Relationship between Human Immunodeficiency Type 1 Infection and Expression of Human APOBEC3G and APOBEC3F

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Background. Human immunodeficiency virus type 1 (HIV-1)–infected individuals with a high viral set point progress to acquired immunodeficiency syndrome (AIDS) more rapidly than those with a low viral set point. It is not entirely clear which host and viral factors are responsible for the viral set point. Host factors that affect virus replication are likely to influence the viral set point. Human APOBEC proteins have been shown to restrict HIV-1 replication.

Methods. This prospective study was conducted to determine the relationship between human APOBEC3G (hA3G) and APOBEC3F (hA3F) levels and the viral set point. Fourteen subjects were classified as having a high viral set point, and 16 were classified as having a low viral set point. We quantified the levels of hA3G and hA3F mRNA in HIV-1–infected, antiretroviral drug–naïve individuals before and after infection.

Results. We found a significant correlation between the hA3G mRNA level and the viral set point. The expression of hA3G and hA3F increased after infection, and the levels of hA3G and hA3F mRNA were significantly higher after infection in the low viral set point group, compared with the high viral set point group.

Conclusions. The results suggest that the level of hA3G expression affects the establishment of the viral set point and may therefore function as a host determinant in the pathogenesis of HIV-1 infection.

The progression of human immunodeficiency virus type 1 (HIV-1) infection to disease has a highly variable duration. Although the median time to development of acquired immunodeficiency syndrome (AIDS) is approximately 8 years, some patients develop AIDS in <3 years, whereas others remain healthy without symptoms for >15 years [1–3]. The reasons for these differences are not fully understood, but the differences are thought to result from the interaction of virus and host factors. For example, heterozygosity of class 1 loci (A, B, and C) delayed progression to AIDS, whereas homozygosity was associated with rapid progression to AIDS [4, 5]. Early HIV-1 infection is characterized by an initial period during which the rate of viral replication is high, leading to very high virus loads ~2 months after infection, followed by a 6–9-month period during which the virus load decreases until achievement of a steady state level, commonly referred to as the viral set point, which is a strong predictor of disease progression [6–8]. Individuals with a high viral set point have been shown to progress to AIDS more rapidly than individuals with a low viral set point [9–11]. The viral set point is thought to be determined by the breadth of HIV-specific immune responses and the extent of viral replication [12].

Ongoing viral replication is considered an important factor in the determination of the viral set point because the composite life span of the virus and virus-producing cells is very short (half-life, 2 days) [13]. In viral dynamics studies by Wei et al. [13], total replacement of wild-type circulating virus in plasma with drug-resistant strains occurred after only 2 weeks, indicating that the HIV-1 viral set point is sustained by a dynamic process that includes continuous rounds of virus infection, replication, and rapid cell turnover [13, 14]. Therefore, any host or viral factor that influences HIV-1 replication likely has an impact on the viral set point.
Two recently discovered host restriction factors in humans, APOBEC3G (hA3G) and APOBEC3F (hA3F), have been shown to restrict HIV-1 replication in vitro [15–20]. hA3G and hA3F exist in 2 forms in the cell, a high-molecular-mass complex and a low-molecular-mass complex; the antiviral activity of the proteins is associated with levels of the low-molecular-mass complexes [21–23]. Moreover, high levels of hA3G mRNA correspond to high levels of high-molecular-mass complex, low-molecular-mass complex, and hA3G-associated antiviral activities [24]. Interestingly, the HIV-1 virion infectivity factor (Vif) has been demonstrated to negatively influence the antiviral activities of hA3G and hA3F [25]. When Vif levels are low or Vif is absent, hA3G and hA3F incorporate into virions and catalyze cytidine deamination, leading to hypermutation from G to A in the viral genome [16, 26, 27]. HIV-1 Vif, in turn, inhibits hA3G and hA3F by inducing their proteasomal degradation and preventing their incorporation into virions.

On the basis of these observations, we hypothesized that high levels of hA3G and hA3F might overcome the neutralizing effects of HIV-1 Vif and reduce the rate of viral replication, decreasing the viral set point. In this study, we explore whether these 2 proteins play a role in determining the viral set point upon HIV-1 infection.

