Anti-APOBEC3G Activity of HIV-1 Vif Protein Is Attenuated in Elite Controllers

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ABSTRACT

HIV-1-infected individuals who control viremia to below the limit of detection without antiviral therapy have been termed elite controllers (EC). Functional attenuation of some HIV-1 proteins has been reported in EC. The HIV-1 accessory protein Vif (virus infection factor) enhances viral infectivity through anti-retroviral factor apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (APOBEC3G) degradation; however, little is known regarding Vif function in EC. Here, the anti-APOBEC3G activities of clonal, plasma HIV RNA-derived Vif sequences from 46 EC, 46 noncontrollers (NC), and 44 individuals with acute infection (AI) were compared. Vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped viruses were generated by cotransfecting 293T cells with expression plasmids encoding patient-derived Vif, human APOBEC3G, VSV-G, and a vifenv-deficient luciferase-reporter HIV-1 proviral DNA clone. Viral stocks were used to infect 293T cells, and Vif anti-APOBEC3G activity was quantified in terms of luciferase signal. On average, the anti-APOBEC3G activities of EC-derived Vif sequences (median log10 relative light units [RLU], 4.54 [IQR, 4.30 to 4.66]) were significantly lower than those of sequences derived from NC (4.75 [4.60 to 4.92], P < 0.0001) and AI (4.74 [4.62 to 4.94], P < 0.0001). Reduced Vif activities were not associated with particular HLA class I alleles expressed by the host. Vif functional motifs were highly conserved in all patient groups. No single viral polymorphism could explain the reduced anti-APOBEC3G activity of EC-derived Vif, suggesting that various combinations of minor polymorphisms may underlie these effects. These results further support the idea of relative attenuation of viral protein function in EC-derived HIV sequences.

IMPORTANCE

HIV-1 elite controllers (EC) are rare individuals who are able to control plasma viremia to undetectable levels without antiretroviral therapy. Understanding the pathogenesis and mechanisms underpinning this rare phenotype may provide important insights for HIV vaccine design. The EC phenotype is associated with beneficial host immunogenetic factors (such as HLA-B*57) as well as with functions of attenuated viral proteins (e.g., Gag, Pol, and Nef). In this study, we demonstrated that HIV-1 Vif sequences isolated from EC display relative impairments in their ability to counteract the APOBEC3G host restriction factor compared to Vif sequences from normal progressors and acutely infected individuals. This result extends the growing body of evidence demonstrating attenuated HIV-1 protein function in EC and, in particular, supports the idea of the relevance of viral factors in contributing to this rare HIV-1 phenotype.
efficiency of EC-derived env sequences (18), reduced protein functions of nef genes (19), and reduced replication capacity of recombinant virus expressing EC-derived gag and pol sequences (20, 21) have been reported in EC, including at the earliest stages of infection (22). Together, these data support virologic factors as additional determinants of elite control and suggest a potentially important role of genotypic and/or functional characteristics of the transmitted virus in the infection course. However, the contribution of genetic and/or functional properties of other HIV accessory proteins to the controller phenotype remains unknown.

Vif (virion infectivity factor) is an accessory protein that is essential for HIV-1 infectivity in primary CD4+ T lymphocytes (23). This viral protein mediates the degradation of the endogenous antiretroviral factor apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (APOBEC3G) in virus-producing cells (24–27). APOBEC3G belongs to the APOBEC family of proteins possessing cytidine deaminase activity (28). In the absence of Vif, APOBEC3G induces a high rate of C-to-U lesions in the first minus strand of cDNA during the process of reverse transcription. This leads to G-to-A hypermutation in the plus-strand DNA, resulting in a potent restriction of viral infectivity (29, 30). Vif inhibits the lethal incorporation of APOBEC3G into virions by targeting it for ubiquitin-mediated degradation in virus-producing cells, via a mechanism involving the assembly of the Cullin5-ElonginB-ElonginC E3 ubiquitin ligase complex (31, 32). Though some studies have reported the presence of mutated or defective vif sequences in LTNPs (33–36), the relationship between Vif genotypic/phenotypic variation and HIV disease progression remains incompletely characterized.

