Treatment with Granulocyte Colony–Stimulating Factor Decreases the Capacity of Hematopoietic Progenitor Cells for Generation of Lymphocytes in Human Immunodeficiency Virus–Infected Persons

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An obstacle to stem cell gene therapy for AIDS is the limited numbers of hematopoietic progenitors available. Granulocyte colony–stimulating factor (G-CSF) is used for mobilization of progenitors, but little is known about the functional characteristics of mobilized progenitors, and immature and T cell progenitors may not be mobilized. This study examined the effect of G-CSF on the function of progenitors. Ten human immunodeficiency virus–infected patients received G-CSF (filgrastim, 300 μg/day) for 5 days. Absolute numbers of immature and T cell progenitors did not increase. The ability of $CD34^+$ progenitor cells to generate lymphocytes was examined by use of thymic organ cultures. The mean number of lymphocytes generated per $CD34^+$ cell on day 0 was 0.72 and on day 4 was 0.09 ($P < .003$). The number of $CD4^+$ cells generated per $CD34^+$ cell was significantly reduced after G-CSF treatment. Thus, G-CSF increased the number of mature progenitor cells but did not increase T cell progenitors.

More than a decade after discovery of the virus that causes AIDS, treatment of human immunodeficiency virus (HIV) remains a challenge. Although antiviral therapy has improved survival in persons with advanced HIV disease, benefits may not be lasting and appear to be limited by the development of drug resistance. Gene therapy has been suggested as a possible treatment for AIDS. Results from early gene therapy trials showed that introduction of genes into cells can be done safely and that, in some cases, foreign genes can be detected in vivo for prolonged periods, albeit at low levels [1–4]. Evidence from initial gene therapy trials for adenosine deaminase deficiency and AIDS has suggested some clinical benefit [1, 4].

For protection of multiple hematopoietic lineages against HIV, stem cell gene therapy for AIDS has been proposed. Human hematopoietic progenitor ($CD34^+$) cells produce all the cells of the immune system involved in AIDS pathogenesis (e.g., $CD4^+$, dendritic, and microglia cells and monocytes/macrophages). Thus, transfer of anti-HIV genes into hematopoietic progenitor cells of HIV-infected persons may be a therapeutic approach to render mature cells arising from progenitor cells resistant to the destructive events associated with HIV infection. Recent reports indicate that transduction of human $CD34^+$ cells with anti-HIV genes inhibits HIV replication in monocytes and lymphocytes produced in vitro [5–11].

Stem cell gene therapy, however, requires sufficient numbers of $CD34^+$ cells and the ability of the cells to produce the cells of interest, primarily $CD4^+$ T cells. To obtain adequate numbers of progenitors, the use of granulocyte colony–stimulating factor (G-CSF) for mobilization of $CD34^+$ cells in HIV-infected patients has been suggested, and $CD34^+$ cells can be mobilized with G-CSF in HIV-infected persons [6, 8, 12–15]. Implicit is the assumption that the peak of total progenitor cell release also represents the peak release of T cell progenitors. Clearly, G-CSF–mobilized peripheral blood possesses a large number of myeloid progenitor cells, but information on the T cell progenitor content is lacking.

In this study, we examined G-CSF mobilization of progenitor cells in 10 HIV-infected patients. Differentiation of T cells from progenitor cells is restricted to the unique microenvironment of the thymus, and several models to facilitate this have been developed [7, 16–25]. In this study, we used an experimental model based on organ culture chimeras to examine the ability of progenitors to differentiate into T cells [18, 25]. We used flow cytometry to determine the phenotype of mobilized progenitors. The phenotype and functional capacity of progenitor cells mobilized by G-CSF were compared with those of unprimed progenitor cells from the same patients collected before G-CSF treatment.

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This study was approved by the local ethical committee (KF 02-194/96). Informed consent was obtained from all patients after the nature and consequences of the study were fully explained.

