Highly Active Antiretroviral Therapy Normalizes the Function of Progenitor Cells in Human Immunodeficiency Virus–Infected Patients

Susanne Dam Nielsen, Annette Kjær Ersbøll, Lars Mathiesen, Jens Ole Nielsen, and John-Erik Stig Hansen

CD34 cells from human immunodeficiency virus (HIV)–infected persons have been described to be impaired in function. The effect of highly active antiretroviral treatment (HAART) on the function of CD34 cells in HIV-infected patients was examined. Numbers and function of CD34 cells from 11 HIV-infected patients were determined prior to HAART and after 2, 4, 8, and 12 weeks of therapy. The mean number of colony-forming units (cells) per milliliter (cfu/mL) was 15.0 prior to HAART vs. 109.8 in healthy controls ($P<.001$). During HAART, the number of cfu/mL increased to 100.3 ($P<.001$). This increase in cfu/mL eliminated the differences between HIV-infected patients and controls. Significant increases in numbers of CD34 cells were not detected. Of importance, the cloning efficiency of CD34 cells increased from 1.7% prior to therapy to a peak at 18.7% ($P=.003$). In conclusion, HAART normalized CD34 cell function in HIV-infected patients and thus might allow de novo production of T lymphocytes from progenitor cells.

Human immunodeficiency virus (HIV) infection is characterized by the progressive loss of CD4+ T lymphocytes. In addition to this hallmark deficiency of CD4 cells, deficiencies of other hematopoietic lineages, which can result in anemia, neutropenia, and thrombocytopenia, have been observed in some patients with HIV infection, suggesting a generalized impairment of hematopoiesis. Several mechanisms have been proposed to account for this, including impairment of growth and functionality of CD34+ progenitor cells. CD34 cells isolated from HIV-infected persons are reduced in numbers and impaired in their ability to give rise to colonies [1–7]. Theoretically, HIV could exert suppressive effects on CD34 cells through direct infection of the CD34 cells or through indirect mechanisms. Although direct infection has been suggested, there remains much controversy surrounding the possible infection of progenitor cells [2–4, 6–16]. Another hypothesis is that HIV mediates alteration of the bone marrow stroma that results in dysregulated hematopoietic growth [3, 17, 18]. Finally, HIV may damage CD34 cells by inducing apoptosis or anergy [19].

Previous studies have shown that impaired hematopoiesis caused by HIV is not reversed by nucleoside analogues such as zidovudine or didanosine [17, 20, 21]. In contrast, some drugs (e.g., zidovudine) used to treat HIV infection might lead to bone marrow suppression [22]. Recently, however, new drugs for control of viral replication have been developed: highly active antiretroviral therapy (HAART) or protease inhibitors. HAART efficiently reduces virus load and increases the CD4 cell count in HIV-infected patients [23–25]. The effect of HAART on progenitor cells and hematopoiesis is not known. The present study was designed to examine the effect of HAART on the number and function of CD34 cells and the numbers of other hematopoietic cells. Furthermore, because zidovudine has been described to exert suppressive effects on CD34 cells, we examined whether this was also the case when zidovudine was used in HAART.

Patients, Materials, and Methods

HAART efficiently reduces virus load and increases the CD4 cell count in HIV-infected patients [23–25]. The effect of HAART on progenitor cells and hematopoiesis is not known. The present study was designed to examine the effect of HAART on the number and function of CD34 cells and the numbers of other hematopoietic cells. Furthermore, because zidovudine has been described to exert suppressive effects on CD34 cells, we examined whether this was also the case when zidovudine was used in HAART.
sentially as described [26]. Briefly, 100 μL of blood was incubated with 10 μL of fluorescence-conjugated monoclonal antibodies at room temperature for 15 min. Erythrocytes were lysed with 2 mL of NH₄Cl buffer at room temperature for 10 min, and the samples were washed and resuspended in PBS supplemented with 1% CellFIX (Becton Dickinson Immunocytometry Systems, San Jose, CA). All samples were analyzed using a FACScan (Becton Dickinson) equipped with a 488-nm, argon-ion laser. Data were processed using CELLQuest software (Becton Dickinson).

