JOURNAL OF VIROLOGY, Feb. 2006, p. 2069–2072 Vol. 80, No. 4
0022-538X/06/$08.00+0 doi:10.1128/JVI.80.4.2069–2072.2006

APOBEC3F and APOBEC3G mRNA Levels Do Not Correlate with Human Immunodeficiency Virus Type 1 Plasma Viremia or CD4$^+$ T-Cell Count

Soo-Jin Cho,[1] Henning Drechsler,[2] Ryan C. Burke,[2] Max Q. Arens,[3] William Powderly,[2] and Nicholas O. Davidson[1]*

Divisions of Gastroenterology[1] and Infectious Diseases,[2] Department of Medicine, and Division of Laboratory Medicine, Department of Pediatrics,[3] Washington University School of Medicine, St. Louis, Missouri 63110

Received 27 September 2005/Accepted 17 November 2005

APOBEC3F and APOBEC3G (hA3F and hA3G) are part of an innate mechanism of antiretroviral defense. The human immunodeficiency virus type 1 (HIV-1) accessory protein Vif targets both proteins for proteasomal degradation. Using mRNA from peripheral blood mononuclear cells of 92 HIV-infected subjects not taking antiretroviral therapy and 19 HIV-uninfected controls, we found that hA3F ($P < 0.001$) and hA3G ($P = 0.016$) mRNA levels were lower in HIV-infected subjects and were positively correlated with one another ($P = 0.003$). However, we found no correlation in the abundance of either hA3F or hA3G mRNA with either viral load or CD4 counts in HIV-infected subjects.

APOBEC3F (hA3F) and APOBEC3G (hA3G) are members of a family of related cytidine deaminases shown to have antiretroviral activity in vitro (1, 8, 9, 11, 14, 23, 29). In the absence of the human immunodeficiency virus type 1 (HIV-1) accessory protein Vif, hA3F and hA3G are incorporated into virions and induce G-to-A hypermutations in the viral genome (1, 8, 12, 14, 27, 29, 30). Vif counteracts hA3F and hA3G by preventing their encapsidation within virions and by inducing their proteasomal degradation (4, 11, 13, 16, 17, 24, 25, 28). However, higher levels of hA3G expression can overcome the antihost effects of Vif (17), suggesting that regulation of hA3G expression may represent a novel target for antiretroviral therapy and modulation of the progression of HIV-1 infection.

The determinants of individual HIV-1 disease progression are incompletely understood (reviewed in reference 2; see also references 18 to 20). Plasma HIV-1 viral load at steady state is highly variable among infected individuals, with RNA levels ranging from 10^3 to 10^6 copies/ml. We examined the hypothesis that individual variations in mRNA expression of hA3F and/or hA3G might account for the differences in viral load, and hence disease progression, in HIV-1-infected individuals. Given that both proteins have been shown in vitro to be active against HIV infection, we also hypothesized that hA3F and hA3G may have a compound effect in anti-HIV defense.

This study was approved by the Institutional Review Board of the Washington University School of Medicine. All subjects provided written informed consent. Plasma HIV viremia (viral load) was quantified (Roche Amplicor 2.0) in the Retrovirus Laboratory in the Department of Pediatrics, Washington University School of Medicine. CD4 counts were determined in the Immunology Laboratory of Barnes-Jewish Hospital (St. Louis, MO). HIV-infected subjects (seropositivity range, 2 months to 17 years; mean, 4.8 years) were not taking antiretroviral therapy for at least 3 months and had CD4 cell counts of >200/μl (http://gastro.wustl.edu/faculty/davidson.html; at this website, see the link to Cho et al., J. Virol. supplementary material Table 1). Peripheral blood mononuclear cells (PBMCs) were isolated and cryopreserved in RNAlater (Ambion). RNA was isolated from 2×10^6 to 5×10^6 cells and DNase treated (DNAfree kit; Ambion). cDNA products were synthesized with random hexamers and Superscript II RNA polymerase (Invitrogen). hA3F (NM_145298) and hA3G (NM_021822) mRNA expression levels were quantified using SYBR Green (ABI) chemistry and target validated primers. Primers for hA3F were 5′-TGGAAGTTGTAAAGCACCACTCA-3′ (forward; nucleotides 665 to 687) and 5′-AGCACCTTTCTGCATGACAATG-3′ (reverse; nucleotides 760 to 739). The primers for hA3G were 5′-GGCTCCACATAAACACGGTTTC-3′ (forward; nucleotides 735 to 756) and 5′-AAGGGAATCACGTCCAGGAA-3′ (reverse; nucleotides 803 to 784). Human β-actin (NM_001101) was used as a normalizing control with the following primers: 5′-CTGGCACCCAGCACAATG-3′ (forward; nucleotides 958 to 975) and 5′-GCCGATCCACACGGAGTACT-3′ (reverse; nucleotides 1026 to 1007). Each 25-μl reaction mixture contained 12.5 μl 2× SYBR Green PCR mix (ABI), 0.25 μl of each 10 μM primer, and 1 μl of 1:4-diluted cDNA products. The reactions were run in an ABI 7000 with one cycle at 50°C