In the present study, the anti-APOBEC3G activity of Vif proteins derived from HIV-1-infected elite controllers was compared to the anti-APOBEC3G activity of those from noncontrollers (NC) and from individuals with acute infection (AI). We observed significant attenuation of anti-APOBEC3G activity of Vif proteins derived from EC that did not appear to be attributable to a common single viral genetic defect in these patients.

MATERIALS AND METHODS

Study subjects and plasma collection. The EC, AI, and NC cohorts have been described in detail elsewhere (10, 20, 37, 38). Briefly, EC were defined as having plasma HIV-1 RNA levels of <50 copies/ml in the absence of antiretroviral therapy at least 3 times over a 12-month period (episodes of plasma viremia of up to 1,000 copies/ml were permitted if they were not consecutive and if they represented the minority of all determinations). EC were recruited from outpatient clinics at local Boston hospitals and were referred from providers throughout the United States. Untreated NC, recruited from Boston hospitals, were defined as having plasma HIV-1 RNA levels above 2,000 copies/ml. Untreated AI were enrolled through a private medical clinic (Jessen-Praxis) in Berlin, Germany, and through Massachusetts General Hospital, Boston, MA. AI was defined according to published criteria (Acute Infection, Early Disease Research Program [AIEDRP] sponsored by NIAID) (39, 40). The estimated date of infection of AI was calculated according to the AIEDRP criteria (41). A total of 47 untreated EC (all with <50 copies/ml plasma; median CD4 count, 850 [interquartile range [IQR], 603 to 1,057] cells/μl), 46 untreated NC (median plasma viral load, 4.89 [IQR, 4.12 to 5.22] log10 copies/ml; median CD4 count, 323 [IQR, 60 to 488] cells/μl), and 44 AI (median plasma viral load, 5.87 [IQR, 5.30 to 6.51] log10 copies/ml; median CD4 count, 453 [IQR, 364 to 650] cells/μl) were included in the analysis. A single representative clone per patient, exhibiting an amino acid sequence identical to that of the original bulk sequence, was selected for subsequent analysis. In cases where bulk sequences exhibited amino acid mixtures, either residue was permitted in the representative clone.

Virologic factors as determinants of elite controllers. The p24 antigen levels in viral supernatants were measured by an HIV-1 antigen capture assay (Focus Diagnostics, Inc., Santa Clara, CA). HIV RNA was eluted in 80 μl of DNase- and RNase-free water and stored at −80°C until use.

Viral RNA isolation. For EC, a mean of 19.7 (range, 4.5 to 35.0) ml plasma was centrifuged for 10 min at 1,500 rpm to remove cell debris. Viral RNA was then concentrated by ultracentrifugation at a relative centrifugal force of 124,000 for 2 h using a SW32 Ti rotor (Beckman Coulter, Fullerton, CA). The supernatant was removed, and HIV RNA was extracted from the resulting pellet using a Qiagen viral RNA minikit (Qiagen Inc., Valencia, CA). For NC and AI, viral RNA was isolated from 0.5 ml of plasma. Viral RNA was eluted in 80 μl of DNase- and RNase-free water and stored at −80°C.

