Mobilization of CD34\(^+\) Progenitor Cells by Granulocyte Colony-Stimulating Factor in Human Immunodeficiency Virus Type 1–Infected Adults

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We conducted a clinical trial to determine the feasibility of growth factor mobilization of CD34\(^+\) progenitor cells in human immunodeficiency virus type 1 (HIV-1)–infected individuals. Eight asymptomatic, HIV-1–infected adults (median CD4\(^+\) T-cell count, 415 cells/μL), received 480 μg/d of granulocyte colony-stimulating factor (G-CSF) for 6 days without evidence of viral activation. Despite concerns that HIV-1 might inhibit hematopoiesis, CD34\(^+\) cells were successfully mobilized to the periphery of all donors, independent of the baseline CD4\(^+\) T-cell count, and the status of antiretroviral therapy. Leukapheresis was performed on day 6, and yielded a median of 194 \(\times\) 10\(^6\) CD34\(^+\) cells per leukapheresis (n = 7). CD34\(^+\)-enriched cells from the leukapheresis were predominately myeloid-committed, but between 0.2% and 1.7% were primitive CD34\(^+\)/CD38\(^-\) progenitors. A median of 21.7% of the mobilized CD34\(^+\) cells were dimly positive for CD4. Consequently, CD34\(^+\)-enriched cells were purified on the cell sorter (mean purity, 97.7% ± 2.4%; n = 7), and examined for HIV-1 DNA. Purified CD34\(^+\) cells from two of seven donors were polymerase chain reaction (PCR)-positive for HIV-1, but only from one of three samples from each donor. We conclude that G-CSF can safely mobilize CD34\(^+\) progenitor cells in HIV-1–infected subjects, and that these cells are suitable for consideration in gene-transfer strategies.

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Flow cytometry. The CD34+ cell frequency in peripheral blood was determined by staining leukocytes present in whole blood with (peridinin chlorophyll protein [PerCP], fluorescein isothiocyanate [FITC]−, or phycoerythrin−) conjugated monoclonal antibodies (mAb) directed against CD45, CD34, and CD14 (Becton Dickinson [BD] Immunocytometry Systems, San Jose, CA). On the first day of G-CSF, a 100-μL aliquot of peripheral blood was stained with 10 μL of each mAb. After the first day, with the increase in the peripheral leukocyte count in response to G-CSF administration, 20 μL of each mAb was used to stain a 50-μL aliquot of blood, to maintain the same degree of CD45 fluorescence. After staining, erythrocytes were lysed (Easy-Lyse; Leuco Technologies, Ballwin, MO) and leukocytes were fixed in 0.5% paraformaldehyde. The six daily samples were batched and analyzed together to reduce interassay variability (having previously confirmed no loss of fluorescence over this time period). Two-color flow cytometry was performed on the FACScan (BD). MNC were identified using the expression of CD45, and the frequency of CD34+ cells was determined among this population. For each analysis, a minimum of 10,000 events was collected and analyzed. The absolute number of CD34+ cells in the peripheral blood was calculated by multiplying the CD34+ cell frequency by the total daily leukocyte count.

Immunophenotypic analysis was performed on CD34-enriched cells derived from the leukapheresis product. Aliquots of 2 × 10⁶ CD34-enriched cells were stained with directly conjugated mAb directed against CD45, CD34, and CD14 (Becton Dickinson [BD] Immunocytometry Systems, San Jose, CA). After the first day of G-CSF, a 100-μL aliquot of peripheral blood was stained with 10 μL of each mAb. After the first day, with the increase in the peripheral leukocyte count in response to G-CSF administration, 20 μL of each mAb was used to stain a 50-μL aliquot of blood, to maintain the same degree of CD45 fluorescence. After staining, erythrocytes were lysed (Easy-Lyse; Leuco Technologies, Ballwin, MO) and leukocytes were fixed in 0.5% paraformaldehyde. The six daily samples were batched and analyzed together to reduce interassay variability (having previously confirmed no loss of fluorescence over this time period). Two-color flow cytometry was performed on the FACScan (BD). MNC were identified using the expression of CD45, and the frequency of CD34+ cells was determined among this population. For each analysis, a minimum of 10,000 events was collected and analyzed. The absolute number of CD34+ cells in the peripheral blood was calculated by multiplying the CD34+ cell frequency by the total daily leukocyte count.