Levels of hA3G and hA3F mRNA in HIV-1–infected individuals are lower than in uninfected individuals [28]. In contrast, other studies showed that hA3G expression is higher in HIV-1–exposed seroconverters than in healthy controls and that long-term nonprogressors express a higher level of hA3G than rapid progressors [29, 30]. However, it is not known whether the levels of hA3G and hA3F increase or decrease upon HIV-1 infection or whether host factors establish the levels. To determine the effect of HIV-1 infection on expression of hA3G and hA3F, we characterized expression of these host restriction factors before and after HIV-1 infection.

SUBJECTS, MATERIALS, AND METHODS

Study population. This study was conducted in a cohort of female commercial sex workers in Dakar, Senegal, who have prospectively studied since 1985, as previously described [31, 32]. Informed consent was obtained from all study subjects, in accordance with the guidelines of the US Department of Health and Human Services for the conduct of clinical research. As part of the prospective study design, HIV-1–negative women are evaluated at quarterly intervals for evidence of HIV infection, which permits identification of the approximate time of seroconversion. For this study, a subset of antiretroviral-naive female commercial sex workers with incident HIV-1 seroconversion was selected from the cohort. In addition, repeat measurements of the virus load 6–30 months after seroconversion were required over a minimum observation period of 6 months. The median virus load for each individual was considered as the viral set point. The time of infection was estimated as the mid-point of the interval between the final seronegative sample and the first seropositive sample. Finally, we dichotomized patients according to their estimated viral set point: <7000 copies/mL was defined as a low viral set point and was observed in 16 women, and >23,000 copies/mL was defined as a high viral set point and was observed in 14 women. Thus, we excluded 5 women with estimated viral set points of 7000–23,000 copies/mL. At the time of sampling, none of the individuals had progressed to AIDS (as defined on the basis of Centers for Disease Control and Prevention criteria). Two peripheral blood mononuclear cell (PBMC) samples were selected from each individual in the high and low viral set point groups; one sample was obtained before HIV-1 infection, and one was collected 6–30 months after seroconversion. The CD4+ cell count was determined by calculating the median value of ≥2 CD4+ cell counts for an individual within 3 years after seroconversion.

Quantification of hA3G, hA3F, and β-actin mRNA. Cryopreserved PBMCs were thawed and rested overnight in RPMI medium supplemented with 10% (vol/vol) of FCS and 1% (vol/vol) of antibiotics without stimulation. RNA was extracted from PBMCs by use of the RNA4PCR kit (Ambion), according to the manufacturer’s instructions. cDNA synthesis was performed using the TaqMan reverse transcription kit (Applied Biosystems). Each reaction tube contained 10 μL of 10× TaqMan reverse-transcriptase buffer, 22 μL (50 mmol/L) MgCl2, 20 μL of dNTPs mixture, 2.5 μL (25 μmol/L) Poly-T primers, 2 μL (20–40 μmol/L) RNase inhibitor, 2.5 μL (50 μmol/L) MultiScribe reverse transcriptase, and 38.5 μL of total RNA sample. The samples were incubated for 10 min at room temperature, 30 min at 48°C, and 5 min at 95°C. The reaction products were stored at −20°C.

We used the TaqMan assay to quantify the hA3G and hA3F mRNA loads. The reactions were performed in a 96-well optical reaction plate (Applied Biosystems), and primers and probes for hA3G, hA3F, and β-actin were provided (TaqMan [Applied Biosystems]). Each reaction contained 2.25 μL of H2O, 12.5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems), 1.25 μL of primers and probes, and 10 μL of cDNA. Each reaction was performed in duplicate. The reactions were run on the ABI Prism 7000 Sequence Detection System (Applied Biosystems). The amplification program was 50°C for 2 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. The levels of both hA3G and hA3F were quantified using standard curves generated using pTcr99A with an hA3G insert (Genbank accession number NM_021822) [33] and pTcr99A with an hA3F insert (Genbank accession number NM_145298) [34]. The quantity of β-actin was determined by a standard curve generated by human genomic DNA. The levels of both hA3G and hA3F mRNA expression were normalized to the expression of the housekeeping gene β-actin. The levels of both hA3G and hA3F mRNA were reported in log form as the median number of
copies (range) of hA3G or hA3F per 100 copies of β-actin (hereafter, log copies).