PCR amplification. HIV-1 Vif was amplified from extracted plasma HIV RNA by nested reverse transcription-PCR (RT-PCR) using first-round primers spanning the 3’ half of HIV-1 genome (forward, GCATT CCGTCAATTCCCAAAAG [HXB2 nucleotides 4648 to 4669]; reverse, GCACCTCAAGGCACGTTTATTGAGCGC [HXB2 nucleotides 9629 to 9604]), followed by second-round primers incorporating restriction sites for cloning (forward, GGCTTACATTGAAAACAGATGGCAGGTG [HXB2 nucleotides 5033 to 5061] [the KpnI restriction site is underlined]; reverse, CTAGTGCCATTCATGTTAGTGCCTCC [HXB2 nucleotides 5619 to 5593] [the MscI restriction site is underlined]). For all of the ECs and 19 of the NCs, first-round amplicons had previously been generated as part of a published study (38). A SuperScript III one-step RT-PCR system with Platinum Taq High Fidelity (catalog no. 12574-030; Invitrogen) and TaKaRa EX Tag DNA polymerase, Hot Start version (catalog no. RR006; TaKaRa Bio Inc., Shiga, Japan), were used to generate first-round and second-round amplicons, respectively. Amplification of second-round PCR products was confirmed by agarose gel electrophoresis.

Proviral DNA constructs, plasmids, and vectors. HIV-1 proviral construct pNL4-3, Vif-deficient HIV-1 proviral indicator construct pNL-Luc-vif(-)−/− (vif−/−), vesicular stomatitis virus glycoprotein (VSV-G) expression vector pHIT/G, and hemagglutinin (HA)-tagged human APOBEC3G expression plasmid pCA-hA3G-HA were described elsewhere (42–45). The GenBank accession number for the human APOBEC3G sequence used for constructs pCA-hA3G-HA is NM_021822.

Vif sequence analysis and cloning. Second-round vif amplicons were (directly) bulk sequenced using a 3130xl genetic analyzer (Applied Biosystems). Viral nucleotide sequences were edited using Sequencer 4.8 (Gene Codes Corporation). Sequences were aligned using ClustalW, and a phylogenetic tree was constructed using the DNA maximum likelihood program (DNAMl) and vif nucleotide sequences, implemented in BioEdit 7.0.9.0 (Ibis Biosciences). HIV-1 subtypes were determined using the REGA HIV subtyping tool (http://hivdb.stanford.edu/).

In addition, second-round vif amplicons were digested with KpnI and MscI and cloned into pCAGGS-FLAG-RRE (a FLAG-tagged Vif expression plasmid containing a Rev-responsive element), as previously described (46). A single representative clone per patient, exhibiting an amino acid sequence identical to that of the original bulk sequence, was selected for subsequent analysis. In cases where bulk sequences exhibited amino acid mixtures, either residue was permitted in the representative clone.

Viron production, APOBEC3G degradation, and viral infectivity assay. The viral infectivity assay for comparing the levels of anti-APOBEC3G activity of Vif has been previously described (46). Briefly, to prepare VSV-G–pseudotyped HIV-1 luciferase reporter viruses, 1.75 × 105 293T cells were cotransfected with 12.5 ng of pCA-hA3G-HA, 50 ng of VSV-G expression plasmid pHIT/G, 4 ng of the patient-derived Vif expression plasmid, and 0.43 μg of an empty vector (to adjust the total DNA amount to reach 1 μg) together with 500 ng of pNL-Luc-vif(-)/−/− using FuGENE 6. The amount of APOBEC3G expression plasmid was optimized to physiologically relevant levels (46). After medium exchange and DNase treatment, pseudoviruses were harvested 48 h following transfection. The p24 antigen levels in viral supernatants were measured by an HIV-1 p24-antigen capture enzyme-linked immunosorbent assay.
(ZeptoMetrix Corporation). To determine the viral infectivity, $3.5 \times 10^4$ 293T cells were incubated with supernatants containing 1 ng p24. After 48 h, luciferase activity was determined by the use of a ONE-Glo luciferase assay system (Promega) using a GloMax 96 Microplate Luminometer (Promega).

