Filgrastim, or granulocyte colony-stimulating factor, reverses neutropenia associated with human immunodeficiency virus type 1 (HIV-1) and cytomegalovirus (CMV) infections. During a trial of anti-CMV retinitis therapies coadministered with antiretroviral therapy, 2–4 plasma specimens of HIV-1 RNA were collected from 36 HIV-1–infected patients receiving filgrastim to prevent neutropenia and from 36 patients not receiving filgrastim. For both groups, the crude mean and mean rate of change of HIV-1 log_{10} RNA levels were similar. Adjustment for covariates (CD4+ T cell lymphocytes, virus load at enrollment, level of neutropenia and antiretroviral therapy [mainly non–highly active antiretroviral therapy], and anti-CMV therapy during follow-up) resulted in a mean log_{10} HIV-1 RNA level for individuals receiving filgrastim versus those not receiving the drug of 5.11 versus 4.87 ($P = .12$) and respective log mean rates of change per month of $-0.08$ versus $-0.21$ ($P = .08$). This latter difference has borderline statistical significance, which suggests that filgrastim may reduce the decline of HIV-1 RNA loads.
cessed within 24 hours of phlebotomy, and plasma was stored at −70°C until testing. The assay used the Amplicor HIV-1 Monitor kit (Roche Diagnostic Systems), which is capable of detecting 400,000–750,000 copies of HIV-1 RNA in plasma [7].

CD4⁺ T cell lymphocyte counts and absolute neutrophil counts (ANCs) were quantified by use of flow cytometry at enrollment and at all subsequent visits; a history of all drug therapy, including filgrastim, was also obtained.

Statistical procedures. All HIV-1 RNA levels were expressed as log₁₀ copies per milliliter. CD4⁺ T cells were categorized as binary levels above or below the median value. Mean levels of viral RNA were calculated with multiple regression analysis, using generalized linear models (PROC GLM; SAS). Filgrastim exposure was recorded as a time-dependent binary variable for each interval between 2 sequential virus load determinations, even if they were not used throughout the entire interval.

Adjusted means and rates of change were calculated for 63 patients, with a total of 145 virus load determinations because missing data on covariates from 9 patients were required by the statistical models used. Covariates were fitted into the simplest regression model and included at the time of enrollment the log₁₀ HIV-1 RNA load, CD4⁺ T cell counts, and Karnofsky Scores and included, for each interval, the follow-up covariates: filgrastim use, ANC, time since enrollment, and the use ≥1 reverse-transcriptase inhibitors (RTIs) together with protease inhibitors (PIs). We utilized log transformed values of HIV-1 RNA in the generalized estimation equation (PROC GENMOD; SAS) to calculate P values of the differences between means of HIV-1 RNA, and the coefficients of their relationship with important covariates, thereby providing robust estimates of the variance and adjusting for the lack of independence of repeated measures.

The mean rates of log₁₀ HIV-1 RNA change per 30.4 days were calculated by the generalized linear model with the aforementioned covariates, excluding time. Comparing the virus load with its immediately preceding value obtained a precise comparison for the smallest available interval of change in virus load, and P values were calculated with PROC GENMOD.

Results

Filgrastim was administered to 36 of the 72 patients, and at least 1 subsequent plasma specimen was obtained for virus load determination during the next 6 months of their follow-up. Thirty-three patients received filgrastim at baseline, 17 received filgrastim intermittently in the first and third intervals between 4 virus load determinations, and 19 received filgrastim in only 1 or in all such intervals during follow-up. Because there was no significant difference between the mean virus load levels associated with filgrastim given to these 2 groups, data from all 36 patients receiving filgrastim were subsequently analyzed together.

Patients who received filgrastim at least once and those receiving none were largely similar with respect to age, sex, injection drug use, time from AIDS diagnosis to enrollment, randomization to receive human monoclonal antibody or placebo, and Karnofsky Score and HIV-1 RNA load at enrollment (table 1). The mean ANC at study entry was higher in those individuals who were administered filgrastim, reflecting its more frequent use (39% vs. 0%) prior to recruitment into the study. Only 4 patients received highly active antiretroviral therapy (HAART). However, either before or during the trial, 53% of those exposed to fil-