RESULTS

Characteristics of the high and low viral set point groups.
The median age of study subjects at seroconversion was 35.5 years (range, 24.0–63.0 years). There was no significant difference between the median age for the low viral set point group (35.0 years [range, 24.0–63.0 years]) and that for the high viral set point group (36.3 years [range, 24.0–50.0 years]; P = .707, by the Mann-Whitney U test). Similarly, the median interval between seroconversion and collection of samples in the low viral set point group (22.0 months [range, 7.0–29.5 months]) was similar to that in the high viral set point group (25.0 months [range, 6.0–29.0 months]; P = .709, by the Mann-Whitney U test). There was no statistically significant difference in the median CD4+ cell count between the high viral set point group (622 cells/mm³ [range, 299–1177 cells/mm³]) and the low viral set point group (734 cells/mm³ [range, 289–1608 cells/mm³]; P = .245, by the Mann-Whitney U test).

Expression of hA3G and hA3F mRNA before and after infection.
Before infection, the median level of hA3G transcripts for the low viral set point group (1.104 log copies [range, −0.954–2.453 log copies]) was higher than that for the high viral set point group (0.023 log copies [range, −0.342–1.889 log copies]), and the difference was statistically significant (P = .046, by the Mann-Whitney U test). Also, the median level of hA3F mRNA transcripts before HIV infection in the low viral set point group (−0.207 log copies [range, −1.668–1.602 log copies]) was not statistically different from the median level of hA3F mRNA transcripts in the high viral set point group (−0.582 log copies [range, −1.954–1.756 log copies]; P = .317, by the Mann-Whitney U test).

After infection, the median level of hA3G mRNA in the low viral set point group (1.930 log copies [range, 0.658–2.899 log copies]) was greater than that in the high viral set point group (1.314 log copies [range, −0.954–2.513 log copies]; P = .020, by the Mann-Whitney U test). The same pattern was seen for hA3F mRNA, for which the median level in the low viral set point group (1.271 log copies [range, −1.277–1.789 log copies]) was higher than that in the high viral set point group (−0.012 log copies [range, −0.886–1.245 log copies]; P = .022, by the Mann-Whitney U test). The increase in expression of hA3G after HIV-1 infection was higher in the low viral set point group (P = .041, by the Mann-Whitney U test), but the difference between the 2 groups was not statistically significant in the case of hA3F (P = .083, by the Mann-Whitney U test). By use of both univariate and multivariate analyses, we established that the expression of hA3G and hA3F did not correlate with age at seroconversion, time after seroconversion, or CD4+ cell count.

Viral set point and expression of hA3G and hA3F mRNA.
To investigate the relationship between APOBEC (hA3G and
hA3F) expression levels and the viral set point, we used both univariate and multivariate linear regression. Other determinants of the viral set point, such as age at seroconversion, time after seroconversion, and CD4+ cell count, were analyzed. In the univariate analysis, the level of hA3G before infection (coefficient, −0.437; \( P = .035 \)) and the levels of hA3G (coefficient, −0.600; \( P = .010 \)) and hA3F (coefficient, −0.611; \( P = .007 \)) after infection were inversely correlated with the viral set point (table 1). In the multivariate model, the hA3G level before infection (coefficient, −0.455; \( P = .023 \)) was inversely correlated with the viral set point level; when one of the study subjects (subject 1813) with undetectable levels of hA3G before infection was included in the analysis, the correlation was not statistically significant (coefficient, −0.361; \( P = .074 \)), but the model was still significant (Prob > F = 0.041). In the multivariate analysis, the inverse correlation between hA3G expression after infection and the viral set point was statistically significant (coefficient, −0.537; \( P = .002 \)).

**Figure 2.** Comparison of human APOBEC3F (hA3F) mRNA transcript levels in high and low viral set point groups (high VL and low VL, respectively). The shaded boxes show the levels of hA3F mRNA before human immunodeficiency virus type 1 (HIV-1) infection, whereas the unshaded boxes show the levels of hA3F mRNA after HIV-1 infection. The levels of hA3F are expressed as the number of log-transformed copies per 100 copies of β-actin. Box plots, median values and interquartile ranges; dots, outliers; whiskers, ranges. \( P \) values were calculated using the Mann-Whitney test for comparison between groups and the Wilcoxon signed rank test for comparison within a group.

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\* Data are measures of the association between HIV-1 RNA load and independent factors in the model.
DISCUSSION

The role of hA3G and hA3F proteins in restricting HIV-1 replication in vivo has been suggested in previous studies [34, 35]. In vivo studies have supported in vitro observations in which hA3G mRNA levels in PBMCs were higher in long-term nonprogressors than in progressors. Because the viral set point correlates with the rate of disease progression [9], we investigated whether there was any relationship between the levels of hA3G and hA3F expression and the viral set point. We found an intriguing relationship between the levels of both hA3G and hA3F mRNA transcripts and the viral set point in the HIV-1–infected individuals we studied.