To determine the percentage of CD34 cells in peripheral blood, the fluorescence of 50,000 cells was measured (CD34 and isotype controls). The fraction of cells expressing CD34 was multiplied by the white blood cell count to determine the absolute number of CD34 cells in peripheral blood. To determine the percentage of CD34 cells coexpressing CD7, CD38, HLA-DR, and Thy-1, we collected 200–2000 cells using a CD34 gate (SSC vs. CD34). Monoclonal antibodies used were isotype control (γ1-fluorescein isothiocyanate (FITC)/γ1-phyceroerythrin (PE), CD34-PE (anti-HPCA-2), 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).

Furthermore, we determined the number of CD4 cells and the number of CD4 cells with naive phenotype, that is, CD4⁺CD45RA⁺CD62L⁺. The fluorescence of 5000 cells was measured. To obtain the absolute number of a lymphocyte population, the fraction of cells in a lymphocyte gate expressing lymphocyte markers was multiplied by the lymphocyte count. Monoclonal antibodies used to determine lymphocytes were isotype control γ1-FITC/γ1-PE/γ1-peridinin chlorophyll (PerCP), CD3-PerCP (Leu-4)/CD4-FTC (Leu-3a)/CD8-PE (Leu2a), and CD4-PerCP (Leu-3a)/CD45RA-FTC (Leu-18)/CD62L (anti-LECAM-1, clone SK11) from Becton Dickinson.

**Table 2.** CD34 cell subset distribution in 11 HIV-infected patients before and during highly active antiretroviral therapy (HAART).

<table>
<thead>
<tr>
<th>Week</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>12</th>
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<tbody>
<tr>
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<td>20</td>
<td>11</td>
<td>11</td>
<td>9</td>
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<tr>
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<td>12</td>
<td>14</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Thy-1⁺</td>
<td>14</td>
<td>16</td>
<td>13</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>CD7⁺</td>
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<td>17</td>
<td>14</td>
<td>31</td>
<td>33</td>
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Absolute numbers of CD34 cells/mL

<table>
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<th>CD3⁺, all subsets</th>
<th>722</th>
<th>1060</th>
<th>1465</th>
<th>1558</th>
<th>1248</th>
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<tbody>
<tr>
<td>CD8⁺</td>
<td>176</td>
<td>131</td>
<td>143</td>
<td>160</td>
<td>90</td>
</tr>
<tr>
<td>HLA-DR⁺</td>
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<td>150</td>
<td>214</td>
<td>225</td>
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</tr>
<tr>
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<td>125</td>
<td>159</td>
<td>201</td>
<td>149</td>
<td>184</td>
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<td>219</td>
<td>207</td>
<td>539</td>
<td>314</td>
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</tbody>
</table>
Impaired function of CD34 cells from HIV-infected patients. In order to determine the number of colony-forming cells in peripheral blood from HIV-infected patients, clonogenic assays were done on methylcellulose medium enriched with stem cell factor. Prior to HAART, the mean number of cfu per $5 \times 10^5$ PBMC was 5.5 (±1.3), and the mean number of cfu/mL of peripheral blood was 15.0 (±4.0) in HIV-infected patients (figure 1A, 1B). For comparison, clonogenic assays were also done using PBMC from 12 healthy blood donors. The mean numbers of cfu per $5 \times 10^5$ PBMC and cfu/mL peripheral blood were 26.5 (±4.5) and 109.8 (±13.9) in healthy donors and thus significantly higher than in HIV-infected persons ($P < .002$ and $P < .001$, respectively; figure 1A, 1B). The cloning efficiency of peripheral blood CD34 cells was determined (figure 1C). In HIV-infected patients, the mean cloning efficiency was 1.7% (±0.6%) prior to HAART. In contrast, the cloning efficiency of CD34 cells from donors was 18.2% (±9.8%) and thus significantly higher than in HIV-infected patients ($P < .01$).

