than were their counterparts with higher TRIM5 α hu expression levels.

We reasoned that HIV-1 infection could be associated with transient or prolonged immune dysregulation of such intrinsic antiviral factors as TRIM5 α hu, as has been documented for other components of the antiviral immune system. We therefore examined the kinetics of TRIM5 α hu mRNA expression in matched HIV-1–negative and –positive samples obtained from the 13 individuals who acquired HIV-1 infection during follow-up. PBMCs were available from these individuals at study enrollment (before infection), within the first 3 months after infection (during the acute phase), and between 3 and 12 months after infection (during the early chronic phase). The levels of TRIM5 α hu noted for the 13 seroconverters before infection, in the acute phase, and in the early chronic phase are shown in figure 4A. Median TRIM5 α hu levels did not significantly differ between any of the 3 phases (preinfection, acute, and early chronic) analyzed, even though there were fluctuations at the individual patient level, with 6 individuals showing a decrease and 7 individuals showing an increase in the TRIM5 α hu level after infection. This result suggests that, in these individuals, TRIM5 α hu levels were stable.

We then investigated whether TRIM5 α hu expression is also stable in samples obtained from the same HIV-1–uninfected individuals at different time points. To do this, we analyzed TRIM5 α hu expression in PBMCs collected from 10 seronegative

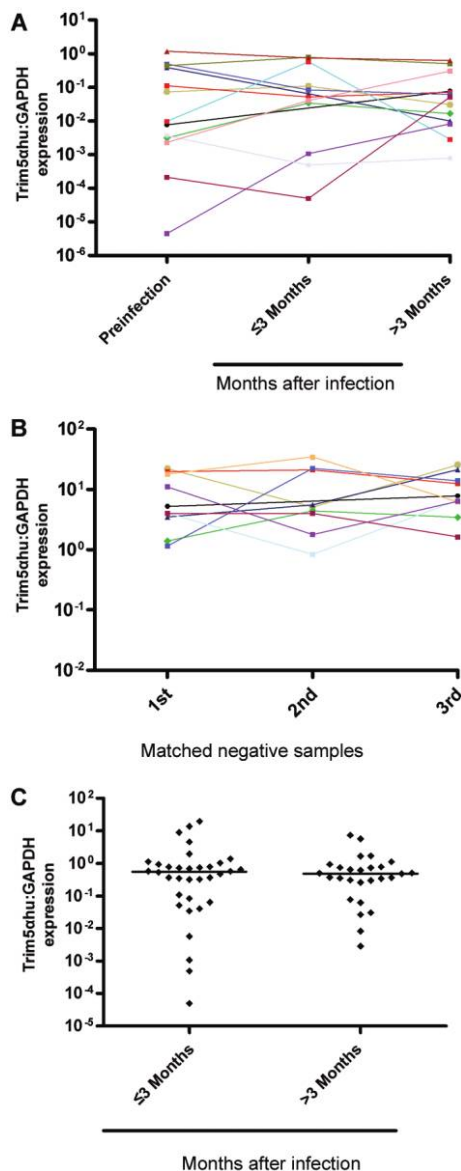


Figure 4. A, Kinetics of human TRIM5 α (TRIM5 α hu) expression in the 13 seroconverters for whom preinfection and postinfection peripheral blood mononuclear cell (PBMC) samples were available. Samples were analyzed before infection, at ≤ 3 months after infection (in the acute phase), and at > 3 months after infection (in the early chronic phase). For the early chronic phase, samples were available at 6 and 12 months after infection, and the average levels of expression were determined and depicted as single data points. There were no significant differences in median levels of TRIM5 α hu mRNA between the 3 phases. The differences between groups were evaluated using Dunn's multiple-comparison test. $P < .05$ denoted statistical significance. B, Kinetics of TRIM5 α hu expression in 10 individuals who remained human immunodeficiency virus type 1 (HIV-1) negative throughout the study. Samples obtained at 3 consecutive visits made at 3-month intervals were analyzed. There were no significant differences in the median levels of TRIM5 α hu messenger RNA between the 3 time points studied. The differences between groups were evaluated using Dunn's multiple-comparison test. $P < .05$ denoted statistical significance. C, Expression of TRIM5 α hu in all samples obtained from infected participants who were analyzed in the acute and early chronic phases of infection. For each participant, multiple samples were available, and the average TRIM5 α hu levels were determined and presented as single values. Data are the normalized ratio of TRIM5 α hu to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The horizontal line denotes the median value. There were no significant differences in expression between different postinfection phases. The differences between groups were evaluated using the Mann-Whitney U test. $P < .05$ denoted statistical significance.

