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

Soo-Jin Cho, Henning Drechsler, Ryan C. Burke, Max Q. Arens, William Powderly, and Nicholas O. Davidson

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

Received 27 September 2005/Accepted 17 November 2005

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, 9, 11, 14, 23, 29). 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 antiviral 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 10³ to 10⁶ copies/ml. We examined the hypothesis that individual variations in mRNA expression of hA3F and/or hA3G might account for 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). CD4 counts were lower in HIV-infected subjects and were positively correlated with one another (P < 0.001). 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.

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Mean mRNA expression (log transformed)</th>
<th>t statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hA3F</td>
<td>4.67 ± 0.23</td>
<td>-3.38</td>
<td>0.001</td>
</tr>
<tr>
<td>hA3G</td>
<td>6.13 ± 0.17</td>
<td>-2.44</td>
<td>0.016</td>
</tr>
</tbody>
</table>

* 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.

All mRNA expression levels were measured as copies per 10,000 copies of beta-actin mRNA and then log transformed (base e) for statistical analyses.

Mean mRNA expression (log transformed) = n = 92.

Mean mRNA expression (log transformed) = n = 19.
(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 /H9252-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 /H9252-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 mRNA. a) hA3F mRNA 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.](http://jvi.asm.org/)

![TABLE 2. hA3F and hA3G mRNA expression levels by sex and race](http://jvi.asm.org/)

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Subject group</th>
<th>mRNA expression analyzed by:</th>
<th>Sex (two-sample ( t ) test)</th>
<th>Race (one-way ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean (M/F) ( ^a )</td>
<td>( t ) statistic</td>
<td>( P ) value</td>
</tr>
<tr>
<td>hA3F</td>
<td>HIV(^+)</td>
<td>4.67/4.66</td>
<td>−0.10</td>
<td>0.92</td>
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<tr>
<td></td>
<td>HIV(^-)</td>
<td>5.06/5.18</td>
<td>0.70</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>4.74/4.74</td>
<td>−0.002</td>
<td>0.99</td>
</tr>
<tr>
<td>hA3G</td>
<td>HIV(^+)</td>
<td>6.17/6.10</td>
<td>−0.64</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>HIV(^-)</td>
<td>6.44/6.45</td>
<td>0.05</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>6.22/6.15</td>
<td>−0.66</td>
<td>0.51</td>
</tr>
</tbody>
</table>

\( ^a \) 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.

![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 mRNA. a) hA3F mRNA 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.](http://jvi.asm.org/)

![TABLE 2. hA3F and hA3G mRNA expression levels by sex and race](http://jvi.asm.org/)
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.
REFERENCES