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analyzed for ICDp24 levels. Results are presented in Fig 1. To determine whether the antigen levels changed over time after the administration of G-CSF, we used a random coefficient growth curve model with intercept and slope. The results of the Wald-type chi-square test failed to demonstrate an association between ICDp24 and the described time points after the administration of G-CSF ($\chi^2 = 34, P = .56$).

Leukocyte response to G-CSF administration. The administration of G-CSF resulted in an abrupt increase in the leukocyte count in all eight subjects in the 24 hours following the first dose. Most of this increase was secondary to an increase in mature and band neutrophils. Substantial individual variation in the peak leukocyte value was noted (range, 17.7 to 43.3 \times 10^3 \text{WBC}/\mu\text{L}), with a median among the eight donors of 30.6 \times 10^3 \text{WBC}/\mu\text{L}. Following cessation of the G-CSF, the WBC count on day 14 was below baseline in six of eight subjects, but showed recovery toward baseline on day 28.

Lymphocyte response to G-CSF administration. Although the percent circulating lymphocytes decreased with G-CSF administration (concomitant with the increase in the percent circulating neutrophils), an increase in the absolute number of lymphocytes was observed. Both T and B lymphocytes contributed to this increase. Among the eight donors, there was a median increase in the number of CD3$^+$ T cells by 1.5-fold, and a median increase in the number of CD19$^+$ B cells by twofold, between days 1 and 6. Following the discontinuation of G-CSF, the lymphocyte number decreased to below baseline values by day 14 in six of the eight donors. These values showed recovery toward baseline on day 28. To determine whether the changes in the absolute number of CD3$^+$ T cells in HIV-1–infected individuals reflected changes in both CD4$^+$ and CD8$^+$ T-cell subsets, we measured the CD4:CD8 ratios among the eight donors. The mean CD4:CD8 ratio before G-CSF administration (0.5 ± 0.1), was unchanged following 6 days of G-CSF (0.5 ± 0.1), and remained so on days 14 and 28. Thus, CD4$^+$ and CD8$^+$ T cells contributed proportionately to the changes in the CD3$^+$ T-cell count.

CD34$^+$ cell response to G-CSF administration. Analysis of daily blood samples demonstrates that G-CSF can mobilize CD34$^+$ cells in the HIV-1–infected individual. Although great variability in the absolute number of CD34$^+$ cells recruited was observed, all eight donors showed a response to growth factor administration. The kinetics of the CD34$^+$ cell response was similar among the donors, with the peak CD34$^+$ cell response occurring on day 5 or day 6 (Fig 2). The median number of CD34$^+$ cells in the peripheral blood before G-CSF administration was 3 cells/\mu\text{L} (range, 2 to 9) and increased to a median of 111 cells/\mu\text{L} at the peak of the response (range, 55 to 401). This resulted in an overall median increase of 37-fold between baseline and peak CD34$^+$ cell number present in the circulation. To determine whether the donor baseline CD4$^+$ T-cell count affected the magnitude of the CD34$^+$ cell response, we regressed the peak CD34$^+$ cell count on the initial CD4$^+$ T-cell count. The simple regression analysis suggested that the CD4$^+$ T-cell count does not have any significant effect on the peak of the CD34$^+$ cell response ($P = .16$).