TABLE 1. hA3F and hA3G mRNA expression levels in HIV-infected and HIV-uninfected subjects analyzed by two-sample *t* test

mRNA	mRNA expression[a]		*t* statistic	*P* value
	HIV^{+b}	HIV^{-c}		
hA3F	4.67	5.13	−3.38	0.001
hA3G	6.13	6.45	−2.44	0.016

[a] All mRNA expression levels were measured as copies per 10,000 copies of β-actin mRNA and then log transformed (base e) for statistical analyses.
[b] Mean mRNA expression (log transformed); $n = 92$.
[c] Mean mRNA expression (log transformed); $n = 19$.

* Corresponding author. Mailing address: Washington University School of Medicine, Division of Gastroenterology, Box 8124, 660 S. Euclid Ave., St. Louis, MO 63110. Phone: (314) 362-2027. Fax: (314) 362-2033. E-mail: nod@wustl.edu.

TABLE 2. hA3F and hA3G mRNA expression levels by sex and race

mRNA	Subject group	mRNA expression analyzed by:					
		Sex (two-sample *t* test)			Race (one-way ANOVA)[c]		
		Mean (M/F)[a]	*t* statistic	*P* value	Mean (C/AA/O)[b]	*F* (ANOVA)	*P* value
hA3F	HIV^+	4.67/4.66	−0.10	0.92	4.58/4.68/4.93	0.47	0.63
	HIV^-	5.06/5.18	0.70	0.50	5.12/5.28/4.99	0.47	0.63
	All	4.74/4.74	−0.002	0.99	4.78/4.71/4.96	0.59	0.56
hA3G	HIV^+	6.17/6.10	−0.64	0.52	6.24/6.10/5.97	0.62	0.54
	HIV^-	6.44/6.45	0.05	0.97	6.47/6.34/6.45	0.15	0.87
	All	6.22/6.15	−0.66	0.51	6.32/6.11/6.26	2.03	0.14

[a] M, male, $n = 49$ (all); F, female, $n = 62$ (all).
[b] C, Caucasian, $n = 35$ (all); AA, African-American, $n = 71$ (all); O, other, $n = 5$ (all).
[c] ANOVA, analysis of variance.

(2 min) followed by 95°C (10 min) and then 40 cycles at 95°C (15 s) followed by 60°C (1 min). Absolute mRNA copy numbers were calculated by generating standard curves using serial dilutions of plasmids containing the desired gene (hA3F or hA3G) or a PCR product (actin). Each sample was run in triplicate. hA3F/hA3G mRNA expression levels were calculated as number of copies per 10,000 copies of β-actin.