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Patients and Methods

Patients and study design. Ten male HIV-infected patients were included in this study [14]. All had 103–323 CD4 cells/μL and were given G-CSF treatment for 5 consecutive days (300 μg of filgrastim/day; Amgen, Thousand Oaks, CA). Blood for laboratory studies was obtained on days 0 and 4 for flow cytometry determination of $CD34^+$ cells and to collect peripheral blood mononuclear cells (PBMC) for thymic organ cultures and colony-forming cell (CFC) assay. As controls, we determined the $CD34^+$ subsets in samples from 10 patients with cancer: non-Hodgkin's lymphoma, 8; Hodgkin's lymphoma, 1; and acute lymphoblastic leukemia, 1. The patients with cancer received G-CSF (10 μg/kg) for 4 or 5 days as part of their treatment. Finally, for comparison, T cell development was examined in samples from 3 healthy donors who were treated with G-CSF as part of allogenic transplantation.

Flow cytometric determination of progenitor cells. Flow cytometric analyses of $CD34^+$ cells were done using blood from all 10 HIV-infected patients drawn before and after G-CSF treatment and from 10 HIV-negative patients with cancer treated with G-CSF. The assay was done as described elsewhere [26]. In brief, 100 μL of blood was incubated with 10 μL of fluorescence-conjugated monoclonal antibodies (MAbs) at room temperature for 15 min. Erythrocytes were lysed with 2 mL of NH_4Cl buffer at room temperature for 10 min, and the samples were washed and resuspended in PBS supplemented with 10% CellFIX (Becton Dickinson Immunocytometry Systems, San Jose, CA). All samples were analyzed by FACScan (Becton Dickinson) equipped with a 488-nm argon-ion laser. The fluorescence of 50,000 cells (CD34 and isotype controls) was measured with logarithmic amplification. A gate was determined on two parameter dot plots (forward scatter [FSC] vs. side scatter [SSC]). This gate was set to exclude cell debris from analyses. To determine the percentage of $CD34^+$ cells coexpressing CD2, CD4, CD7, CD38, HLA-DR, and Thy-1, we collected 200–2000 cells using an acquisition CD34 gate (low SSC vs. intermediate-to-high CD34 expression) [26, 27]. Data were processed by use of CELLQuest software (Becton Dickinson). To determine the absolute $CD34^+$ cells in peripheral blood, the percentage of cells expressing CD34 was multiplied by the white blood cell count. MAbs used to determine phenotypes were isotype control γ_1-fluorescein isothiocyanate (FITC)/γ_1-phycoerythrin (PE), CD34-PE (anti-HPCA-2), CD2-FITC (Leu-5b), CD4-FITC (Leu-3a), CD7-FITC (Leu-9), CD38-FITC (Leu-17), and anti–HLA-DR–FITC (clone L243) from Becton Dickinson and Thy-1-FITC (clone 5E10) from Pharmingen (San Diego).

Colony assays for progenitor cells. Colony assays were done using PBMC from all 10 HIV-infected patients obtained before and 4 days after initiation of G-CSF treatment. CFCs were grown in methylcellulose medium (stem cell colony–forming unit [cfu] kit; Baxter Healthcare, Deerfield, IL) in accordance with the manufacturer's instructions. In brief, 8×10^5 PBMC in 1 mL of dilution medium was mixed with 3 mL of cfu culture medium to allow plating at a concentration of 2×10^5 PBMC/mL. Dilution medium contained Iscove's modified Dulbecco's medium supplemented with 20% fetal bovine serum; culture medium consisted of Iscove's modified Dulbecco's medium, 5% GCT-conditioned medium, 36% modified fetal bovine serum, 10^{-4} *M* 2-mercaptoethanol, 0.778 U/mL erythropoietin, and 0.8% methylcellulose. We added 100 ng/mL stem cell factor (Genzyme, Cambridge, MA), and the cell suspension was aliquoted in 1-mL triplicates in 35-mm culture plates (Nunc, Roskilde, Denmark). The plates were incubated for 14 days in a humidified incubator at 37°C and 5% CO_2. Colonies (granulocyte-macrophage cfus) of >50 cells were counted by use of an inverted microscope.

The median number of colonies counted in triplicate was the number of CFCs/2×10^5 PBMC. The number of CFCs/mL of peripheral blood was calculated by the following equation: CFC/mL = [$5 \times$ (CFC/2×10^5 PBMC)] $\times$ [no. of mononuclear cells (monocytes + lymphocytes) $\times 10^6$/mL peripheral blood]. Finally, we determined the cloning efficiency of $CD34^+$ cells by the equation: cloning efficiency (%) = (CFC/mL/number of $CD34^+$ cells/mL) $\times$ 100.