Analysis of CD34$^+$ cells in the leukapheresis product. Seven donors were leukapheresed on day 6 of G-CSF. The volume of the leukapheresis product among the seven subjects averaged approximately 55 mL, and contained a median of 0.67% (range, 0.4% to 1.7%) CD34$^+$ cells. Numbers of mononuclear and CD34$^+$ cells in the leukapheresis product are summarized in Table 2.

To characterize CD34$^+$ progenitor cells mobilized to the peripheral blood, we enriched the cells on an immunoaffinity column. The purity of the CD34$^+$-enriched fraction averaged 70% ± 7% CD34$^+$ cells (n = 7), with an overall yield of 36% ± 11% of the CD34$^+$ cells calculated to have been loaded on the column. The G-CSF–mobilized CD34$^+$ cells uniformly coexpress low levels of the CD45 marker (Fig 3), similar to marrow-derived CD34$^+$ cells. To estimate the proportion of early CD34$^+$CD38$^-$ progenitor cells in the G-CSF–mobilized population, three-color FACS analysis was performed using antibodies directed against CD34, CD45, and CD38 molecules. Between 0.2% and 1.7% of the G-CSF–mobilized cells are primitive CD34$^+$CD38$^-$ cells (Table 3 and Fig 3), which approximates the level found in marrow. Analysis of CD34$^+$ cells for coexpression of my-
Table 2. Leukapheresis Product From HIV-1 Infected Individuals Following 6 Days of G-CSF

<table>
<thead>
<tr>
<th>Donor No.</th>
<th>Dose G-CSF (μg/kg/d)</th>
<th>Day 6 WBC Count (10^9 cells/μL)</th>
<th>% CD34+ Cells</th>
<th>MNC No. (× 10^9)</th>
<th>Total CD34+ Cell No. (× 10^9)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.1</td>
<td>18.8</td>
<td>0.46</td>
<td>19</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>5.2</td>
<td>24.7</td>
<td>0.40</td>
<td>22</td>
<td>88</td>
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<td>4</td>
<td>5.5</td>
<td>14.9</td>
<td>0.59</td>
<td>11</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>5.8</td>
<td>27.1</td>
<td>0.67</td>
<td>29</td>
<td>194</td>
</tr>
<tr>
<td>6</td>
<td>5.9</td>
<td>26.7</td>
<td>0.91</td>
<td>22</td>
<td>200</td>
</tr>
<tr>
<td>7</td>
<td>6.8</td>
<td>33.2</td>
<td>0.71</td>
<td>29</td>
<td>206</td>
</tr>
<tr>
<td>8</td>
<td>7.4</td>
<td>43.3</td>
<td>1.70</td>
<td>20</td>
<td>340</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>26.7</td>
<td>0.67</td>
<td>22</td>
<td>194</td>
</tr>
</tbody>
</table>

Abbreviation: MNC, mononuclear cells.

* Total number of CD34+ cells in each leukapheresis product was determined by multiplying the MNC count by the proportion of CD34+ cells in the leukapheresis (established by FACS analysis).

eoloid-associated antigens (CD13 and CD33) demonstrated that the majority of G-CSF–recruited CD34+ cells are committed to the myeloid lineage (Table 3 and Fig 3), as is characteristic of PBPC.21

Mobilized CD34+ cells were also examined for the expression of lymphocyte markers, including CD19, CD8, and CD4 (Table 3). A median of 0.5% of mobilized CD34+ cells coexpress the B-cell marker, CD19, a median of 4% (range, 1.0% to 8.8%) coexpress the CD8 molecule, and a median of 21.7% (range, 6.8% to 27.9%) coexpress the CD4 molecule, although the CD4 fluorescence was dim (Fig 3). CD4 coexpression was not a feature of the particular anti-CD4 mAb used, as the observation was consistent when either of two different mAbs were used (data not shown).

