CD34\(^+\) Progenitor Cells From Asymptomatic Patients Are Not a Major Reservoir for Human Immunodeficiency Virus-1

By Thomas F. Neal, H. Kent Holland, Charles M. Baum, Francois Villinger, Aftab A. Ansari, Rein Saral, John R. Wingard, and William H. Fleming

Controversy exists as to whether hematopoietic progenitor cells are infected by human immunodeficiency virus-1 (HIV-1) in vivo. Most studies have focused on patients with acquired immunodeficiency syndrome (AIDS)/AIDS-related complex, and little data are available on asymptomatic patients with well preserved CD4\(^+\) T-cell counts. To determine if CD34\(^+\) hematopoietic progenitor cells are infected early in the course of HIV-1 disease, we evaluated 10 asymptomatic HIV-1 seropositive (HIV-1\(^+)\) patients. The CD34\(^+\) cell fraction was purified by a two-step procedure consisting of both affinity chromatography and fluorescence-activated cell sorting that resulted in a median purity of over 99%. Using conventional and nested polymerase chain reaction (PCR) assays, we evaluated the presence and frequency of HIV-1 proviral DNA. Both bone marrow mononuclear cells and CD34\(^+\) cells from all 10 patients were strongly positive for the HIV-1 pol and/or gag gene sequences. In contrast, sorted CD34\(^-\) cells from only two of 10 patients were positive, and the number of copies of proviral DNA in these samples was estimated to be from 2 to 5 per 250,000 cells. To test the in vitro functional capacity of CD34\(^+\) progenitors, these cells were assayed in both methylcellulose and long-term stromal culture. We found no significant reduction in the number of colony-forming unit-erythroid (CFU-E), burst-forming unit-erythroid (BFU-E), or colony-forming unit-granulocyte/macrophage (CFU-GM) colonies, or in the frequency of cobblestone area forming cells from limit dilution analysis in HIV-1\(^+\) asymptomatic patients. Pooled methylcellulose colonies generated from CD34\(^+\) cells were HIV-1\(^-\) in nine of 10 samples. All progeny from long-term cultures of CD34\(^+\) cells were HIV-1\(^-\). We conclude that the CD34\(^+\) hematopoietic progenitor compartment is not infected in the majority of asymptomatic HIV-1\(^+\) patients, and that these cells may represent a suitable target for strategies designed to protect developing CD4\(^+\) T cells from infection.

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PERIPHERAL BLOOD cytopenias and dysmyelopoiesis are well-described, but poorly understood, phenomena in most patients with advanced human immunodeficiency virus-1 (HIV-1) infection.\(^2\)\(^1\) The etiology of hematopoietic dysfunction is often multifactorial and may be related to HIV-1 infection, antiretroviral medication, antimicrobial therapy, concurrent viral, fungal, or Mycobacterial infection, and bone marrow (BM) infiltration from lymphoma.\(^3\) The precise role of HIV-1 in these hematologic abnormalities has never been clearly elucidated, but several mechanisms have been proposed including: (1) infection of primitive hematopoietic progenitors\(^4\); (2) infection of BM stromal cells leading to dysregulation of hematopoiesis\(^5\); (3) HIV-1 structural/regulatory proteins that suppress hematopoiesis\(^6\);\(^7\);\(^8\);\(^9\);\(^10\); and (4) T-cell–mediated suppression of normal hematopoiesis.\(^11\);\(^12\)

When CD34\(^+\) progenitors from HIV-1\(^+\) patients are evaluated by polymerase chain reaction (PCR), most\(^13\);\(^14\);\(^15\) but not all\(^1\) investigators have shown low levels of infection. The patients examined in these studies have been heterogeneous with many having symptomatic HIV-1 infection and variable numbers of CD4\(^+\) T cells. Because of the inherent difficulties in obtaining a highly purified population of CD34\(^+\) cells, the positive PCR results have frequently been attributed to contamination from HIV-1–infected CD34\(^-\) cells, such as CD4\(^+\) T cells or monocytes. Further complicating this issue is the variation in the sensitivity of the PCR assays in these studies, which ranged from 1:1000 to 1:100,000. In vitro incubation of enriched CD34\(^+\) progenitors from normal volunteers with HIV-1 has been shown to lead to productive infection in long-term liquid cultures.\(^16\);\(^17\) Using this system, reverse transcriptase and p24 antigen have consistently been detected after 2 to 4 weeks in culture. However, it remains unclear if HIV-1 infects CD34\(^+\) cells directly or if a contaminating cell population secondarily infects the progeny of the CD34\(^+\) cells. Recent studies have shown the presence of low levels of a functional CD4 receptor on subpopulations of CD34\(^+\) cells raising the possibility that these cells may be targets for HIV gp120.\(^18\)