HAART normalizes the function of CD34 cells from HIV-infected patients. During HAART, the mean numbers of cfu per $5 \times 10^5$ PBMC and cfu/mL of peripheral blood in HIV-infected patients gradually increased (figure 1A, 1B). Thus, 4 weeks after the start of treatment, a significant increase was seen in the numbers of cfu per $5 \times 10^5$ PBMC and cfu/mL ($P < .02$). The mean number of cfu per $5 \times 10^5$ PBMC increased from 5.5 (±1.3) prior to therapy to a peak value of 25.5 (±4.9) after 12 weeks ($P = .0002$), and the mean cfu/mL increased from 15.0 (±4.0) to 100.3 (±20.6) after 12 weeks ($P < .0001$). The increases in the numbers of cfu per $5 \times 10^5$ PBMC and cfu/mL eliminated the differences between HIV-infected patients and healthy donors (figure 1A, 1B).

Of importance, HAART normalized the cloning efficiency of CD34 cells from HIV-infected patients (figure 1C). Thus, the cloning efficiency of CD34 cells increased from 1.7% (±0.6%) prior to therapy to a peak of 18.7% (±9.0%) after 8 weeks of HAART ($P = .003$). This increase eliminated the difference that was found between HIV-infected patients and donors prior to therapy (figure 1C). Of interest, a small decrease in cloning efficiency was detected between week 8 (18.7% ± 9.0%) and week 12 (12.4% ± 4.1%) of HAART. Because zidovudine has been reported to have suppressive effects upon CD34 cells, we examined whether there was a different development in cloning efficiency between the 6 patients that received HAART without zidovudine and the 5 patients that had zidovudine included in their HAART (figure 2). The mean increase in cloning efficiency from baseline to week 12 of HAART was 10.7% (±4.0%). In the patients who did not receive zidovudine, the mean increase was 15.7% (±6.6%), while the cloning efficiency in patients who received zidovudine increased only 4.6% (±1.9%). Due to the low number of patients, this difference was not significant ($P = .17$). However, these results suggest that the use of zidovudine might explain the decrease in cloning efficiency seen after week 8 of HAART.

**Figure 1.** No. of colony-forming units (cfu) was determined in 11 HIV-infected patients during initial 12 weeks of highly active antiretroviral therapy and for comparison in 12 healthy blood donors. A. Nos. of cfu/$5 \times 10^5$ peripheral blood mononuclear cells (PBMC) prior to therapy (week 0) and at weeks 2, 4, 8, and 12. B. Calculated nos. of cfu/mL peripheral blood. C. Cloning efficiency of circulating CD34 cells (no. of [cfu/mL]/no. of CD34 cells/mL) × 100. All results are means (±SE).
The effect of HAART on the distribution of peripheral blood progenitor subsets. To further characterize circulating CD34 cells during HAART, the CD34 cell subset distribution was determined using flow cytometry. The fraction of CD34 cells that did not coexpress the maturity markers CD38 and HLA-DR was determined. Furthermore, we analyzed CD34 cells for coexpression of Thy-1, which is a marker of immaturity, and for coexpression of CD7, which characterizes T cell progenitors. Data on the subset distribution are presented in table 2. There was some variation from week to week and from patient to patient, and no significant differences were found regarding percentages of CD34 coexpressing HLA-DR, Thy-1, or CD7. In contrast, there was a significant decrease in the percentage of CD34 cells that did not coexpress CD38 from week 0 to week 12 (P < .01). Thus, we found a relative decrease in the population of immature CD34 cells. However, due to a minor increase in the absolute number of CD34 cells, there was no significant decrease in absolute numbers of immature CD34 cells (table 2).

The effect of HAART on virus load and CD4 cell count. HAART had a dramatic effect on virus load. The mean copy number was 195.3 × 10^3/mL (±120.1) prior to HAART, and a significant reduction in virus load was detected after 2 weeks of therapy (2.4 × 10^3 copies/mL ± 1.3, P < .001). After 12 weeks of therapy, the mean copy number was 1.2 × 10^3/mL (±1.1), and in 7 of the patients, virus load was <200 copies/mL.

