CONCISE COMMUNICATION

Granulocyte Colony-Stimulating Factor Increases CD4+ T Cell Counts of Human Immunodeficiency Virus–Infected Patients Receiving Stable, Highly Active Antiretroviral Therapy: Results from a Randomized, Placebo-Controlled Trial

Hassan Aladdin,¹ Henrik Ullum,¹
Susanne Dam Nielsen,² Christina Espersen,¹
Lars Mathiesen,² Terese L. Katzenstein,¹ Jan Gerstoft,¹
Peter Skinhoj,¹ and Bente Klarlund Pedersen¹

¹Department of Infectious Diseases, Rigshospitalet, and ²Department of Infectious Diseases, Hvidovre Hospital, Copenhagen, Denmark

Thirty human immunodeficiency virus (HIV)–infected patients with CD4+ T cell counts <350 cells/mm³ who had received stable, highly active antiretroviral therapy (HAART) for at least 24 weeks were randomized to receive either placebo or granulocyte colony-stimulating factor (G-CSF; 0.3 mg/mL 3 times a week) for 12 weeks. Blood samples were collected at specified time points. G-CSF treatment enhanced the total lymphocyte count (P = .002) and increased CD3+ (P = .005), CD4+ (P = .03), and CD8+ (P = .004) T cell counts as well as numbers of CD3+CD16−CD56− NK cells (P = .001). The increases in CD4+ and CD8+ cell counts resulted from increases in CD45RO+ memory T cells and cells expressing the CD38 activation marker. Lymphocyte proliferative responses to phytohemagglutinin and Candida antigen decreased, whereas NK cell activity and plasma HIV RNA did not change during G-CSF treatment. After 24 weeks, all immune parameters had returned to baseline values. This study suggests that G-CSF treatment of HIV-infected patients receiving stable HAART increases the concentration of CD4+, CD8+, and NK cells without inducing changes in the virus load.
either 1 or 2 of the following protease inhibitors—saquinavir, ritonavir, indinavir, or nelfinavir. Patients had received HAART for a median of 31 months (range, 20–34). G-CSF (filgrastim; AMGEN [Roche], Copenhagen, Denmark) was administered subcutaneously at a dose of 0.3 mg/mL 3 times a week for 12 weeks. The placebo group was treated with 2 mL of 0.3 mg/mL NaCl solution. All medications other than HAART (e.g., prophylaxis for Pneumocystis carinii pneumonia [PCP]) remained unchanged throughout the study. Blood samples were collected for quantification of virus load and for immune monitoring at enrollment and at weeks 4, 12, and 24. Additional virus load measures were obtained at weeks 2, 6, and 8.

**CD4** cell count and plasma HIV RNA. CD4+ lymphocyte counts were measured in whole blood by flow cytometry (Becton Dickinson, San Jose, CA), and HIV RNA was quantified in plasma by means of a standardized reverse-transcriptase polymerase chain reaction assay (Amplicor HIV-1 Monitor; Roche Diagnostic Systems, Branchburg, NJ). The detection level was 20 copies/mL.

**Flow cytometry.** Whole blood was collected from patients and incubated for 15 min with antibodies. Expression of CD4 and CD8 was determined by phycoerythrin (PE)–Texas Red (ECD)–conjugated anti-CD4 (clone SFC124D11 [Th4]; Immunotech, Miami) and carboxyamine-5 (Cy5)–conjugated anti-CD8 (clone DK25; Dako, Copenhagen). T cell subsets were further characterized by PE-conjugated CD45RA (clone 4KB5; Dako), PE-conjugated CD45RO (clone UC71; Dako), fluorescein isothiocyanate (FITC)–conjugated CD2L (clone DREG56; Immunotech), FITC-conjugated CD38 (clone AT13/5; Dako), or PE-conjugated CD28 (clone L293; Becton Dickinson). NK cells were identified by Cy5-conjugated CD3 (clone UCH1; Dako) in combination with PE-Cy5-conjugated CD56 (clone MY31; Becton Dickinson) and FITC-conjugated CD16 (clone NK15; Becton Dickinson). Labeled cells after incubation were lysed for 10 min with FACS lysing solution (Becton Dickinson) and centrifuged for 5 min at 700 g. The pellet was resuspended in 0.5 mL of 3% PBS and analyzed by flow cytometry (Epics XL-MCL; Coulter, Hialeah, Florida).