**Levels of APOBEC3G degradation by different Vif isolates.** To determine the APOBEC3G degradation efficiency of different Vif proteins, the levels of intracellular and virion-associated APOBEC3G were evaluated using representative Vif clones from 5 EC and 5 NC. A total of $7 \times 10^5$ 293T cells were cotransfected with 50 ng of pCA-hA3G-HA, 200 ng of VSV-G expression plasmid pHIT/G, 16 ng of the patient-derived Vif expression plasmid, and 1.73 μg of an empty vector (to adjust the total DNA amount to reach 4 μg) together with 2 μg of pNL-Luc-vif(-)env(-) using FuGENE 6. Forty-eight hours posttransfection, pseudotyped virus was harvested and cells were lysed in 400 μl of radioimmunoprecipitation assay (RIPA) buffer with protease inhibitor (Complete; Roche). Western blot analysis was performed using the cell lysate and purified virions. Monoclonal antibodies specific for HA (Cell Signaling Technology, Inc.), p24 (Abcam), and β-actin (Sigma-Aldrich) were used. Western blot band intensity was quantified with ImageJ 1.48 (National Institutes of Health).

**Vif protein expression analysis.** To confirm Vif protein expression, 293T cells were cotransfected with 200 ng of RRE-carrying FLAG-tagged patient-derived Vif expression plasmid, 200 ng of Rev expression plasmid (pCA-Rev [47]), and empty vector to reach a total of 1 μg of DNA, using the FuGENE 6 transfection reagent (Roche Applied Science). Western blot analysis was performed using extracts from the 293T cells. Antibodies specific for FLAG and β-actin were used. Note that a larger amount (200 ng) of Vif expression plasmid was used to confirm the expression of Vif proteins than was used in the viral infectivity assay (4 ng).

**Sequence analysis and statistics.** Statistical analysis of continuous variables was performed using the Mann-Whitney U test in GraphPad Prism 6 (GraphPad Software, Inc.). Correlations were evaluated using Spearman’s rank coefficients in GraphPad Prism 6 (GraphPad Software, Inc.). Comparisons of categorical values were performed using Fisher’s exact test computed in JMP Pro 9 (SAS Institute). A q value approach was employed to correct for multiple comparisons (48). In our analyses of viral genetic correlates of EC, P values of <0.05, corresponding to q values of <0.4 (indicating a 40% false-discovery rate), were considered statistically significant.

**RESULTS**

**Phylogenetic analysis of HIV-1 vif from patients with different disease statuses.** Vif genes from 137 HIV-positive individuals with different disease statuses (47 EC, 46 NC, and 44 AI) were successfully amplified and sequenced. With the exception of a single EC infected with HIV-1 subtype A1 (subsequently excluded from analysis), all harbored HIV-1 subtype B. Phylogenetic analysis revealed no gross clustering of viral sequences from the different patient groups (EC, NC, and AI), indicating that the extreme viremia control exhibited by the EC patients is not explained simply by recent shared ancestry (Fig. 1). No contamination was suspected.

**The anti-APOBEC3G activity of Vif proteins derived from EC was attenuated compared to that of Vif proteins from NC or AI.** Patient-derived Vif genes were cloned into FLAG-tagged mammalian expression plasmids and resequenced. For each patient, a single clone whose amino acid sequence was identical to the original bulk plasma HIV RNA sequence was selected for study. Verification of Vif protein expression in the plasmid-transfected cells was performed on a randomly selected panel of 11 EC and 10 NC vif genes by immunoblotting using anti-FLAG antibodies. These experiments revealed no substantial differences in Vif protein expression levels between EC-derived Vif and NC-derived Vif (Fig. 2). To assess the anti-APOBEC3G activity of patient-derived Vif proteins, VSV-pseudotyped virions harboring patient-derived Vif protein, human APOBEC3G protein, and a luciferase-reporter vif-env-defective HIV-1 NL4-3 DNA genome were used to infect 293T cells. Vif anti-APOBEC3G activity was measured in terms of