PCR analysis of sorted CD34+ cells, mobilized in HIV-1-infected individuals. The expression of CD4 on the surface of some of the mobilized CD34+ cells prompts the question of whether these cells are infected with HIV-1 in the infected host. To address this, we purified populations of CD34+ cells on the cell sorter, and analyzed DNA extracted from these populations for HIV-1 by PCR. Sorted CD34+ cells had a mean purity of 97.7% ± 2.4% (range, 92.9% to 99.3%). Peripheral blood MNC and CD34+ cells from all seven donors were positive by PCR for HIV-1 (data not shown). However, purified CD34+ cell DNA from five of the seven donors was negative by PCR for HIV-1 (data not shown). However, purified CD34+ cell DNA from five of the seven donors was negative by PCR for HIV-1 (data not shown). However, purified CD34+ cell DNA from five of the seven donors was negative by PCR for HIV-1 (data not shown). However, purified CD34+ cell DNA from five of the seven donors was negative by PCR for HIV-1 (data not shown).

Fig 3. Subset analysis of G-CSF–mobilized CD34+ cells. CD34-enriched leukapheresis cells from donor 8 were examined for expression of CD45, CD38, CD13/CD33, CD19, CD8, and CD4 (BD anti-CD4). Top panels represent enriched leukapheresis cells stained with directly conjugated isotype matched control mAb.
Table 3. Phenotypic Characteristics of G-CSF-Mobilized CD34⁺ Cells From HIV-1-Infected Donors

<table>
<thead>
<tr>
<th>Donor No.</th>
<th>% CD13/33⁻</th>
<th>% CD38⁻</th>
<th>% CD19⁺</th>
<th>% CD8⁻</th>
<th>% CD4⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.7</td>
<td>0.2</td>
<td>1.0</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7.7</td>
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<td>22.3</td>
</tr>
<tr>
<td>3</td>
<td>5.6</td>
<td>0.2</td>
<td>0.7</td>
<td>8.6</td>
<td>8.9</td>
</tr>
<tr>
<td>4</td>
<td>5.4</td>
<td>0.3</td>
<td>0.4</td>
<td>1.3</td>
<td>27.9</td>
</tr>
<tr>
<td>5</td>
<td>7.1</td>
<td>0.2</td>
<td>0.4</td>
<td>3.5</td>
<td>24.4</td>
</tr>
<tr>
<td>6</td>
<td>2.4</td>
<td>0.6</td>
<td>1.2</td>
<td>4.4</td>
<td>11.1</td>
</tr>
<tr>
<td>7</td>
<td>2.9</td>
<td>0.1</td>
<td>0.6</td>
<td>5.4</td>
<td>21.7</td>
</tr>
</tbody>
</table>

All values are expressed as the percentage of peripheral blood CD34⁺ cells that either lack (CD13/33, CD38) or coexpress (CD19, CD8, CD4) the designated marker(s).

Abbreviation: ND, not done.

DISCUSSION

This study demonstrates that CD34⁺ cells can be safely mobilized to the peripheral blood of HIV-1-infected donors following the administration of G-CSF. That progenitor cells can be mobilized in individuals with CD4⁺ T-cell counts greater than 200/μL provides some insight into the interaction between HIV-1 and the hematopoietic system. While studies suggest that hematopoietic progenitors may be reduced in the infected individual (because of direct infection or secondary to infection of hematopoietic regulatory cells), the response of CD34⁺ progenitors to G-CSF is not impaired in the HIV-1-infected individual. This is true even in our donors with lower CD4⁺ T-cell counts. Indeed, the donor with the highest CD34⁺ cell response had the third lowest baseline CD4⁺ T-cell count (380 cells/μL). Moreover, the CD34⁺ cell responses to G-CSF are preserved in HIV-1-infected subjects in degrees remarkably similar to those described for uninfected, healthy donors. Interestingly, the magnitude of the CD34⁺ cell response among our subjects was greater than that described in two studies of healthy donors receiving G-CSF. It is possible that these higher values simply represent the spectrum of responses that will become more evident as greater numbers of donors are evaluated. Alternatively, they may reflect technical differences in determining the frequency of CD34⁺ cells in the peripheral blood. If found to be a consistent feature of HIV-1-infected subjects, these increased responses may be a reflection of a system driven to cope with sustained lymphocyte destruction.