Thymic organ cultures. Progenitor cells in PBMC preparations from 7 HIV-infected patients (patients 1, 2, 4, 6–8, and 10 [14]) obtained prior to G-CSF treatment and after 4 days of G-CSF treatment were analyzed for ability to differentiate into T cells in thymic organ cultures. For comparison, this assay was also done by use of PBMC from 3 healthy donors who had been treated with G-CSF.

RAG-1 knockout mice were maintained and bred in microisolator cages in the animal care facility at the Netherlands Cancer Institute. Fetal thymus lobes were removed from 14- or 15-day-gestation mouse fetuses and placed in organ cultures as described elsewhere [18, 25]. In brief, fetal murine thymus lobes were placed in an organ culture system on 25-mm-thick filters (0.45-μm pore size; Millipore, Bedford, MA) supported on surgical gelfoam (Upjohn, Kalamazoo, MI). Organ cultures were grown in Dulbecco's MEM (Sigma, St. Louis) with 20% fetal bovine serum (Life Technologies, Paisley, UK), 1 mg/mL penicillin, 1 U/mL streptomycin, and 3.4 g/L sodium bicarbonate and were maintained at 37°C with 5% CO_2. After cryopreserved cells were thawed and counted, viability was assessed by trypan blue dye exclusion. Then, 5×10^5 to 1×10^6 total viable PBMC were placed on each thymus lobe by direct application of broken pellets of cells in 0.2-μL aliquots until the designated total donor cells per lobe was reached. After 14 days in culture, lobes were enzymatically digested in collagenase, and the cells that were removed were counted and stained for flow cytometry.

Cells from collagenase digestion were stained with the following MAbs: CD3-FITC, CD4-tricolor, CD8-PE, CD45-tricolor, MHCI-PE, MHCII-FITC, and isotype controls. All MAbs were from Caltag (Burlingame, CA). Cells were analyzed by flow cytometry (FACScan; Becton Dickinson), using CellQuest software. Cells were gated on the basis of FSC characteristics. Gated cells were analyzed for the expression of $CD4^+$ and $CD8^+$ cell subsets: $CD4^+CD8^-$, $CD4^-CD8^+$, $CD4^+$, $CD8^+$, and $CD4^-CD8^-$. Each subset was further analyzed for the expression of CD3. Total human cells were calculated by multiplying cell number by percentage of $CD45^+$ MHC^+ cells. Flow cytometry data from thymic organ cultures were calculated on a per lobe basis by multiplying the frequency of a given T cell subset by the total human cells produced per lobe as assessed by CD45 and MHC staining.

Statistical methods. All data points are mean ($\pm$SE). When variation was found, logarithmic transformations of the measurements were done prior to further statistical analyses. Differences between patients at days 0 and 4 and between patients and donors

were evaluated by use of a paired and an unpaired *t* test, respectively. A 5% significance level was used in all analyses.

Results

Mobilization of progenitor cells by G-CSF in HIV-infected patients. We examined the effect of G-CSF on absolute numbers of $CD34^+$ cells and CFCs in 10 HIV-infected patients. Before G-CSF treatment, the number of circulating $CD34^+$ cells was 1.50 ± 0.11 cells/μL. The absolute number of circulating progenitor cells increased significantly during G-CSF treatment [14]. Thus, on day 4, there were 16.08 ± 0.87 $CD34^+$ cells/μL ($P < .002$). $CD34^+$ cells peaked on day 5 (20.01 cells/μL) after initiation of G-CSF treatment. This increase in circulating $CD34^+$ cells was paralleled by a significant increase in CFCs (figure 1). On day 0, the mean CFC/mL count was 33.6 ± 10.4. During G-CSF treatment, the mean CFC/mL increased to 497.0 ± 122.2 ($P < .01$). Finally, the function of the mobilized mature $CD34^+$ cells was evaluated by cloning efficiency. Before G-CSF treatment, the cloning efficiency was 9.9% ± 4.8%. G-CSF did not affect the cloning efficiency, and after 5 days of G-CSF treatment, the cloning efficiency was 7.3% ± 2.9% ($P = .99$).