Background staining was determined by use of the following negative controls: FITC-PE-Cy5-conjugated IgG1 (clone 679.1-Mc7; Immunotech) and ECD-conjugated IgG1 (clone 679.1-Mc7; Immunotech). Lymphocytes were distinguished from monocytes on the basis of their forward versus right-angle light scatter and were controlled by an FITC-PE–conjugated CD45–CD14 (clone Mc7; Immunotech) and ECD-conjugated IgG1 (clone 679.1Mc7; Immunotech). NK cell assays. NK cytotoxicity was assayed against K562 in a 4-h 1μCr release assay [4]. Briefly, target cells were labeled with 1μCr (NEN, Boston) for 45 min and washed 3 times with cold RPMI 1640. Unstimulated effector BMNC were then added at effector : target ratios of 50 : 1, 25 : 1, 12.5 : 1, and 6.25 : 1. Effector cells stimulated with IL-2 were used at an effector : target ratio of 50 : 1.

Spontaneous release never exceeded 10%, and NK cell activity was calculated as 100 × [experimental release – spontaneous release]/(maximum release – spontaneous release).

**Results**

**Clinical and patient characteristics.** This study was not designed to gather data about adverse events or side effects associated with the use of G-CSF; however, all patients in the group treated with G-CSF reported bone pain and flu-like symptoms. One G-CSF–treated patient developed PCP immediately after stopping treatment with G-CSF. The patient had not been receiving PCP prophylaxis during the study; however, he had discontinued prophylaxis 2 months before study entry because he had a persistent CD4+ cell count >200 cells/mm³. Three patients in the G-CSF group were noncompliant because of side effects and discomfort with injecting themselves and were withdrawn from the study.

**Baseline characteristics.** There were no significant differences between the groups receiving G-CSF and placebo with respect to baseline concentrations of lymphocyte subpopulations, HIV RNA (table 1), neutrophil counts (figure 1), IL-2 production, lymphocyte proliferative responses, or NK cell activity (table 1).

**Neutrophil and lymphocyte concentrations.** G-CSF treatment induced significant increases in concentrations of both neutrophils and lymphocytes. Twelve weeks after discontinuation of G-CSF, neutrophil and lymphocyte counts returned to baseline values (figure 1).

**Plasma HIV RNA concentration.** The concentration of HIV-1 RNA did not differ significantly between treatment groups and did not change in response to G-CSF treatment (table 1).

**Concentration of T cell subpopulations.** Concentrations of CD4+, CD8−, and CD3− T cells decreased in response to treatment with G-CSF (figure 1). The increase in number of CD4+
cells resulted from an increase in the number of memory CD4\(^+\) cells. G-CSF did not induce any significant changes in naive CD4\(^+\) cells or in the expression of CD28 or CD38 on CD4\(^+\) T cells (table 1). The increase in CD8\(^+\) cell numbers resulted from a combination of increases in numbers of memory CD8\(^+\) T cells and activated cells expressing the CD38 marker. Other subsets of CD8\(^+\) T cells exhibited no significant changes. Thus, 12 weeks after treatment, all values were similar to pretreatment findings.

**IL-2 production.** The production of IL-2 in supernatants PHA-stimulated for 24 h decreased at 4 and 12 weeks in response to treatment with G-CSF. After 12 weeks of follow-up, the concentration of IL-2 had returned to baseline values (table 1). However, a considerable although not significant reduction in IL-2 production was also observed during follow-up in the placebo group.