The mean CD4 cell count increased significantly during HAART (figure 3). At week 0, the mean CD4 cell count was 148/μL (±33), and after 4 weeks of therapy, it was 213/μL (±40) (P < .0004). The CD4 cell count peaked at 241/μL (±27) after 12 weeks of therapy (P < .0001). To determine the number of naive CD4 cells, we analyzed CD4 cells for the coexpression of CD45RA and CD62L using flow cytometry. The fraction of naive CD4 cells was 20% (±5%) prior to HAART, and no significant changes in percentage of naive CD4 cells were detected during 12 weeks of therapy. Due to the increase in CD4 cell count, there was an increase in the mean number of naive cells from 30/μL (±12) at week 0 to 53/μL (±12) after 12 weeks of therapy (P < .0001) (figure 3). However, although increased numbers of naive CD4 cells were detected, the HIV-infected patients still had significantly fewer naive cells than did healthy donors (53 naive CD4 cells/μL in HIV-infected patients after 12 weeks of HAART vs. 304/μL in donors, P < .0001). Previous studies have shown that CD4 cells exported from the thymus express the surface antigen CD45RA. We therefore examined whether there was a correlation between functionality of CD34 cells and the number of naive CD4 cells. Of interest, a significant correlation was found between the cloning efficiency of CD34 cells and the number of naive CD4 cells (r = .66, P < .03).

The effect of HAART on white blood cell counts. There were few changes in white blood cell counts during HAART. Thus, there were no significant changes in numbers of granulocytes and monocytes. Furthermore, the numbers of granulocytes and monocytes in HIV-infected patients did not differ significantly from those of healthy donors. An increase in the mean number of lymphocytes from 91.11 × 10^3/L (±21) at week 0 to 1.46 × 10^3/L (±15) at week 12 was detected.
(P = .002). The mean lymphocyte count in donors was 1.65 × 10^9/L (± 0.15), which was significantly higher than the lymphocyte count in patients at week 0 (P < .01). This difference, however, was eliminated after 4 weeks of HAART. During the study period, there were no changes in the CD8 cell count due to HAART (figure 3). Furthermore, the CD8 cell count in patients did not differ significantly from that in donors (466 CD8 cells/μL in HIV-infected patients vs. 453 CD8 cells/μL in donors).

Discussion

We demonstrate that CD34 cells from HIV-infected patients are functionally impaired, thus confirming results obtained by others. During 12 weeks of HAART, there was an increase in the number of CD34 cells, and the functional impairment of CD34 cells was fully reversed. Several studies have shown that when clonogenic assays are performed with bone marrow or peripheral blood samples from HIV-infected patients, the number of colonies formed are reduced compared with those of controls, suggesting a proliferative defect of committed CD34 cells [1–7]. Some studies, however, have found that the number of colonies formed with samples from HIV-infected patients is indistinguishable from that formed with control samples [10, 11]. However, most patients with severely reduced numbers of CD4 cells have greatly reduced numbers and growth of CD34 cells, and it has been suggested that reduced cloning efficiency is related to disease progression [5–7, 12]. In the present study, patients had a mean CD4 cell count of 148, and the demonstrated impairment of CD34 cell functionality confirms previous observations.

Several mechanisms have been proposed to account for the impaired function of CD34 cells from HIV-infected patients. Direct infection of CD34 cells by HIV has been suggested [6, 8, 10, 13]. However, much controversy surrounding the possible HIV infection of CD34 cells remains, and numerous studies have shown that the majority of CD34 cells are not infected by HIV [2–4, 7, 9, 11, 12, 14, 15]. Although CD34 cells in vivo are not infected by HIV, simple exposure of CD34 cells to HIV and HIV proteins has been shown to induce apoptosis or to decrease CD34 cell function [2, 16, 19]. Other findings indicate that the decrease in hematopoietic cell production following HIV infection in vivo might result from effects on the bone marrow stroma [3, 18, 27]. Furthermore, indirect mechanisms of viral suppression of hematopoiesis as a result of cytokine (e.g., tumor necrosis factor-α) induction by HIV might exist [28]. In the present study, we demonstrated that HAART not only has an effect on the CD4 cell count, but HAART also normalizes the function of CD34 cells. Theoretically, HAART could exert this effect by reducing virus load or viral proteins or by indirect mechanisms due to normalization of bone marrow stroma or cytokine production. At present, we have no evidence to support any of these mechanisms, and further studies to clarify this should be done.