![FIG 1 Phylogenetic tree of patient-derived vif nucleotide sequences. Maximum likelihood phylogenetic trees were computed using bulk plasma HIV RNA-derived vif nucleotide sequences. Green, red, and blue lines indicate AI, NC, and EC-derived sequences, respectively. All but one patient-derived vif were subtype B; the single subtype A sequence was excluded from all further analyses. No gross clustering was observed by patient phenotype (EC, NC, or AI).](https://jvi.asm.org/content/89/9/4994/F1)

![sequence alignment](https://jvi.asm.org/content/89/9/4994/F1)
luciferase activity (represented as relative light units [RLU]) 48 h later. Overall, we observed a relatively broad range of Vif anti-APOBEC3G activities across all patient groups studied (Fig. 3). Nevertheless, on average, Vif proteins derived from EC displayed significantly reduced activities (median log_{10} RLU, 4.54 [IQR, 4.30 to 4.66]) compared to those from NC (4.75 [4.60 to 4.92], P < 0.0001) and those from AI (4.74 [4.62 to 4.94], P < 0.0001). In contrast, no significant difference between the activities of AI- and NC-derived Vif sequences was observed.

**Vif-mediated infectivity correlates inversely with cell-associated APOBEC3G levels.** To examine whether differences in Vif-mediated infectivity were associated with levels of viral particle-associated or intracellular APOBEC3G, we performed Western blot analysis using lysates of purified virions and of producer cells expressing Vif proteins from 5 representative EC and 5 representative NC. When Vif proteins were coexpressed, APOBEC3G could not be detected in viral particles (data not shown). However, analysis of APOBEC3G levels in virion-producer cells revealed lower APOBEC3G levels in cells expressing NC-derived Vif than in those expressing EC-derived Vif (Fig. 4A), consistent with reduced Vif function in the latter patient group. Importantly, Vif-mediated infectivity was significantly inversely associated with cell-associated APOBEC3G levels (normalized by Pr55gag) (Spearman’s r = −0.68; P = 0.035) (Fig. 4B). Together, these data indicate that EC-derived Vif isolates have a decreased ability to promote intracellular proteasomal degradation of APOBEC3G proteins.

**Vif functional motifs were conserved in EC.** We next examined known Vif functional motifs for evidence of mutations that could explain the relative attenuation of EC-derived Vif proteins. These included the SLQYLA motif (located between codon 144 and codon 148), critical for Elongin C binding (49, 50), the HCCH motif (comprised of residues 108H, 114C, 133C, and 139H), the TQX5ADX2I motif (located at residues 96 to 107), critical for interaction with Cullin5 (51–54), the C-terminal PPLP motif (located between codon 161 and codon 164), required for multimerization of Vif (55–57), and the tryptophan (W) residues at positions 11, 21, 38, 79, and 89, essential for the selective suppression of APOBEC3G and APOBEC3F (58). However, with the exception of three NC who harbored polymorphisms in the SLQYLA motif, all other motifs were 100% conserved in all patients (Fig. 5). Therefore, defects in these functional motifs are unlikely to explain the elite control.

**No common Vif amino acid mutations explain elite control.** We next investigated the potential existence of common Vif mutations that account for elite control by comparing the frequencies of all amino acids observed in Vif sequences from EC to the frequencies of those observed in NC and AI (as a single combined group), using Fisher’s exact test. At a P value of <0.05 (corresponding to q < 0.4), differential amino acid frequencies of the groups were observed at 4 sites (codons 30, 39, 47, and 159; total, 6 polymorphisms) (Table 1). However, at all of these sites, the consensus HIV subtype B amino acid (defined at [http://www.hiv.lanl.gov/content/sequence/NewAlign/align.html](http://www.hiv.lanl.gov/content/sequence/NewAlign/align.html)) was observed at a higher frequency in EC than in non-EC. The high prevalence of consensus amino acids at these positions in EC-derived Vif sequences indicates that their lower function (relative to NC and AI) cannot be explained by a single common substitution.