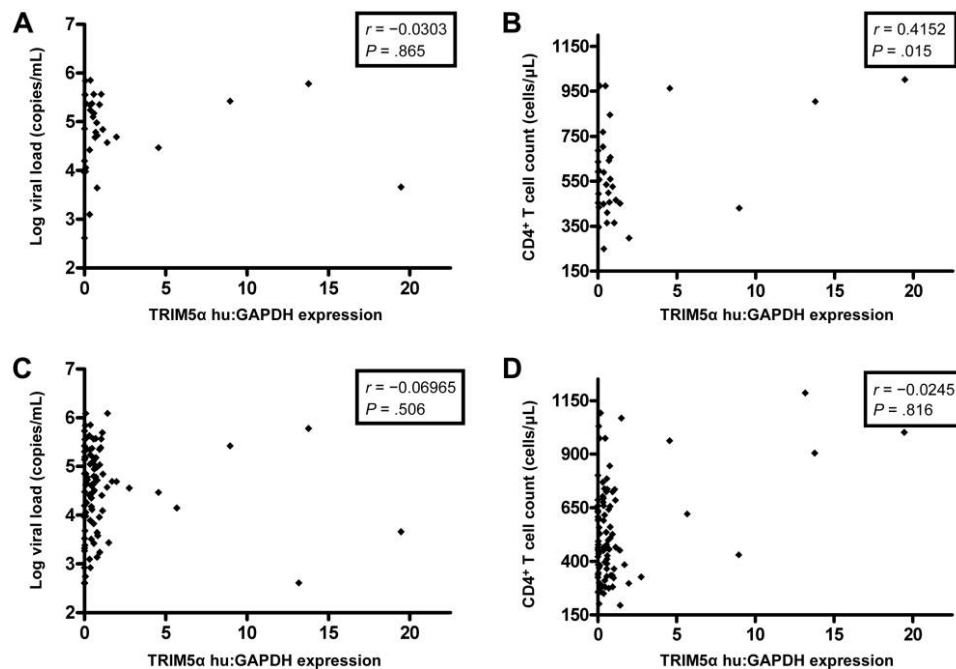


Figure 5. Analysis of the correlation between human TRIM5 α (TRIM5 α hu) messenger RNA levels and plasma human immunodeficiency virus type 1 (HIV-1) load (A) and CD4⁺ T cell counts (B) during the acute phase of infection and between plasma HIV-1 load (C) and CD4⁺ T cell counts (D) during the early chronic phase of infection.

individuals at 3-month intervals. As shown in figure 4B, there was minimal fluctuation in TRIM5 α hu mRNA levels. The observation of steady median TRIM5 α hu mRNA levels after infection held true, even when a larger sample size of all infected samples (including those for which matched preinfection samples were not available) was analyzed (figure 4C). In addition, because we had a small sample of participants with matched preinfection and postinfection samples ($n = 13$), we investigated whether this group of individuals was unique, compared with other individuals with primary infection, in terms of TRIM5 α hu expression. We compared postinfection levels of TRIM5 α hu mRNA noted in the acute and early chronic phases of infection in these 13 individuals versus those in individuals without matched preinfection samples. Although the 13 individuals with matched samples had median TRIM5 α hu mRNA levels that were lower than those of the individuals without matched HIV-negative samples, the differences were not statistically significant ($P = .07$ and $P = .06$, respectively).

To determine whether TRIM5 α hu gene expression had any functional implications for viral control during primary infection, we assessed whether there was a correlation between TRIM5 α hu mRNA levels and HIV-1 loads or CD4⁺ T cell counts, on the basis of data for the 38 HIV-1-infected subjects. To avoid bias, for each HIV-positive subject, data from only 1 time point was included in this analysis. Furthermore, because HIV-1 loads can fluctuate dramatically during the acute phase of HIV-1 infection, and because factors involved in viral control may differ according to the phase of infection, we analyzed data

obtained within 3 months after infection (the acute phase) separately from data obtained 3–12 months after infection (the early chronic phase), when the viral load is likely to have stabilized. During the acute phase of infection, there was no correlation between the levels of TRIM5 α hu expression and the HIV-1 load (figure 5A), but there was a significant but weak positive correlation with CD4⁺ T cell counts (figure 5B). During the early chronic phase of infection, there was no correlation between TRIM5 α hu mRNA levels and the plasma HIV-1 load or the CD4⁺ T cell count (figure 5C and 5D).

DISCUSSION

Innate and adaptive antiviral immune mechanisms are crucial for the control of HIV-1 replication, although the virus has evolved mechanisms to thwart these processes [13–16]. Host cells also possess intrinsic antiviral restriction factors that play an important role in host antiviral defense [17, 18]. TRIM5 α hu is a host restriction factor with a largely unknown influence on HIV-1 restriction in vivo.

In the present study, we found that levels of TRIM5 α hu expression were higher in HIV-1-negative individuals than in HIV-1-infected individuals. This result suggested 2 possibilities: either TRIM5 α hu is dysregulated subsequent to HIV-1 infection (by direct down-regulation, targeted killing of cells, or translocation of TRIM5 α hu-enriched cells away from peripheral blood) or those who acquired HIV-1 infection had lower levels of TRIM5 α hu at baseline. To discriminate between these possi-

bilities, we used samples from a longitudinal follow-up cohort and compared TRIM5 α hu mRNA levels in preinfection samples obtained from 13 individuals who subsequently became seropositive with similar samples obtained from 44 nonseroconverters who were in the same cohort. Interestingly, the data showed that nonseroconverters had significantly higher TRIM5 α hu mRNA levels.