Zidovudine is a commonly used drug in the treatment of HIV infection. However, zidovudine has suppressive effects on hematopoiesis [22]. Studies have shown that although zidovudine can reduce viral replication in vitro, the addition of zidovudine to in vitro cultures does not normalize CD34 cell function [17, 20, 21]. In contrast, the addition of zidovudine to HIV-infected cultures has been reported to mediate synergistic reduction in CD34 cell function [17]. Data presented in this study suggest that the use of HAART with zidovudine has a negative impact on the CD34 cell function. However, zidovudine is an efficient drug, and so far the impact of an impaired CD34 cell function in HIV-infected patients has not been determined. It could be argued that although the function of CD34 cells was reduced, patients in the present study did not have leukopenias. Furthermore, when CD34 cell function was normalized, there was no increase in numbers of granulocytes or monocytes. However, circulating progenitors might be affected earlier than those of the bone marrow due to the high lymphocyte–to–stem cell ratio in peripheral blood. Thus, impairment of CD34 cell function might represent the first lesion created by HIV on progenitor cells [1].

Gene therapy has been suggested as a possible treatment for patients with AIDS [29–31]. In theory, stem cell gene therapy offers several advantages. The cardinal features of hematopoietic stem cells, self-renewal and pluripotency, represent distinct advantages of this approach. In light of the extensive proliferation of progeny from stem cells and rapid turnover of CD4 cells in HIV-infected persons, transduction of even a small fraction of stem cells with a protective gene might lead to expansion of a significant population of cells resistant to HIV [32]. However, challenges remain to reconstituting immunity in HIV-infected persons by stem cell gene therapy, among them the impairment of CD34 cell function. Furthermore, only primitive CD34 cells that lack CD38 or HLA-DR can give rise to each of the hematopoietic cell lineages [33, 34], making these cells the ideal target cell for gene therapy. Alternatively, T cell progenitors, that is, CD34 cells coexpressing CD7, could be used as target cells in gene therapy [35, 36]. During HAART, there were no changes in absolute numbers of primitive CD34 cells or T cell progenitors. Since the function of mature CD34 cells increased during HAART, we would expect that the function of primitive CD34 cells and T cell progenitors increased as well. Thus, if thymic activity was present in the patients, CD4 cells should be generated from CD34 cells. Unfortunately, thymopoiesis is impaired in HIV-infected persons [20, 37]. However, it was recently demonstrated that following HAART, new thymopoiesis was initiated in HIV-infected thymic implants [38]. Thus, HAART normalizes both CD34 cell function and thymopoiesis. These findings could have positive implications for the concept of stem cell gene therapy.

Expression of CD45RA and CD62L were used to charac-
terize naïve CD4 cells [39, 40]. HIV infection is characterized by a progressive loss of CD4 cells. Along with a decline in CD4 cells, absolute numbers of naïve CD4 cells decline as well [23]. A recent study of the effects of HAART demonstrated that following HAART, the number of naïve CD4 cells increased slowly and, in some patients, not at all [23]. In the present study, a minor increase in the mean number of naïve CD4 cells was detected. Since HAART does restore the CD34 cell function and thymopoiesis, the small increase in absolute number of naïve CD4 cells might be due to thymopoiesis.

In conclusion, we have demonstrated that HIV-infected patients have reduced numbers of cfu/mL and impaired function of CD34 cells compared with healthy controls. However, HAART normalizes CD34 cell function in HIV-infected patients. Furthermore, a small increase in naïve CD4 cells was detected. These findings suggest that antiviral therapy might allow de novo production of T lymphocytes from progenitor cells. These fully functional progenitor cells with the potential for generating T cells may be the ideal target cells for gene therapy against AIDS.

Acknowledgments

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References