Data from 92/100 consecutively enrolled HIV-infected (suppl. Table 1 at http://gastro.wustl.edu/faculty/davidson.html) and 19 HIV-uninfected subjects (suppl. Table 2 at http://gastro.wustl.edu/faculty/davidson.html) were analyzed. Eight HIV-infected subjects were excluded for not meeting inclusion criteria or missing samples. Mean (± standard deviation) hA3F expression levels (copies per 10,000 copies of β-actin mRNA) were 122 (±60) and 179 (±67) for HIV-infected and -uninfected subjects, respectively. Mean hA3G expression levels were 547 (±419) and 668 (±215) for HIV-infected and -uninfected subjects, respectively. hA3F/hA3G values and viral load were log transformed so that all parameters were normally distributed. Using two-sample *t* tests, both hA3F and hA3G values were significantly lower in HIV-infected compared to uninfected subjects ($P = 0.001$ and 0.016 for hA3F and hA3G, respectively) (Table 1). There were no significant differences in hA3F or hA3G expression between males and females or by race (Table 2). We found no correlation between hA3F or hA3G mRNA abundance and viral load (Fig. 1a and b), CD4 count

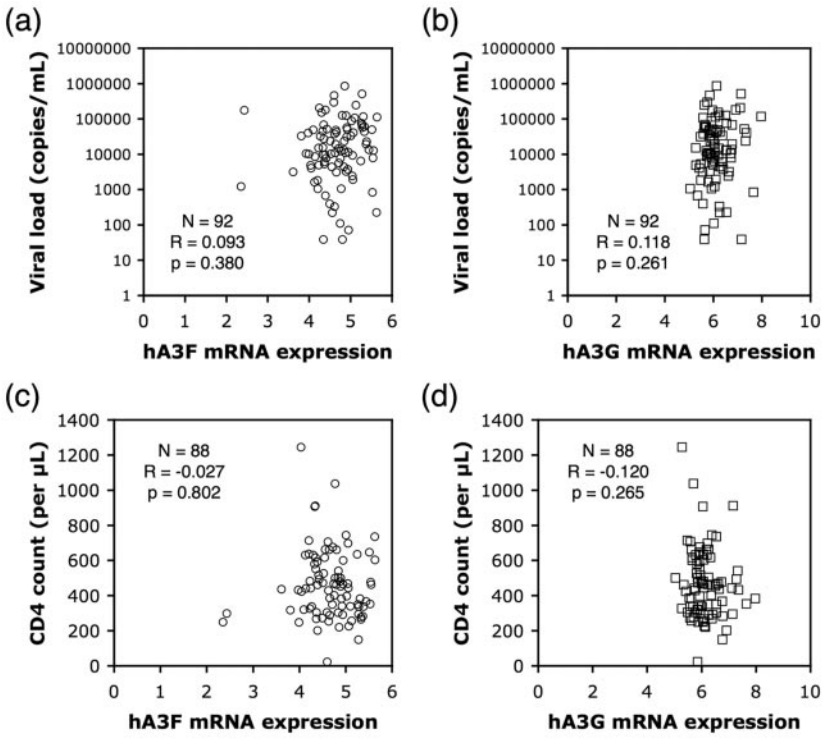

FIG. 1. hA3F and hA3G mRNA expression levels in PBMCs of HIV-infected subjects do not correlate with viral load or CD4 count. All mRNA expression levels were calculated as copies of hA3G/hA3G per 10,000 copies of β-actin and log transformed (base e) prior to analysis; log-transformed values are shown. Viral load was also log transformed (base e) prior to calculation of the correlation coefficient. N, number of subjects; R, Pearson's correlation coefficient; p, *P* value.