Table 1. Patient (n = 72) characteristics at baseline and follow-up and their association with virus load.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Filgrastim (n = 36)</th>
<th>No filgrastim (n = 36)</th>
<th>P</th>
<th>Coefficient</th>
<th>P (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at enrollment, years</td>
<td>39.5</td>
<td>40.7</td>
<td>.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex, %</td>
<td>89</td>
<td>92</td>
<td>.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since AIDS diagnosis, mean months (SD), median [range]</td>
<td>36.7 (26.1) 33.1 [1.1–114.1]</td>
<td>27.5 (20.2) 20.1 [0.1–79.3]</td>
<td>.12</td>
<td>−0.03</td>
<td>.44 (−0.09 to 0.04)</td>
</tr>
<tr>
<td>CD4⁺ T cell count/mm³ (median)</td>
<td>11.9 (7.0)</td>
<td>14.3 (7.0)</td>
<td>.56</td>
<td>0.10</td>
<td>.39 (−0.13 to 0.33)</td>
</tr>
<tr>
<td>Injection drug use, %</td>
<td>6</td>
<td>0</td>
<td>.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Karnofsky Score, mean (median)</td>
<td>78 (80)</td>
<td>79 (80)</td>
<td>.57</td>
<td>0.09</td>
<td>1.4 (−0.03 to 0.21)</td>
</tr>
<tr>
<td>ANC, mean cells/µL (median [range])</td>
<td>2780 (1997) [200–8148]</td>
<td>1813 (1612) [432–5000]</td>
<td>.02</td>
<td>0.40</td>
<td>&lt;0.01 (0.14 to 0.66)</td>
</tr>
<tr>
<td>RTI therapy, %</td>
<td>94</td>
<td>92</td>
<td>.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV-1 RNA level, mean log₁₀ copies/mL (median)</td>
<td>5.26 (5.30)</td>
<td>5.28 (5.28)</td>
<td>.92</td>
<td>0.80</td>
<td>&lt;0.01 (0.61 to 0.99)</td>
</tr>
<tr>
<td>Follow-up therapy during trial, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filgrastim</td>
<td>50</td>
<td>50</td>
<td>.00</td>
<td>0.24</td>
<td>0.12 (−0.06 to 0.54)</td>
</tr>
<tr>
<td>RTI</td>
<td>64</td>
<td>47</td>
<td>.16</td>
<td>−0.09</td>
<td>0.50 (−0.36 to 0.18)</td>
</tr>
<tr>
<td>Ever RTI plus PI</td>
<td>53</td>
<td>42</td>
<td>.35</td>
<td>−0.79</td>
<td>&lt;0.01 (−1.23 to −0.36)</td>
</tr>
<tr>
<td>GCL</td>
<td>78</td>
<td>67</td>
<td>.29</td>
<td>0.03</td>
<td>1.23 (−0.33 to 0.39)</td>
</tr>
<tr>
<td>FOS</td>
<td>28</td>
<td>22</td>
<td>.22</td>
<td>0.28</td>
<td>0.87 (0.13 to 0.69)</td>
</tr>
<tr>
<td>GCL and FOS</td>
<td>31</td>
<td>25</td>
<td>.60</td>
<td>−0.57</td>
<td>0.02 (−1.06 to 0.09)</td>
</tr>
<tr>
<td>MSL109</td>
<td>53</td>
<td>50</td>
<td>.81</td>
<td>0.03</td>
<td>0.36 (−0.24 to 0.30)</td>
</tr>
<tr>
<td>GCL, FOS, and MSL109</td>
<td>17</td>
<td>8</td>
<td>.29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. ANC, absolute neutrophil count; CI, confidence interval; FOS, foscarnet; GCL, ganciclovir; HIV, human immunodeficiency virus; MSL109, human anti–cytomegalovirus antibody; PR, protease inhibitor; RTI, reverse transcriptase inhibitors.

P b-coefficients for major covariates with their significance levels in relation to mean virus load from multiple regression analysis using the generalized estimating equation.

P b P value for log transformed virus loads in generalized estimating equation model with limited covariates.

n 66.

n 71.

n 69.
Filgrastim and HIV-1 RNA

Figure 1. Human immunodeficiency virus (HIV) RNA levels over time and adjusted rate of change (bold lines) for 36 individuals not receiving filgrastim (A) and 36 individuals receiving filgrastim (B). Rates were adjusted for baseline variables of CD4+ T cells and follow-up variables of preceding virus load, therapy with reverse-transcriptase and protease inhibitors, ganciclovir with foscarnet, and neutropenia.  

The coefficients reflecting the relationship between the mean virus load and individual covariates at baseline and during each interval are displayed in table 1. For filgrastim, this value indicated that, on average, the virus load was 1.7-fold (0.24 log) higher for those receiving this drug than for those receiving none, but the $P$ value was not significant ($P = .12$). The coefficient for therapy $\geqslant 1$ RTIs and a PI indicated that virus load during follow-up for patients receiving these drugs was, on average, one-sixth ($-0.79$ log) of the virus load of those not receiving therapy ($P < .01$).

The HIV-1 RNA levels shown in figure 1 chart 36 individuals treated with filgrastim between 2 determinations of virus load and 36 patients not receiving the drug. There is no statistically significant difference with respect to filgrastim use between the unadjusted mean levels of HIV-1 RNA for the 69 patients with virus load determinations at enrollment (table 1) or all 72 patients, including the latters’ unadjusted mean rates of change during follow-up (table 2).