To determine if CD34\(^+\) cells harbor proviral DNA early in the course of HIV-1 infection, we studied 10 asymptomatic HIV-1\(^+\) patients with no history of an acquired immunodeficiency syndrome (AIDS) defining illness. We used a two-step purification procedure consisting of a CD34\(^+\) immunoaffinity column followed by fluorescence-activated cell sorting to identify a highly purified population of CD34\(^+\) cells. These purified cells were then examined for the presence of HIV-1 proviral DNA by: (1) direct polymerase chain reaction (PCR) analysis; (2) PCR analysis of clonogenic methylcellulose colonies; and (3) PCR analysis of surviving nonadherent cells in long-term stromal culture. Single-round PCR amplification allowed for the quantitation of 10,000 to less than 10 HIV-1 proviral DNA copies per 250,000 cells, and the nested PCR was able to consistently detect a single copy of the HIV-1 genome in a population of 250,000 cells. We report here that low copy numbers of HIV-1 proviral DNA were detected by direct PCR analysis in the CD34\(^+\) cells from only two of 10 asymptomatic seropositive patients. The functional capacity of these CD34\(^+\) cells, as measured by methylcellulose assay and long-term stromal culture limit-dilution, was within normal limits in all cases.

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MATERIALS AND METHODS

BM samples. After obtaining Institutional Review Board (IRB) approval from Emory University Hospital, informed consent was obtained from each patient and posterior iliac crest BM samples (10 to 40 mL) were aspirated into a heparinized 10-mL syringe. We studied 10 asymptomatic HIV-1+ volunteers at the Infectious Disease Clinic at Ponce de Leon Center; Grady Health Care System, Atlanta, Georgia. Bone marrow samples (20 to 30 mL) from seven HIV-1+ normal allogeneic bone marrow transplant donors were provided by the Emory University Bone Marrow Transplant Program.

CD34+ cell purification. BM mononuclear cells (BMMCs) were isolated by density gradient separation using ficoll-hypaque (Histopaque-1077, Sigma Chemical Co, St Louis, MO). Interphase BMMCs were removed and washed twice with 1% bovine serum albumin and incubated for 25 minutes at room temperature with a biotinylated murine antihuman CD34 monoclonal antibody (12.8; CellPro, Bothel, WA). After incubation, the cells were placed onto a continuous flow Ceptrate LC34 column (CellPro) containing avidin-coated beads. The CD34- cells in the effluent were collected, and after mechanical agitation of the column, the CD34+ cells were similarly collected. The cells were centrifuged for 5 minutes at 1,500 RPM and resuspended in RPMI/IMDM/10% (250 mL) Iscove's modified Dulbecco's medium; 250 mL of RPMI-1640; 50 mL of 10% fetal calf serum; 5 mL of 1 mmol/L sodium pyruvate; 5 mL of 2 mmol/L L-glutamine; 5 mL of 100 U/mL penicillin 0.1 mg/mL streptomycin; and 500 mL of 5 x 10^-3 mol/L 2-mercaptoethanol). The highly enriched CD34+ cells and CD34- cells were incubated for 30 minutes with anti-CD34-PE (Becton Dickinson Immunocytometry Systems, San Jose, CA) and further purified with a Becton Dickinson Vantage Cell Sorter. Nonviable cells were excluded using propidium iodide (<5% of each sample).

Hematopoietic progenitor assays. BMMCs were cultured at concentrations of 100,000 and 300,000 cells per mL and the CD34+ cells (postcolumn and postsort) were cultured at concentrations of 3,000 and 10,000 cells per mL. The BMMCs and CD34+ cells were incubated in 1 mL of Iscove's Methylcellulose Ready-Mix with erythropoietin and DNA template of 2.5 x 10^5 cells per sample. The reactions were then overlaid with three drops of mineral oil and subjected to 35 cycles of denaturation (94°C, 45 seconds), annealing (55°C, 60 seconds), and elongation (72°C, 60 seconds) with a final incubation at 72°C for 7 minutes to complete partial copies (Perkin Elmer 480 thermocycler, Norwalk, CT). For nested pol amplifications, 5 mL of the product of the first round of amplification were reamplified using the JA18/19 primer pairs.