Once mobilized to the peripheral blood compartment, the CD34⁺ cells from our subjects were easily collected via leukapheresis in numbers (median, 194 × 10⁶; n = 7) comparable to those of uninfected individuals (mean, 119 ± 65 × 10⁶; n = 7). Although the number of mobilized CD34⁺ cells that will achieve prompt engraftment is not precisely defined, between 0.5 and 5 × 10⁹ cells/kg of patient body weight has been suggested as a threshold range. The number of mobilized CD34⁺ cells collected in our study would fall within this range. The composition of the mobilized CD34⁺ cells was also similar to that described for cells mobilized from uninfected individuals, including the proportions of early CD34⁺CD38⁺ progenitor cells. Insofar as this phenotype reflects hematopoietic potential, G-CSF-mobilized cells in the infected individual will likely have similar reconstitution capability as those mobilized from the uninfected host. It has been suggested that a higher proportion of early progenitors are present among cells mobilized with GM-CSF; however, we elected to use G-CSF because GM-CSF (and not G-CSF) has been reported to enhance HIV-1 production in monocyte cultures. However, neither cytokine has been associated with consistent alteration of viral expression in vivo. We observed no sustained increase in serum antigen levels in this study, despite an increase in the number of circulating T lymphocytes in response to G-CSF. It is interesting to note that proportional increases in both CD4⁺ and CD8⁺ T cells contributed to the increase in T-cell numbers in the HIV-1-infected individual, similar to the responses reported in uninfected cancer patients receiving G-CSF.

The preservation of the CD34⁺ cell response to G-CSF in HIV-1-infected individuals might not exclude the possibility that these cells are infected with HIV-1, especially given the expression of dim levels of surface CD4 by a fraction of the G-CSF-mobilized CD34⁺ cells. CD34⁺CD4⁺ progenitor cells are not unique to the HIV-1-infected individual, and have been described in studies of murine and human hematopoietic progenitor cells. In our study, PCR of sorted CD34⁺ cells from five of seven HIV-1-infected donors was negative for HIV-1 proviral DNA. A serial dilution of 8E5/LAV cells (containing one HIV-1 proviral DNA copy per cell) demonstrated that our nested HIV-1 PCR technique could consistently detect a single HIV-1 proviral genome in viral.
25,000 cells. Thus, fewer than 10 proviral copies were present in the 250,000 mobilized CD34+ cells analyzed from donors with negative HIV-1 PCR results. Two donors had a positive PCR on the first aliquot of sorted CD34+ cells, but had negative PCR signals from the next two aliquots of CD34+ cells collected from the same sort. Each PCR was performed on 250,000 CD34+ cells, with purities of 98.3% and 99.2% (donors 4 and 7, respectively). That only one of three samples was positive suggests that either a very small proportion of CD34+ cells was infected (since the positive signal was not detected in the other two samples with 250,000 CD34+ cells), or that a small number of contaminating CD4+ cells was present in the positive samples. Serial dilutions of CD4+ T cells from donors 4 and 7 demonstrated that as few as 625 or 1,250 CD4+ T cells, respectively, could be consistently detected in a population of 250,000 cells using our nested PCR technique. Perhaps such small numbers of CD4+ T cells were present in the first aliquot of sorted cells collected from these two donors. The results of this study are similar to recent studies of marrow CD34+ cells from HIV-1-infected individuals,9 and are consistent with the notion that CD34+ progenitor cells are not infected with HIV-1. Thus, G-CSF can safely mobilize CD34+ progenitor cells in the HIV-1-infected individual, and these cells represent an appropriate target for gene-transfer studies in the infected individual.

ACKNOWLEDGMENT

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