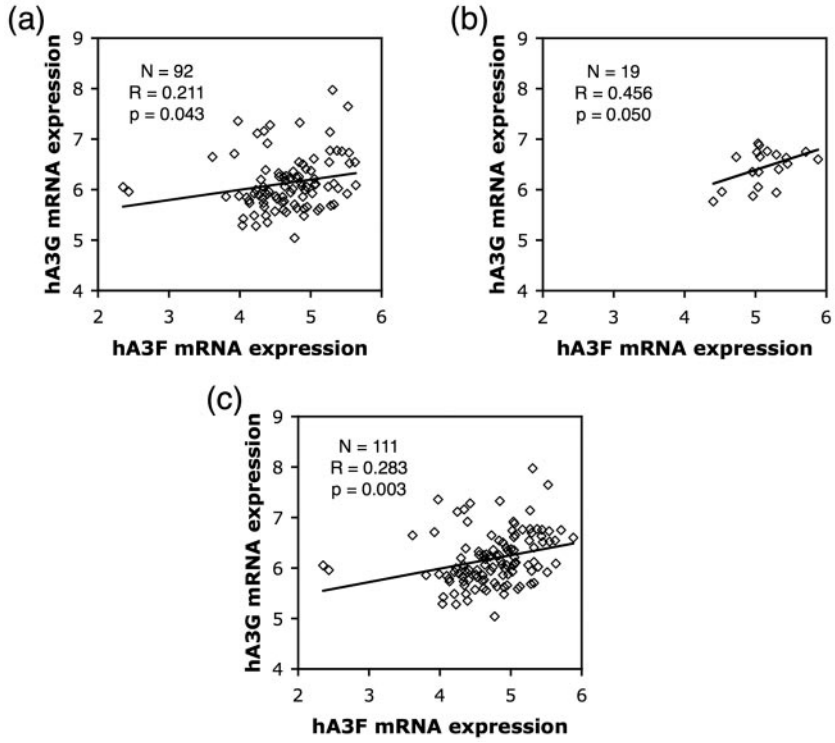

FIG. 2. hA3F and hA3G mRNA levels show a positive, linear correlation. (a) HIV^+ subjects only; (b) HIV^- subjects only; (c) all subjects. All mRNA expression levels were calculated as copies of hA3F/hA3G per 10,000 copies of β-actin and log transformed (base e) prior to analysis; log-transformed values are shown. R, Pearson's correlation coefficient; p, *P* value.

(Fig. 1c and d), CD4 percentage (data not shown), or age (data not shown). Furthermore, multiple regression analyses showed no significant combined effect of hA3F and hA3G on either viral load or CD4 count (data not shown). However, hA3F and hA3G mRNA abundances revealed a positive, linear relationship (Pearson's correlation coefficient of 0.283; $P = 0.003$) (Fig. 2), in agreement with previous studies in which hA3F and hA3G showed similar patterns of expression in tissues and were postulated to be coordinately regulated (1, 12, 26). It should be noted that our cross-sectional survey was not aimed at identifying long-term nonprogressors and, therefore, did not include sufficient numbers to allow valid statistical evaluations of this subgroup. All statistical analyses were repeated, and the results were validated with nonparametric tests using nontransformed values for hA3F/hA3G and viral load (suppl. Tables 3 and 4 at http://gastro.wustl.edu/faculty/davidson.html).

Jin et al. also analyzed the expression of hA3G in the PBMCs of 25 HIV-infected subjects and found that hA3G expression was higher in HIV-uninfected than in HIV-infected subjects, in agreement with our findings (10). However, in stark contrast to our study, these authors found statistically significant correlations between hA3G expression and viral load (inverse) as well as CD4 cell count (positive). A plausible explanation for this divergence may be that Jin et al. stimulated PBMCs with anti-CD3 and anti-CD28 antibodies prior to RNA extraction. A recent study by Chiu et al. found that in resting $CD4^+$ T cells, hA3G is enzymatically active and present in a low-molecular-mass complex which protects against HIV infection (3). In contrast, when $CD4^+$ T cells were costimulated with anti-CD3 and anti-CD28 antibodies, hA3G expression was induced within an inactive, high-molecular-mass hA3G complex, resulting in cells highly permissive for HIV infection. We propose that examining unstimulated PBMCs is more representative of the "physiologic" steady state of T cells in vivo, presumably reflecting the population actively resisting HIV infection and replication. Additionally, our subjects were specifically selected to include only those whose HIV status was at steady state in the absence of antiretroviral therapy.

In conclusion, our results do not predict an informative role for hA3F or hA3G in HIV-1 disease progression. Furthermore, the observed differences in hA3F and hA3G mRNA expression between HIV-infected and -uninfected individuals remain to be explained. hA3G has received considerable attention as an innate mechanism of cellular defense against retroviral infection and as a potential target for antiretroviral therapy. While it is clear that hA3G can counteract multiple retroviruses as well as retrotransposons in vitro (6, 7, 15, 22), its usefulness as a clinical marker of infection remains unclear. Further studies to elucidate the clinical utility of hA3G and hA3F may include the examination of $CD4^+$ T cells rather than PBMCs and follow a larger cohort over time to examine the predictive relationship on progression to AIDS. APOBEC3B (hA3B), another member of the APOBEC3 gene cluster on chromosome 22 (9), may also have anti-HIV effects (5, 21), and this target may be evaluated in future studies.