The amplified fragments were electrophoresed through 1.6% agarose gels and transferred to Nylon membranes (Hybond-N, Amersham, Arlington Heights, IL). After ultraviolet (UV) cross-linking of the DNA to the membranes, blots were prehybridized with BLOTTO for 2 hours at 45°C and hybridized with their respective digoxigenin-labeled internal oligoprobe (Genius 6; Boehringer Mannheim Corp, Indianapolis, IN) for a minimum of 5 hours. The blots were then washed twice in 2x SSC at 45°C for 15 minutes before being developed using an antidigoxigenin-alkaline phosphatase conjugate and Lumiphos (Boehringer) followed by exposure to Kodak XAR2 film (Eastman-Kodak, Rochester, NY). The sensitivity of each PCR assay was verified by detection of a positive signal in the 1 to 2 copy range (10^-3 to 10^-5 copies) using a quantitative curve derived from the amplification products of the 8ES/5LV dilution. A quantitative curve was derived from the amplification products of the 8ES/5LV dilutions using a densitometer. The number of copies present in each experiment sample was then evaluated by comparison to a standard curve generated for each PCR reaction.

Primers. SK38 primer, ATATATCCACCTATCCGATTAAGAGAAT; SK39 primer, TTTTGGTCCTGCTTAATGCAGAC; SK19 probe, ATTTCTGATTAATAATTTGATAGTAATGATACTGAC; JA17 primer, TCCCCCTTITTATTTGATGG; JA18 primer, CCTGGCTTIAATTITTTTCTGG; JA18 primer, GGAAACAAAATGATAGG; JA19 primer, ATTATTTGATACAGGTTAGG; JA21 primer, ACTCATAGAATCTGGCCACATAA; PC03 primer, ACAACACTGTTGTTCATAGC; PC04 primer, CAACACTCATCAGCTCAGCC.

RESULTS

Clinical characteristics of HIV-1+ patients. The HIV-1+ patients enrolled in this study were asymptomatic with no history of an opportunistic infection or any other AIDS defining illness. All patients were receiving antiretroviral therapy. Eight patients were male and two patients were female. The age ranged from 21 to 49 with a median age of 34 years. The median CD4+ T-cell count was 323 cells/mm^3 (range, 124 to 483), the median white blood cell (WBC) count was 4.3 x 10^3/mm^3, the median platelet count was 201 x 10^9/
CD34 cells were analyzed of sorted CD34- (B1 and CD34' (Cl cells is shown. The percent purity of each fraction is indicated.

Table 1. HIV Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex/Age</th>
<th>WBC (x10^3/mm³)</th>
<th>Platelet (x10^3/mm³)</th>
<th>HB (g/dL)</th>
<th>CD4 (cells/mm³)</th>
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<td>1</td>
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<td>223</td>
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<td>483</td>
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<td>M/30</td>
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<td>14.8</td>
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<td>10</td>
<td>M/38</td>
<td>5.1</td>
<td>238</td>
<td>14.3</td>
<td>279</td>
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</tbody>
</table>

All patients were asymptomatic, on antiretroviral therapy, and had no history of any AIDS-defining illness.

mm³, and the median hemoglobin concentration was 14.3 g/dL. All hematologic parameters and patient demographics are listed in Table 1.

**CD34+ cell purification.** The number of BMMCs separated on the LC34 Ceprate column ranged from 0.3 to 2.0 × 10^6. The number of CD34+ cells collected postcolumn ranged from 0.5 to 3.0 × 10^6. The median purity of the CD34+ cell fraction after the column was 88% (range, 66% to 94%). Further enrichment with the Vantage cell sorter yielded a median purity of 99.8% (range, 95.5% to 99.9%). Viability as assessed by propidium iodide was typically >99%. Reanalysis of the CD34+ cells postcolumn and the sorted CD34+ and CD34- cell fractions is shown in Fig 1.