The HIV-1 RNA mean and mean rate of change during follow-up were adjusted for 63 patients, 31 of whom received filgrastim (table 2). These 63 patients had a distribution of characteristics and antiretroviral therapy equivalent to the entire group of 72 patients in table 1. The adjusted mean difference between those exposed and unexposed to filgrastim use between the unadjusted mean levels of HIV-1 RNA for the 69 patients with virus load determinations at enrollment (table 1) or all 72 patients, including the latters’ unadjusted mean rates of change during follow-up (table 2).

The adjusted mean rate of HIV-1 RNA decline per month for those not treated with filgrastim was 2.6 log-fold greater than the monthly decline for those receiving G-CSF, but this difference was of borderline statistical significance ($P = .08$; table 2). These adjusted rates summed all segmental changes in HIV-1 load, regardless of how short the time span covered, and are modeled in figure 1.

Discussion

We found no marked differences in the unadjusted mean HIV-1 load or mean rate of decline of HIV-1 RNA between patients receiving and not receiving filgrastim during follow-up. These results are consistent with those of Kuritzkes et al. [1], who reported a similar pattern of virus load levels in patients randomized to receive filgrastim over a 24 week period. In another study of 18 patients with AIDS, a higher dose of filgrastim administered daily for 1 week, along with leukopheresis to stimulate and harvest CD34+ cells, raised HIV-1 RNA load $>0.6 \log_{10}$, in all but one patient [2]. Hermans et al. [8] found no difference in the unadjusted change in HIV-1 p24 antigen with and without filgrastim treatment.

Our results suggest that, after adjustment for potential confounding variables, the mean HIV-1 RNA load for patients receiving filgrastim, compared with those never receiving fil-
Filgrastim, was similar, and their rate of decline of HIV-1 RNA load was somewhat less, although this difference is of borderline significance ($P = .08$). We are aware of only 1 other report of the rate of change of mean HIV load during administration of filgrastim to patients receiving HAART that cited a 6 log-fold greater decline in the unadjusted rate of virus load [9]. In our study, filgrastim use permitted the continuation of anti-CMV and antiretroviral therapy without the commonly associated neutropenia. The decline in HIV-1 load overall in our patients during follow-up likely represents the suppression of HIV-1 replication by the introduction of PIs, treatment of CMV that decreases HIV-1 p24 antigen suppressing CMV replication, and the up-regulation of HIV-1 [10–11].

Although the earlier report of GM-CSF in 1 HIV-infected patient not receiving antiretroviral therapy (an unlikely current clinical situation) correlated with increases in plasma and cerebrospinal fluid HIV-1 RNA, 2 randomized trials of GM-CSF in patients with advanced AIDS noted declines in HIV-1 RNA, compared with no decline in patients not receiving GM-CSF [4, 12–13].

Our results deserve cautious interpretation. A larger study is necessary to show a statistically significant difference in rates of the magnitude we observed, since a study of 36 patients per group and a 2-sided type I error level of 5% provides an 80% power to detect a 1.9-fold increase in rates. Because filgrastim in this study was not randomly assigned, it was necessary to adjust for differences in treatment and other known confounding variables between the 2 treatment groups, despite almost identical mean virus load levels and disease status at enrollment. However, there may well be unknown confounding variables affecting our conclusions that only randomization could have neutralized. The borderline difference in adjusted mean rate of change with filgrastim use we report is conservative because the statistical modeling in our analysis underestimated any actual effect of continuous exposure to filgrastim on virus load. Individuals may have received filgrastim for less than an entire interval between virus load determinations, but the statistical model calculating the rate of change in virus load assumed treatment throughout each interval. This model also adjusted for multiple observations and combined differences in virus load across the shortest possible time intervals. However, since the use of filgrastim with HAART in another study produced a decline in virus load greater than we observed, contemporary patients receiving HAART and filgrastim may experience greater declines in HIV-1 RNA levels [9]. Finally, any reduction in the decline in HIV-1 load from filgrastim suggested here is inconsistent with a marked survival advantage of filgrastim use we reported elsewhere [14].

Fortunately, the problem of neutropenia due to natural HIV-1 infection and treatment of coinfections is infrequent with HAART drug regimens. However, HIV-1 multidrug resistance continues to increase and could herald the return of an increased incidence of these same opportunistic infections with their attendant therapy and neutropenia [15]. Because this study suggests that during the introduction of treatment with PIs or anti-CMV therapy filgrastim use may influence virus load, future investigations should examine HIV-1 replication with HAART and administration of cytokine growth factors.

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References