Mobilization of immature progenitors in HIV-infected patients and HIV-negative controls. To determine whether administration of G-CSF altered the progenitor subset distribution, the phenotype of progenitors in HIV-infected patients before and after G-CSF mobilization was analyzed by flow cytometry. The results are summarized in table 1. Immature progenitor cells can be found in the $CD34^+CD38^-$, $CD34^+$HLA-DR^-, and $CD34^+$Thy–1^+ subsets of progenitors [28–31]. After treatment with G-CSF, there were significant decreases in the percentage of $CD34^+CD38^-$ and $CD34^+$HLA-DR^- cells ($P < .001$ and $< .003$, respectively). Although the percentage of $CD34^+$ Thy-1^+ cells seemed to decrease, this was not significant ($P = .38$). For comparison, the distribution of progenitor subsets was analyzed in blood samples from 10 HIV-negative patients with cancer who had been treated with G-CSF for 4 or 5 days (table 1). Compared with HIV-negative patients with cancer, the HIV-infected patients had a significantly lower fraction of immature $CD34^+CD38^-$ cells (2.7% vs. 13.8%, $P < .003$). In contrast, there were no differences in percentages of $CD34^+$HLA-DR^- and $CD34^+$Thy-1^+ cells between HIV-infected patients and HIV-negative donors. Because of the increase in absolute $CD34^+$ cells during G-CSF mobilization, the populations of immature progenitors tended to increase (figure 2*A*). Absolute $CD34^+$Thy-1^+ cells increased significantly ($P < .04$). There were no significant changes in absolute $CD34^+CD38^-$ and $CD34^+$HLA-DR^- cells during G-CSF administration (figure 2*A*). In contrast, there were dramatic increases in mature progenitors ($CD34^+CD38^+$ and $CD34^+$HLA-DR^+ cells, $P < .0003$ and $< .0002$, respectively).

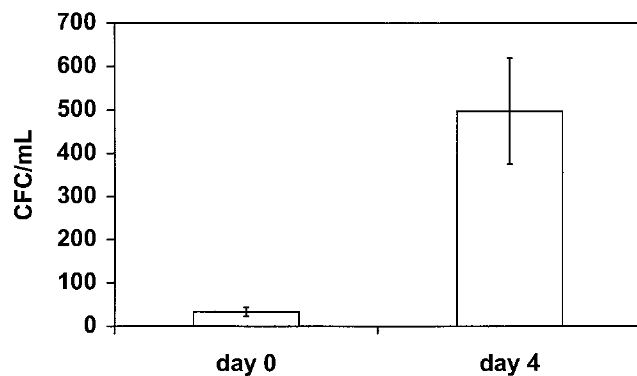

Figure 1. Absolute number of colony-forming cells (CFCs)/mL before granulocyte colony–stimulating factor (G-CSF) and after 4 days of G-CSF treatment ($P < .01$). Data are mean ± SE.

Table 1. Mean progenitor subset distribution in peripheral blood before and after treatment with granulocyte colony–stimulating factor (G-CSF) in 10 human immunodeficiency virus (HIV)–postive men and after G-CSF mobilization in 10 HIV-negative patients with cancer.

Patient group/progenitor subset distribution	HIV positive		HIV-negative donors,
	None	4 days	4 or 5 days
Immature progenitor cells, %			
$CD34^+CD38^-$	21.6	2.8	13.8
$CD34^+$HLA-DR^-	31.8	5.7	3.7
$CD34^+$Thy-1^+	18.1	5.0	6.3
T cell progenitors, %			
$CD34^+CD7^+$	14.0	3.2	2.3
$CD34^+CD2^+$	16.9	2.9	2.5
$CD34^+CD4^+$	6.6	1.6	1.6

Mobilization of T cell progenitors in HIV-infected patients. A concern with the use of G-CSF–mobilized blood in stem cell gene therapy for AIDS is that mobilized blood does not contain T cell progenitors. T cell progenitors may be $CD34^+$ cells coexpressing CD2 or CD7 [25, 32]. Furthermore, previous studies have shown that a subset of progenitors expresses low levels of CD4 [33, 34]. Therefore, we used flow cytometry to determine the fraction of $CD34^+$ cells coexpressing CD2, CD4, and CD7. After G-CSF treatment, there were significant decreases in the percentage of progenitors that coexpressed CD7 and CD2 (table 1). The percentage of $CD34^+CD7^+$ cells decreased from 14.0% ± 2.5% to 3.2% ± 0.9% ($P < .003$), and the percentage of $CD34^+CD2^+$ cells decreased from 16.9% ± 3.7% to 2.9% ± 0.6% ($P < .01$). The percentage of $CD34^+CD4^+$ cells seemed to decrease as well but not significantly. When the percentages of T cell progenitors in mobilized blood from HIV-infected patients and from HIV-negative patients with cancer were compared, there was no difference between the two groups.