We acknowledge that the small size of the sample of seroconverters who had matched preinfection samples was a limitation of this study; therefore, this finding needs to be interpreted cautiously. However, we can be fairly confident about the findings for this cohort, because of the stringent criteria for determination of HIV infection status that were used in this study, which included 2 antibody tests and a confirmatory RNA PCR assay. Furthermore, analysis revealed that the postinfection TRIM5 α hu mRNA levels of the 13 seroconverters did not significantly differ from those of other seroconverters for whom preinfection samples were unavailable; this finding suggests that these 13 individuals were not unique in having low TRIM5 α hu levels by chance. If confirmed in other settings, the observation that low levels of TRIM5 α hu are associated with a higher likelihood of HIV-1 acquisition has important implications for the role of TRIM5 α hu as a mediator of relative resistance to HIV-1 infection. We can speculate that enhancing the expression of TRIM5 α hu could be used as a novel strategy to protect against HIV-1 infection.

We also investigated whether HIV-1 infection is associated with dysregulation of TRIM5 α hu expression. We showed that, in matched preinfection and postinfection samples, there was no significant difference in the median level of TRIM5 α hu expression. Median levels of TRIM5 α hu mRNA remained relatively steady throughout primary HIV-1 infection, although the levels did fluctuate from one time point to another within individuals. TRIM5 α hu has previously been shown to be an interferon (IFN)- α -regulated gene [19], and IFN- α is transiently down-regulated during primary HIV-1 infection [20]. We did not find a similar trend for TRIM5 α hu dysregulation. We do not interpret this finding to suggest that TRIM5 α hu is not IFN- α regulated, given strong previously reported evidence for this [19, 21]. Instead, we believe that this observation implies that there are complex transcriptional regulatory mechanisms for TRIM5 α in vivo, as has been recently suggested elsewhere [6].

Previous studies show that another intrinsic antiviral factor, APOBEC3G, is down-regulated in HIV-1-infected PBMCs, compared with uninfected PBMCs [22–24]. Our study extends these findings to another intrinsic antiviral factor. In addition, we analyzed matched HIV-1-infected and HIV-1-uninfected samples obtained from the same subjects, and we characterized the kinetics of expression during primary HIV-1 infection. Our data show that HIV-1 infection is not associated with down-regulation of TRIM5 α hu; rather, the expression of TRIM5 α hu is lower in high-risk individuals who develop

HIV-1 infection. It will be important to investigate whether this finding can be extended to other intrinsic antiviral factors, such as APOBEC3G.

Finally, we reasoned that intrinsic antiviral factors are very likely to be involved as a first line of defense and that their effects might be more pronounced during primary HIV-1 infection. During the acute phase of infection, there was no significant correlation between TRIM5 α hu mRNA levels and plasma HIV-1 load, but there was a significant weak correlation with CD4⁺ T cell counts. In the early chronic phase of infection, we found no correlation between TRIM5 α hu mRNA levels and plasma viral loads or CD4⁺ T cell counts. We therefore conclude that TRIM5 α hu levels do not appear to contribute significantly to the rapid control of viral replication observed after peak viremia in acute infection, although the observation of a positive correlation of TRIM5 α hu mRNA levels with CD4⁺ T cell counts during the acute phase of infection is intriguing and may require further investigation in a larger sample size. Our results do not rule out the possibility of a long-term cumulative antiviral effect of TRIM5 α hu, as has been suggested by the recent identification of TRIM5 α capsid escape mutants [11]; however, this can be addressed only with a larger, longer-term longitudinal cohort.

In conclusion, this first description of the expression of TRIM5 α levels in association with HIV-1 pathogenesis in vivo has shown that TRIM5 α hu mRNA levels are lower in PBMCs from HIV-1–positive subjects than in those from HIV-1–negative subjects. In addition, we have shown that these lower TRIM5 α mRNA levels are most likely the result of seroconverters having significantly lower TRIM5 α hu levels at baseline (before infection), compared with nonseroconverters. These results suggest that high TRIM5 α hu levels could be protective against infection. Furthermore, our data suggest that HIV-1 infection is not associated with dysregulation of TRIM5 α hu at the transcriptional level. Finally, we found no negative correlation between TRIM5 α hu levels and plasma HIV-1 load or consistent positive correlation with CD4⁺ T cell counts, and we conclude that TRIM5 α hu levels have minimal or no effects on viral replication during primary infection. Further studies examining the contribution of TRIM5 α hu to viral pathogenesis in different cell/tissue types, at different phases of infection, and in a wide variety of subjects are clearly needed. However, the results of this study suggest that the interaction between HIV-1 and TRIM5 α hu may significantly affect the outcome of exposure to HIV-1 and that this interaction may therefore be an attractive target for the development of novel antiretroviral prophylactics.

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