This work was supported by an American Heart Association Predoctoral Fellowship granted to S. Cho (no. 0415398Z), NIH grants HL-38180 and DK-56260 and DDRCC grant DK-52574 to N. O.

Davidson, and by the discretionary fund of the St. Louis AIDS Clinical Trials Unit ACTU and NIH grant AI-25903 at Washington University.

We also thank the staff of the St. Louis ACTU for their help in recruiting patients, Laura Blair for her dedicated technical assistance, and Diana Nurutdinova and Lisa Mahnke for their helpful discussions.

REFERENCES

1. **Bishop, K. N., R. K. Holmes, A. M. Sheehy, N. O. Davidson, S. J. Cho, and M. H. Malim.** 2004. Cytidine deamination of retroviral DNA by diverse APOBEC proteins. Curr. Biol. **14:**1392–1396.
2. **Bonhoeffer, S., G. A. Funk, H. F. Gunthard, M. Fischer, and V. Muller.** 2003. Glancing behind virus load variation in HIV-1 infection. Trends Microbiol. **11:**499–504.
3. **Chiu, Y. L., V. B. Soros, J. F. Kreisberg, K. Stopak, W. Yonemoto, and W. C. Greene.** 2005. Cellular APOBEC3G restricts HIV-1 infection in resting $CD4^+$ T cells. Nature **435:**108–114.
4. **Conticello, S. G., R. S. Harris, and M. S. Neuberger.** 2003. The Vif protein of HIV triggers degradation of the human antiretroviral DNA deaminase APOBEC3G. Curr. Biol. **13:**2009–2013.
5. **Doehle, B. P., A. Schafer, and B. R. Cullen.** 2005. Human APOBEC3B is a potent inhibitor of HIV-1 infectivity and is resistant to HIV-1 Vif. Virology **339:**281–288.
6. **Dutko, J. A., A. Schafer, A. E. Kenny, B. R. Cullen, and M. J. Curcio.** 2005. Inhibition of a yeast LTR retrotransposon by human APOBEC3 cytidine deaminases. Curr. Biol. **15:**661–666.
7. **Esnault, C., O. Heidmann, F. Delebecque, M. Dewannieux, D. Ribet, A. J. Hance, T. Heidmann, and O. Schwartz.** 2005. APOBEC3G cytidine deaminase inhibits retrotransposition of endogenous retroviruses. Nature **433:**430–433.
8. **Harris, R. S., and M. T. Liddament.** 2004. Retroviral restriction by APOBEC proteins. Nat. Rev. Immunol. **4:**868–877.
9. **Jarmuz, A., A. Chester, J. Bayliss, J. Gisbourne, I. Dunham, J. Scott, and N. Navaratnam.** 2002. An anthropoid-specific locus of orphan C to U RNA-editing enzymes on chromosome 22. Genomics **79:**285–296.
10. **Jin, X., A. Brooks, H. Chen, R. Bennett, R. Reichman, and H. Smith.** 2005. APOBEC3G/CEM15 (hA3G) mRNA levels associate inversely with human immunodeficiency virus viremia. J. Virol. **79:**11513–11516.
11. **Kao, S., M. A. Khan, E. Miyagi, R. Plishka, A. Buckler-White, and K. Strebel.** 2003. The human immunodeficiency virus type 1 Vif protein reduces intracellular expression and inhibits packaging of APOBEC3G (CEM15), a cellular inhibitor of virus infectivity. J. Virol. **77:**11398–11407.
12. **Liddament, M. T., W. L. Brown, A. J. Schumacher, and R. S. Harris.** 2004. APOBEC3F properties and hypermutation preferences indicate activity against HIV-1 in vivo. Curr. Biol. **14:**1385–1391.
13. **Liu, B., P. T. Sarkis, K. Luo, Y. Yu, and X. F. Yu.** 2005. Regulation of Apobec3F and human immunodeficiency virus type 1 Vif by Vif-Cul5-ElonB/C E3 ubiquitin ligase. J. Virol. **79:**9579–9587.