In HIV-infected patients, minor increases in absolute T cell progenitors were found after G-CSF treatment (figure 2*B*), but these were not significant. Thus, although G-CSF induced a 10-fold increase in circulating $CD34^+$ cells, only minor changes

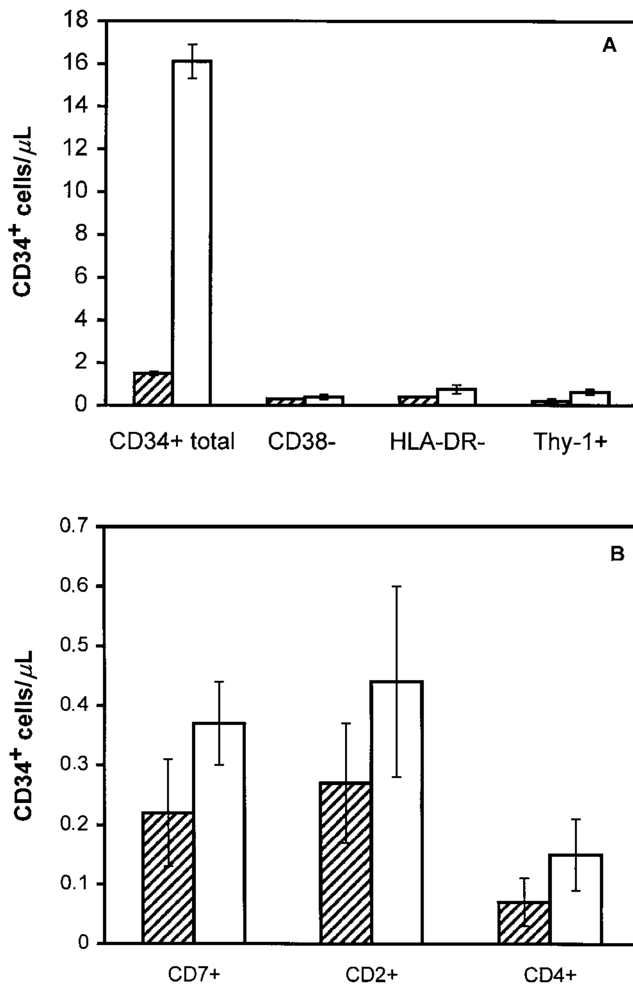

Figure 2. Absolute number of progenitor cells before granulocyte colony–stimulating factor (G-CSF) treatment (hatched bars) and after 4 days of G-CSF mobilization (open bars). *A,* increase in total $CD34^+$ cells ($P < .002$) and in $CD34^+Thy\text{-}1^+$ cells ($P < .04$). There were no significant changes in numbers of $CD34^+CD38^-$ or $CD34^+HLA\text{-}DR^-$ cells. *B,* effect of G-CSF mobilization on number of T cell progenitors (not significant). Data are mean ± SE.

in absolute numbers of circulating T cell progenitors were determined.

Effect of G-CSF on the ability of progenitor cells to generate CD4 cells in thymic organ culture. To evaluate the ability of mobilized progenitors to generate lymphocytes, PBMC from 7 HIV-infected patients obtained before and after G-CSF treatment were analyzed for ability to generate T cells in thymic organ cultures. In each case, 5×10^5 to 1×10^6 total PBMC were placed on each thymic lobe. Because of G-CSF, the percentage of $CD34^+$ cells in PBMC preparations increased from 0.08% ± 0.03% on day 0 to 0.47% ± 0.10% on day 4 ($P <$ .007).