**Protective HLA alleles were not associated with anti-APOBEC3G activity of Vif protein in EC.** Viremia control is strongly associated with expression of particular HLA class I alleles (B*27, B*51, B*57, etc.) (2, 6, 8, 9). As such, we examined associations between HLA alleles expressed in a minimum of 5 EC or NC patients and the anti-APOBEC3G activity of their Vif proteins, using the Mann-Whitney U test (Fig. 6). AI patients were not examined, as sufficient within-host evolution might not have oc-
curred at this early stage for host HLA effects to be detected. No significant differences in Vif function were observed between EC or NC patients harboring protective HLA alleles (B*27, B*51, B*57) and those lacking these alleles. Of note, higher Vif function was associated with HLA-A*03 in EC ($p = 0.007, q = 0.30$). Together, these results suggest that immune pressures mediated by canonical “protective” HLA class I alleles are unlikely to be playing a major role in the reduced Vif activity in EC, though the modest statistical power of our data set must be noted.

**DISCUSSION**

In this study, we found that the average anti-APOBEC3G activity of Vif proteins derived from elite controllers was significantly lower than that of Vif proteins derived from noncontrollers and individuals with acute HIV infection, despite comparable levels of Vif protein expression in the controller and noncontroller patient groups. However, this functional reduction was unlikely to be explained by a common single Vif mutation among EC and did not correlate with the HLA class I alleles expressed by the host.

Previous studies evaluating EC-derived viruses demonstrated that the *in vitro* replication capacity associated with Gag (20) or Pol (21), the entry efficiency of Env (18), and the function of Nef from EC (19) were attenuated compared to those from NC. As within-host HIV genetic diversity (and *in vitro* replicative fitness) tends to increase during the natural course of HIV-1 infection (59, 60), at a rate that is dependent on within-host viral replication, it is possible that the higher level of intrahost viral evolution in NC (who have high viral loads) than in EC (who have undetectable viral loads) could serve as a confounder. Specifically, if transmitted viruses are generally less fit than those isolated later in infection (61), we reasoned that it was necessary to also compare the function of EC-derived viral sequences to the function of those derived from acutely infected individuals. If they were comparable, this would suggest that the lower function of EC-derived sequences was likely due to limited within-host evolution over the disease course. But if the function of EC-derived *vif* sequences was lower than that of AI-derived *vif* sequences, then this would be compatible with acquisition of unusually attenuated viral strains and/or with selection of unusual function-reducing mutations over the disease course. It is for this reason that our study included AI as an additional comparison group.

Importantly, we demonstrated that the anti-APOBEC3G activities of Vif proteins in EC were significantly lower than those of Vif proteins in noncontrollers, regardless of whether the latter patients were assessed in acute infection or chronic infection. This indicates that reduced Vif function in EC does not simply reflect reduced intrahost viral evolution in these patients. Though the cross-sectional nature of our study does not allow us to discern whether this attenuated Vif function is a cause or a consequence of viremia control and the clinical implications remain to be determined, our results nevertheless suggest that transmitted/founder viruses acquired by EC are already unusually attenuated compared to those acquired by individuals who subsequently progress clinically and/or that Vif function in EC is further attenuated over the infection course as a result of adaptation to host-driven pressures. It is also important that, while Vif function in EC was on average significantly lower than that in noncontrollers, there was substantial functional overlap between the two groups. In a previous study using the same assay (46), a 0.5 log10 RLU decrease in anti-APOBEC3G activity resulted in a significant increase in G-to-A hypermutation frequency, suggesting that an *in vitro* functional reduction of this magnitude could be biologically relevant. Eleven (24%) of our 46 EC-derived *vif* isolates exhibited anti-AP
OBEC3G activities that were at least $-0.5 \log_{10}$ RLU lower than the median anti-APOBEC3G activity of NC, indicating that the in vitro reductions observed in this study were of a magnitude that could be biologically relevant. In order to investigate the mechanisms by which Vif function is attenuated in EC, Vif amino acid sequences were analyzed. However, no major mutations were observed within known functional motifs, and no common single polymorphisms were observed that could explain EC status. We speculate that the relative Vif defects observed in EC may attributable to combinations of minor polymorphisms, though further investigations using higher-powered data sets will be necessary to confirm this.