14. **Mangeat, B., P. Turelli, G. Caron, M. Friedli, L. Perrin, and D. Trono.** 2003. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature **424:**99–103.
15. **Mariani, R., D. Chen, B. Schrofelbauer, F. Navarro, R. Konig, B. Bollman, C. Munk, H. Nymark-McMahon, and N. R. Landau.** 2003. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell **114:**21–31.
16. **Marin, M., K. M. Rose, S. L. Kozak, and D. Kabat.** 2003. HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation. Nat. Med. **9:**1398–1403.
17. **Mehle, A., B. Strack, P. Ancuta, C. Zhang, M. McPike, and D. Gabuzda.** 2004. Vif overcomes the innate antiviral activity of APOBEC3G by promoting its degradation in the ubiquitin-proteasome pathway. J. Biol. Chem. **279:**7792–7798.
18. **Mellors, J. W., C. R. Rinaldo, Jr., P. Gupta, R. M. White, J. A. Todd, and L. A. Kingsley.** 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science **272:**1167–1170.
19. **O'Brien, T. R., P. S. Rosenberg, F. Yellin, and J. J. Goedert.** 1998. Longitudinal HIV-1 RNA levels in a cohort of homosexual men. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol. **18:**155–161.
20. **Raboud, J. M., J. S. Montaner, B. Conway, L. Haley, C. Sherlock, M. V. O'Shaughnessy, and M. T. Schechter.** 1996. Variation in plasma RNA levels, CD4 cell counts, and p24 antigen levels in clinically stable men with human immunodeficiency virus infection. J. Infect. Dis. **174:**191–194.
21. **Rose, K. M., M. Marin, S. L. Kozak, and D. Kabat.** 2005. Regulated production and anti-HIV type 1 activities of cytidine deaminases APOBEC3B, 3F, and 3G. AIDS Res. Hum. Retrovir. **21:**611–619.
22. **Schumacher, A. J., D. V. Nissley, and R. S. Harris.** 2005. APOBEC3G hypermutates genomic DNA and inhibits Ty1 retrotransposition in yeast. Proc. Natl. Acad. Sci. USA **102:**9854–9859.
23. **Sheehy, A. M., N. C. Gaddis, J. D. Choi, and M. H. Malim.** 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature **418:**646–650.
24. **Sheehy, A. M., N. C. Gaddis, and M. H. Malim.** 2003. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. Nat. Med. **9:**1404–1407.
25. **Stopak, K., C. de Noronha, W. Yonemoto, and W. C. Greene.** 2003. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol. Cell **12:**591–601.
26. **Wiegand, H. L., B. P. Doehle, H. P. Bogerd, and B. R. Cullen.** 2004. A second human antiretroviral factor, APOBEC3F, is suppressed by the HIV-1 and HIV-2 Vif proteins. EMBO J. **23:**2451–2458.
27. **Yu, Q., R. Konig, S. Pillai, K. Chiles, M. Kearney, S. Palmer, D. Richman, J. M. Coffin, and N. R. Landau.** 2004. Single-strand specificity of APOBEC3G accounts for minus-strand deamination of the HIV genome. Nat. Struct. Mol. Biol. **11:**435–442.
28. **Yu, X., Y. Yu, B. Liu, K. Luo, W. Kong, P. Mao, and X. F. Yu.** 2003. Induction of APOBEC3G ubiquitination and degradation by an HIV-1 Vif-Cul5-SCF complex. Science **302:**1056–1060.
29. **Zhang, H., B. Yang, R. J. Pomerantz, C. Zhang, S. C. Arunachalam, and L. Gao.** 2003. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. Nature **424:**94–98.
30. **Zheng, Y. H., D. Irwin, T. Kurosu, K. Tokunaga, T. Sata, and B. M. Peterlin.** 2004. Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication. J. Virol. **78:**6073–6076.