In thymic organ culture, the total number of lymphocytes generated per lobe did not differ significantly between day 0 and day 4 samples (0.22×10^4 cells ± 0.11 vs. 0.21×10^4 ± 0.08; $P = .85$). Of importance, the numbers of $CD4^+$ cells generated per lobe were not significantly different between samples obtained on day 0 and day 4 (0.09×10^4 cells ± 0.06 vs. 0.07×10^4 ± 0.03; $P = .90$). Furthermore, there were no significant differences in development of CD8 cells or double-positive cells between days 0 and 4 (figure 3). Each subset was further analyzed for the expression of CD3. Cells that expressed little or no CD3 were considered immature, whereas cells that expressed moderate-to-high levels of CD3 were considered mature. There were no differences regarding development of mature or immature cells between days 0 and 4. The ability of mobilized PBMC from HIV-infected patients to generate T lymphocytes in thymic organ culture was also compared with that of 3 healthy donors who had been treated with G-CSF. Mobilized PBMC from healthy donors seemed to generate more CD4 cells than mobilized PBMC from HIV-infected patients (0.13×10^4 cells ± 0.07 vs. 0.07×10^4 ± 0.03); however, perhaps because of the few subjects, this did not reach statistical significance. Pretreatment samples from the 3 healthy donors were not available, but the numbers of T cells generated were compared with those of unprimed healthy donors. Mobilized PBMC containing high numbers of $CD34^+$ cells did not generate more T cells than unprimed PBMC in healthy donors, thus confirming the pattern observed in HIV-infected patients.

The percentage of $CD34^+$ cells in PBMC preparations increased from days 0 to 4, and since the number of PBMC placed on each thymic lobe was unchanged, the number of $CD34^+$ cells placed on each lobe was higher after G-CSF mobilization.

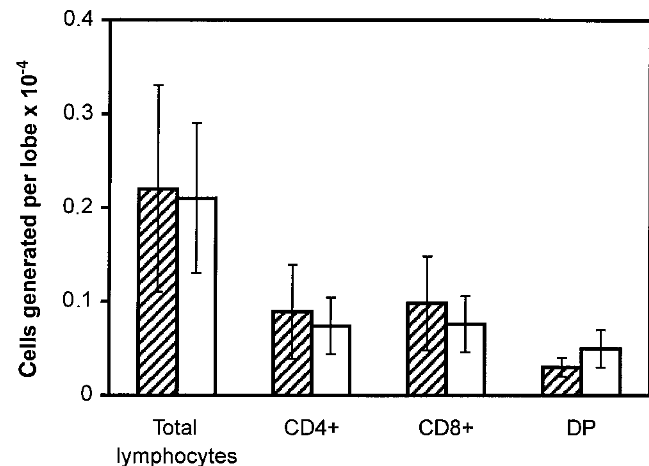

Figure 3. Absolute number of lymphocytes generated per fetal thymus lobe before granulocyte colony–stimulating factor (G-CSF) treatment (hatched bars) and after 4 days of G-CSF mobilization (open bars). In each case, 5×10^5 to 1×10^6 total viable PBMC were placed on each lobe. There were no significant differences between numbers of total lymphocytes or $CD4^+$, $CD8^+$, or double-positive (DP) cells generated before and after G-CSF mobilization. Data are mean ± SE.

The number of lymphocytes developed per CD34$^+$ cell in thymic organ culture was determined (figure 4). Total human cells generated per CD34$^+$ cell on day 0 was 0.72 ± 0.31 and on day 4 was 0.09 cells ± 0.04 ($P < .003$). Furthermore, the numbers of CD4$^+$ and CD8$^+$ cells generated per CD34$^+$ cell were significantly decreased when PBMC obtained after G-CSF treatment was used compared with PBMC obtained before G-CSF treatment ($P < .02$ and <.01 for CD4$^+$ and CD8$^+$ cells, respectively). Finally, the numbers of double-positive cells per CD34$^+$ cell that developed in organ culture was also lower with PBMC obtained after G-CSF treatment ($P < .02$).

Discussion

In this study, we demonstrated mobilization of CD34$^+$ progenitor cells with G-CSF in HIV-infected patients. However, G-CSF mainly increased the absolute numbers of mature progenitors capable of producing myelomonocytic cells. There was a relative decrease in the T cell progenitor population, as determined by flow cytometry. This observation was confirmed when progenitors were grown in thymic organ cultures. Importantly, G-CSF did not change the absolute number of T cell progenitors.