Among EC, certain HLA class I alleles are overrepresented, in particular, HLA-B*57, B*27, and B*51, suggesting that HIV-1-specific cytotoxic T-lymphocyte (CTL) responses restricted by these alleles are crucial for viremia control (9, 10, 12). CTL escape mutations restricted by these protective HLA alleles can also reduce viral replication capacity (20, 62–68). Accordingly, the in vitro replication capacities of recombinant viruses expressing Gag and Pol, as well as the protein function of Nef sequences, have been reported to be attenuated in EC, especially in HLA-B57-positive (HLA-B57/H11001) individuals (19–21). In contrast, in the present study, no association was found between attenuated Vif function and HLA class I alleles, including HLA-B*57, though the relatively modest statistical power of the present data set is noted. As reported previously, the magnitude and breadth of the HIV-specific CTL responses targeting accessory/regulatory proteins (Tat, Rev, Vpr, Vif, and Vpu) were lower than those of the HIV-

![FIG 5](vif_frequency.png)

**FIG 5** Vif amino acid frequencies in EC and non-EC. The top row of capital letters represents the HIV-1 Vif subtype B consensus (Cons B) amino acid sequence. For all codons that were less than 100% conserved in our data set, the frequencies of observed amino acids in EC and non-EC are shown below the consensus data. Known conserved Vif functional motifs are highlighted in gray. Codon positions 1 to 7 and 185 to 192 were the second PCR primer sites.

| TABLE 1 Vif polymorphisms associated with patient phenotype (EC versus non-EC)a |
|-----------------|-------|----------------|-----------------|-------|------------------------|-----------------|----------|
| Amino acid residue | No. (%) of EC with indicated polymorphism | No. (%) of EC without indicated polymorphism | No. (%) of non-EC with indicated polymorphism | No. (%) of non-EC without indicated polymorphism | P value | q value | Subtype B consensus |
| 030H | 1 (2) | 45 (98) | 20 (22) | 70 (75) | 0.0019 | 0.21 | Y |
| 030Y | 45 (98) | 1 (2) | 69 (74) | 21 (23) | 0.0010 | 0.21 | Y |
| 039F | 28 (61) | 18 (39) | 30 (32) | 60 (65) | 0.0032 | 0.24 | F |
| 047T | 40 (87) | 6 (13) | 54 (58) | 36 (39) | 0.0015 | 0.21 | T |
| 159I | 32 (70) | 14 (30) | 29 (31) | 61 (66) | <0.0001 | 0.0045 | I |
| 159R | 4 (9) | 42 (91) | 29 (31) | 61 (66) | 0.0027 | 0.24 | I |

a All polymorphisms, regardless of their observed frequency in our data set, were analyzed. Polymorphisms with a P value of <0.05 and q value of <0.4 are reported. EC, elite controller.
FIG 6 Association between HLA class I allele expression and Vif activity. Box and whisker plots depict the median (vertical line), interquartile range (box), and range (whiskers) of log_{10} RLU (Vif activity), stratified by the HLA class I and whisker plots depict the median (vertical line), interquartile range (box), and range (whiskers) of log_{10} RLU (Vif activity), stratified by the HLA class I allele and range (whiskers) of log_{10} RLU (Vif activity), stratified by the HLA class I allele observed in a minimum of 5 individuals. The dotted vertical line indicates the median Vif activity for each patient group. The asterisk next to A*03 (EC) indicates that EC expressing A*03 exhibited significantly higher Vif activities than those not expressing A*03 (Mann-Whitney U-test; \( P = 0.007, q = 0.30 \)). All other comparisons were non-statistically significant at a \( P \) of <0.05 and a \( q \) of <0.4.

specific CTL responses targeting Gag, Pol, and Nef in EC, viremic controllers, and chronic progressors (10, 11). Therefore, the discordance with the findings in the previous studies on other HIV proteins may be explained by lower levels of CTL responses to the Vif protein.