There was an inverse relationship between the levels of hA3G and hA3F mRNA and the viral set point after HIV-1 infection. The levels of hA3G and hA3F mRNA after infection in the low viral set point group were higher than in the high viral set point group ($P = .020$ for hA3G and $P = .022$ for hA3F, by the Mann–Whitney U test). Our results support previous findings that HIV-1–infected individuals with high viremia expressed less hA3G mRNA than those with low viremia [35]. This inverse relationship between viremia and APOBEC (hA3G and hA3F) expression suggests a role of APOBEC proteins in controlling viremia.

We further explored the association between viral set point and APOBEC mRNA, using univariate and multivariate linear regression (table 1). There was a statistically significant inverse correlation between the level of hA3G and the viral set point (table 1), associating hA3G with the pathogenesis of HIV-1 infection. These results complement the recent work of Mallal et al. [36], who investigated the effect of hA3G- and hA3F-mediated HIV-1 sequence hypermutation on pretreatment viremia. Their study showed that patients with hypermutated sequences had significantly lower virus load than those with nonhypermutated sequences, even after adjustment for the CD4+ cell count and the human leukocyte antigen profile. In another study, Jin et al. [35] linked hA3G to the virus load in an examination of an abundance of hA3G mRNA in primary human cells isolated from patients infected with HIV-1. This study found that the levels of hA3G mRNA are higher in long-term nonprogressors (who have a low virus load) than in progressors (who have a high virus load). Our findings and the observations described above support in vitro studies that demonstrated that hA3G and hA3F can still mutate HIV-1 sequences, even if a functional Vif is present [37, 38]. These observations implicate hA3G as a clinically relevant host restriction factor that may play a role in determining the pathogenesis of HIV-1 infection.

The association between hA3G and the pathogenesis of HIV-1 infection might be due to the fact that hA3G restricts HIV-1 replication [17–19, 34, 39]. Therefore, it is possible that high levels of hA3G inhibit the replication rate of HIV-1, both before and after the onset of specific immune responses, thus contributing to a decrease in the viral set point. Furthermore, low replication rates in individuals with a high level of hA3G expression might limit viral diversity and, therefore, enhance the efficacy of adaptive immunity in clearing viruses, resulting in a low viral set point [40–42].

Apart from showing a difference in expression of hA3G and hA3F between the low and high viral set point groups, this study also revealed an increase in the levels of both hA3G and hA3F mRNA upon HIV-1 infection in each study group. The increase in expression of hA3G was significantly higher in the low viral set point group ($P = .041$, by the Mann–Whitney U test).

Viral regulation of hA3G expression has been reported previously by Komohara et al. [43], who recently showed that individuals who were hepatitis C virus (HCV) positive expressed more hA3G than those who were HCV negative. Another recently published report showed that the expression of hA3G was significantly increased in HIV-exposed seronegative individuals, compared with healthy control subjects [44]. It is not clear, however, whether the increase in hA3G in this study was brought about by HIV exposure or whether the individuals naturally expressed high levels of hA3G as a predetermined host factor. Our study demonstrates for the first time that the expression of both hA3G and hA3F increase upon HIV-1 infection, especially in individuals who develop a low viral set point. The increase in expression seems to be triggered by either infection with or exposure to HIV-1, but it is not proportional to the virus load, as evidenced by the larger increase in expression of hA3G and hA3F in the low viral set point group, compared with the high viral set point group. Interestingly, the level of interferon-α (IFN-α) has been shown to increase upon HIV-1 infection in some individuals [24, 45–47]. Because IFN-α elevates the levels of hA3G and hA3F expression [24, 48–50], increased levels of this cytokine may be responsible for the observed increase in expression of hA3G and hA3F.

In conclusion, this study supports the hypothesis that hA3G and hA3F may be important host factors in the pathogenesis of HIV-1 infection. We show that hA3G mRNA is significantly correlated with establishment of the viral set point and that there is an increase in expression of hA3G and hA3F after HIV-1 infection. The magnitude of increase for both hA3G and hA3F was significantly greater in the low viral set point group. The increase in expression of hA3G and hA3F after infection suggests that these 2 proteins might be part of an innate immune response against HIV-1 infection. Further studies are needed to elucidate how APOBEC proteins are regulated and to shed light on how APOBEC might be incorporated in novel prophylactic and therapeutic interventions.
Acknowledgment

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