**Proliferative responses.** Changes from baseline levels and between treatment groups were detected by assays of 3- and 7-day in vitro proliferative responses to PHA, IL-2, pokeweed mitogen, and Candida antigen. In the G-CSF group, PHA- and Candida antigen-stimulated cells showed a decreased proliferative capacity at 12 weeks and at 4 and 12 weeks, respectively (table 1).

**NK cell concentrations.** Concentrations of CD3\(^+\)CD16\(^-\)CD56\(^-\) NK cells increased by >50\% in response to G-CSF treatment but had returned to baseline values 12 weeks after cessation of treatment (table 1). However, NK cell activity did not change in response to treatment with G-CSF (table 1).

### Discussion

This study showed that G-CSF administered 3 times weekly for 12 weeks not only has an effect on numbers of neutrophils but also has a marked effect on concentration and function of lymphocyte subsets. We observed increases in concentrations of circulating CD4\(^+\) and CD8\(^+\) T cells, which could be attributed to increases in memory T cells as well as to CD8\(^+\) cells coexpressing the activation marker CD38. When a fixed number of BMNC were stimulated with Candida antigen or PHA, the proliferative responses to them declined in the group treated with G-CSF. The finding of decreased PHA-stimulated lymphocyte proliferation may be partly attributed to the larger number of memory cells present in the cultures [11]. However, because these cells normally contain the largest recall antigen proliferative potential [11], the additional finding of impaired Candida antigen—stimulated proliferation may imply a functional defect in the new memory cells during G-CSF treatment. Although the number of NK cells increased during G-CSF treatment, this was not reflected in an increased NK cell function. Tendencies to decreased NK cell function during G-CSF...
Figure 1. Concentrations (mean ± SD) of neutrophils (A), lymphocytes (B), and CD3+ (C), CD4+ (D), and CD8+ (E) T cells in patients infected with human immunodeficiency virus who received either granulocyte colony-stimulating factor (G-CSF) or placebo 3 times a week for 12 weeks. *P (analysis of variance) is given in upper left-hand corner of each panel; *significantly different from baseline (P < .05).
treatment may indicate that NK cells recruited during G-CSF treatment were functionally defective.

Whether the effect of recombinant G-CSF on lymphocytes results from a central or a peripheral effect is unknown. If G-CSF causes expansion of the lymphocyte pool by inducing proliferation of lymphocytes in the circulation, lymphocytes from patients treated with G-CSF may be close to their proliferative end stage. Thus, when assayed and stimulated in vitro, they may rapidly undergo apoptosis. The finding that 1 patient developed PCP can be explained in theory by a G-CSF–induced anergy.

Hartung et al. [7] found that G-CSF (filgrastim) in vitro partially restored IL-2 production. In the present study, we were not able to confirm this. However, the observed decrease in IL-2 production during G-CSF therapy needs to be interpreted with great caution, because similar changes were observed in the placebo group. The finding of decreased IL-2 production in our study suggests that the effect of G-CSF on lymphocyte function in vivo does not result from a direct effect of G-CSF on the cells. The finding of increased numbers of CD4+ T cells, but decreased lymphocyte function, also has been seen in studies in which IL-2 was given continuously to patients infected with HIV [12]. This is also in accordance with a previous report on anti-inflammatory effects of G-CSF in healthy HIV-negative adults [13]. These findings, together with ours, suggest that additional functional studies including other regimens should be done before leukocyte growth factors are applied in clinical trials.

In conclusion, this study is the first randomized, placebo-controlled study to demonstrate that treatment with G-CSF enhances the CD4+ T cell count in patients infected with HIV. However, the additional findings of decreased proliferative response to some stimulants and decreased and increased in vitro production of IL-2, as well as lack of effect after cessation of treatment, question the likelihood that G-CSF will have clinical benefits in nonneutropenic HIV-infected patients. We suggest that future studies of this patient population evaluate the effect of intermittent administration of G-CSF before large-scale studies with clinical end points are eventually performed. Further studies are also needed to address whether the effects of G-CSF on lymphocyte function described in this report may apply to other patient groups.

References