The present study featured several limitations. First, as a marker for anti-APOBEC3G activity of Vif in vitro, we analyzed Vif-mediated infectivity that is mediated by multiple mechanisms, including promotion of the degradation of APOBEC3G (24–26, 31, 32), prevention of encapsidation of APOBEC3G (69–71), and interaction with other APOBEC3G proteins (APOBEC3DE/3F/3H) (72–74). We failed to evaluate the levels of virion encapsidation of APOBEC3G, since encapsidated APOBEC3G was not detected in the presence of Vif protein under our physiologically optimized in vitro conditions. At the least, we found that Vif-induced intracellular degradation of APOBEC3G could be one of the possible mechanisms of attenuated Vif function in EC. Although we did not evaluate Vif’s interactions with other APOBEC3G proteins, the intrinsic potencies of other APOBEC3 proteins (such as APOBEC3DE/3F/3H) appear to be lower than those of APOBEC3G (46, 72–75). Moreover, we did not observe interpatient differences in Vif-mediated infectivity in control experiments where the APOBEC3G expression plasmid was not delivered into 293 T cells (data not shown). As such, our method properly evaluates Vif’s in vitro activity against its principal target, APOBEC3G.

Second, differences in sampling dates could confound Vif functional assessments in two ways: via intrapatient viral evolution over the infection course and/or via interpatient viral evolution over the epidemic’s course. The AI patient group was included in the study to indirectly address the first confounder (as intrapatient evolution is minimized in AI due to the limited elapsed time since infection). With respect to the latter confounder, we observed no correlation between calendar date of sampling and Vif-mediated infectivity in each patient group (Spearman’s \( r = -0.19 \) in EC, \( r = -0.09 \) in NC, and \( r = 0.06 \) in AI; all \( P > 0.05 \)). This suggests that our results are not substantially confounded by the population-level evolution of HIV-1 over the epidemic’s course, though further study is warranted to further investigate the evolution of Vif sequence and function at a population level. Third, only a single Vif clone from each subject was studied (though we took care to select a clone identical to the bulk plasma viral sequence in each case). Given the large genetic diversity in NC, a potential bias could not be ruled out, especially in cases where the bulk sequence featured amino acid mixtures (only one of which was represented in the selected clone). To further address this, we repeated the Vif functional assessments using a randomly selected clone, which yielded results consistent with the primary analysis (data not shown). Therefore, we feel that our observed results are unlikely due to selection biases associated with selection of a single clone per patient.

Fourth, our use of an in vitro assay to evaluate the anti-APOBEC3G function of patient-derived HIV Vif proteins does not allow us to investigate host-related factors such as APOBEC3G polymorphisms and cellular expression levels. Unfortunately, assessment of APOBEC3G levels or polymorphisms was not possible, as peripheral blood mononuclear cells (PBMCs) were not available for the studied patients. A previous report indicated that in vivo APOBEC3G mRNA levels correlated inversely with the level of viremia (76), although the data from another report are conflicting (77). Likewise, attempts to identify SNPs within APOBEC3G associated with HIV disease progression have not yielded any significant hits (78). As such, the potential impact of host factors should be addressed in future studies as well.

In conclusion, we observed that the average anti-APOBEC3G activity of Vif derived from elite controllers was significantly reduced compared to the activity of those derived from noncontrollers, regardless of the infection stage. This reduced activity was unlikely to be explained by common Vif mutations in EC; as such, further study will be required to fully elucidate the genotypic mechanisms underlying the attenuated Vif function in EC. Nevertheless, these results extend the growing body of evidence supporting the idea of relative attenuation of viral protein function in EC-derived HIV sequences.

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