Efforts are under way to develop gene therapy for the treatment of AIDS. To establish protection in both myeloid and lymphoid lineages, it has been suggested that therapeutic anti-HIV genes be transduced into hematopoietic progenitor cells. The capacity of hematopoietic stem cells for self-renewal may offer the potential for a lifelong supply of cells carrying a therapeutic gene. Furthermore, because of the multiple rounds of cell division that occur during hematopoiesis, stable introduction of a gene into even a small number of progenitor cells may result in a significant expansion of cells derived from transduced progenitors. It is conceivable that monocytes/macrophages or T cells protected from the cytopathicity associated with HIV infection may have a selective advantage over unprotected cells, and mature protected cells may therefore accumulate in the circulation [35].

There are, however, several obstacles to overcome before stem cell gene therapy for AIDS can be realized. At present, much work is being done to develop methods for high levels of gene transfer efficiency and lasting expression of the transduced genes [10, 17, 36]. Of importance, HIV diminishes the capacity of progenitor cells to generate new cells of myeloid and lymphocyte lineages [9, 18, 22, 23, 26, 34, 37–41]. The mechanisms behind these deficiencies remain unclear, and several non–mutually exclusive hypotheses may be proposed: HIV may directly infect the progenitors; HIV may infect regulatory cells, including T cells, macrophages, and stromal cells, and indirectly result in suppression of hematopoiesis; HIV structural or regulatory proteins may suppress hematopoiesis; and antiretroviral medication may induce hematopoietic dysfunction [9, 23, 26, 37–43]. In addition, this study shows that im-

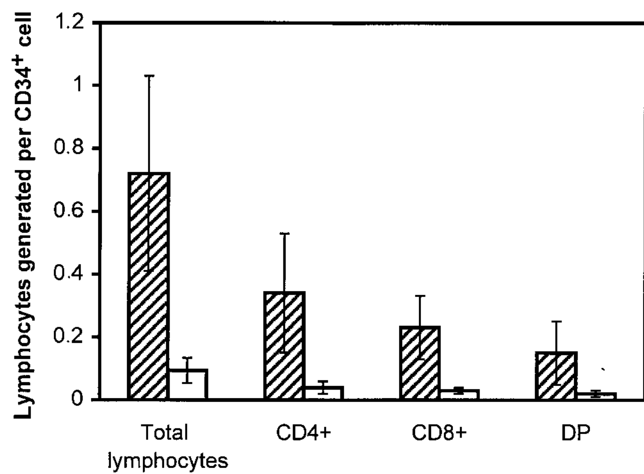

Figure 4. Absolute number of total lymphocytes and CD4$^+$, CD8$^+$, and double-positive (DP) cells generated per CD34$^+$ cell grown in thymic organ culture before granulocyte colony–stimulating factor (G-CSF) treatment (hatched bars) and after 4 days of G-CSF mobilization (open bars). Numbers of lymphocytes generated per CD34$^+$ cell were significantly decreased after G-CSF treatment ($P < .02$ and <.01 for CD4$^+$ and CD8$^+$ cells, respectively). Data are mean ± SE.

mature progenitors (CD34$^+$CD38$^-$ cells) are reduced in HIV-infected persons compared with HIV-negative patients with cancer (2.7% vs. 13.8%, respectively). This finding could reflect increased frequencies of these cells in patients with cancer because of postchemotherapy effects rather than decreased frequencies in HIV-infected persons. However, in a previous study that compared progenitors from HIV-infected subjects with those of healthy controls [38], persons infected with HIV also had significantly fewer CD34$^+$CD38$^-$ cells (1.7% vs. 14%, respectively [38]), suggesting that the use of chemotherapy does not explain the whole difference. Finally, stem cell gene therapy strategies require that thymic stromal elements remain functionally intact to support the maturation of progenitors into mature CD4$^+$ cells. The thymus in adults infected with HIV is generally thought to be inactive, because of both age-related involution and viral destruction (reviewed in [33]). However, a recent study found abundant thymic tissue in many HIV-infected adults, and the presence of thymic tissue was associated with a higher CD4 cell count [44]. Furthermore, the treatment of HIV-infected patients with highly active antiretroviral therapy may normalize the function of both progenitors and the thymus [23, 26, 45] (D.R.C., S.R., N.G. Pakker, et al., unpublished data).

Another obstacle to stem cell gene therapy is the limited number of CD34$^+$ cells available for transduction. The use of G-CSF for mobilization of CD34$^+$ cells prior to transduction has been proposed [6, 8, 12–15]. G-CSF was initially assumed to be lineage-specific; however, G-CSF mobilizes progenitors of several hematopoietic lineages [27, 46–49]. A concern with the use of growth factor–treated progenitor cells is that un-

wanted differentiation may have occurred. The lack of sufficient immature progenitors in the graft may, in turn, result in decreased marrow-seeding efficiency and a decrease in long-term repopulation efficiency [50]. In this study, we determined the fraction and absolute numbers of immature progenitors. After G-CSF treatment, there were significant decreases in percentages of immature progenitors compared with unprimed blood. The absolute numbers of immature $CD34^+$ cells tended to increase, but the increase in absolute $CD34^+CD38^-$ and $CD34^+$HLA-DR^- cells was not significant. In contrast, dramatic increases were found in the mature progenitor subsets. Thus, G-CSF mainly mobilized mature progenitors. These results are in accordance with previous studies in HIV-negative patients, which showed that $CD34^+CD38^+$ and $CD34^+$HLA-DR^+ progenitors are responsible for the major release during G-CSF mobilization but that immature $CD34^+$ cells are mobilized as well [27, 46, 49].

Until now, it was unknown whether G-CSF mobilizes T cell progenitors. Progenitor cells expressing $CD34^+CD2^+$ and $CD34^+CD7^+$ are candidates for the T cell progenitor [32, 51]. Previous studies showed that $CD34^+CD7^+$ cells are required for T cell development in thymic organ cultures [25]. The effect of G-CSF on these cell populations was determined. After treatment with G-CSF, there were significant decreases in percentages of T cell progenitors, thus confirming the results of Steen et al. [27]. Furthermore, G-CSF treatment did not significantly change the absolute numbers of T cell progenitors in peripheral blood. The ability of mobilized PBMC to generate T cells in thymic organ cultures was determined. Despite the large increase in the fraction of $CD34^+$ cells in PBMC preparations after G-CSF mobilization, the number of lymphocytes and $CD4^+$ cells generated in thymic organ cultures did not differ significantly when the numbers of PBMC harvested before and after G-CSF treatment were compared. Furthermore, the total lymphocytes and $CD4^+$ cells generated per $CD34^+$ cell were significantly decreased after G-CSF. Thus, G-CSF does not increase the absolute number of circulating T cell progenitors available for subsequent harvest. However, we cannot rule out that G-CSF mobilizes T cell progenitors that move from the circulation equally rapidly.

After G-CSF treatment, peripheral blood contained a lower fraction of T cell progenitors than unprimed blood. However, T cell progenitors were maintained in constant numbers after G-CSF treatment, and both unprimed and G-CSF–treated progenitors could give rise to immature and mature lymphocytes with normal phenotype. Others have found that G-CSF–mobilized progenitors can give rise to mature lymphocytes [2, 7, 20]. However, it is not clear whether this development represents development from T cell progenitors present before G-CSF treatment or from G-CSF–mobilized progenitors. In contrast, G-CSF mobilized myeloid progenitors in large numbers, and inhibition of HIV replication in monocytic cells derived from transduced G-CSF–mobilized $CD34^+$ cells has been demonstrated [6–11]. Thus, G-CSF mobilization of progenitors may be of benefit in gene therapy protocols if the aim is to target multiple hematopoietic lineages; however, benefits seem limited if the primary aim is to target T cells. Non–lineage-specific cytokines such as stem cell factor or flt-3 ligand might be an alternative to increase the number of T cell progenitors available for harvest and subsequent gene marking and should be tested in vivo.

In conclusion, mobilization of progenitors with G-CSF is possible in HIV-infected persons. However, the mobilized progenitors are mainly mature myeloid progenitors and are relatively depleted of immature progenitor cells and T cell progenitors. Furthermore, although G-CSF–treated progenitors were capable of generating immature and mature lymphocytes, the number of total human cells and $CD4^+$ cells generated per $CD34^+$ cell in thymic organ cultures was significantly decreased when G-CSF–mobilized PBMC were compared with unprimed PBMC. These findings indicate that G-CSF mobilization of progenitors may be of limited benefit in gene therapy protocols if the aim is to target T cells.

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