**Methylcellulose colony assays.** BMMCs from HIV-1+ patients (n = 5) yielded 93 ± 78 CFU-E colonies (per 10^5 cells cultured), 31 ± 25 BFU-E colonies, and 45 ± 23 CFU-GM colonies. CD34+ cells postcolumn yielded 6,005 ± 5,263 CFU-E colonies (per 10^5 cells cultured), 894 ± 614 BFU-E colonies, and 1,555 ± 839 CFU-GM colonies. As shown in Table 2, there was no difference in the median number of colonies generated with BMMCs, and CD34+ cells postcolumn from HIV-1+ patients when compared with uninfected controls. The enrichment in the number of methylcellulose colonies generated with CD34+ cells compared with BMMCs was 64, 28, and 34-fold for CFU-E, BFU-E, and CFU-GM colonies, respectively. CD34+ cells postsort yielded 4,326 ± 2,114 CFU-E colonies (per 10^5 cells cultured), 797 ± 328 BFU-E colonies, and 1,761 ± 849 CFU-GM colonies. There was a trend toward a reduction in the number of colonies generated by the CD34+ cells from the HIV-1+ patients postsort, but this was not statistically significant. Although the number of colonies generated by CD34+ cells from HIV-1+ patients and normal controls were similar in the methylcellulose assay, we did not observe differences in colony morphology. Typically the colonies from HIV-1+ patients were not as large, and the CFU-E and BFU-Es appeared to contain less hemoglobin (data not shown).

**Long-term stromal cultures and limiting dilution analysis.** Figure 2 illustrates the enrichment in growth positive wells in long-term stromal culture when CD34+ cells postsort and BMMCs are analyzed by limiting dilution analysis. Data from HIV-1+ patients and HIV-1- controls showed no significant differences. Using Poisson statistics, the frequency of clonogenic cells derived from CD34+ cells postsort was 1 in 200 compared with 1 in 3,000 in BMMCs. This 15-fold enrichment in the number of growth positive wells in long-term stromal culture limit dilution analysis is comparable to that shown in the normal adult BM CD34+ cells (Fig 2).

**HIV-1 gag and pol detection in CD34+ cells.** BMMCs and CD34+ cells from all 10 patients were strongly PCR+ for the HIV-1 pol and/or gag gene sequences. In contrast, sorted CD34+ cells from eight of 10 patients were PCR- (Table 3 and Fig 3). Sorted CD34+ cells from two patients were initially PCR+. To confirm these results, we repeated the assay on two additional BM aspirates on each of these patients on two separate occasions (ie, each of these patients had a total of three BM aspirates on three different occasions). In both patients, a positive result was obtained in two of three samples. Using an 8E5 cell line serial dilution curve,
(Fig 4) the number of copies of proviral DNA in this population of sorted CD34+ cells was estimated to be from two to five copies per 250,000 cells.

**HIV gag and pol detection in cultured CD34+ cells.** To determine whether relatively mature lineage committed progenitor cells were infected with HIV-1, we examined clonogenic methylcellulose colonies for the presence of HIV-1 proviral DNA. In each of the five patients tested, we pooled CFU-GMs (five colonies) and CFU-E/BJU-Es (10 colonies) from sorted CD34+ cells placed into methylcellulose cultures. The number of cells in the five pooled CFU-GM colonies and 10 pooled CFU-E/BJU-E colonies was approximately 7,500 and 75,000 cells, respectively. All pooled CFU-GMs were PCR-. Only one of five of the CFU-E/BJU-Es was weakly PCR+ at a copy number of less than 2 copies for the entire sample of approximately 75,000 cells. As each of these colonies arose from a single cell, at least 1 copy of the HIV-1 genome should be present in all progeny. An HIV-1 copy number of less than 2 suggests this positive signal is most likely caused by contamination from an HIV-1-infected mononuclear cell or a CD4+ T cell.

CAFcs are primitive hematopoietic cells that are able to grow in culture for several weeks to months. We analyzed nonadherent cells from five pooled growth positive wells (approximately 90,000 cells) from long-term stromal cultures in each of the five HIV-1+ patients tested. The two patients whose CD34+ cells were originally PCR+ in two of three samples were included in this analysis. Pooled colonies from long-term stromal cultures of CD34+ cells were all HIV-1 (five of five), whereas those from CD34- cells were all HIV-1+.

**DISCUSSION**

The results of this study show that hematopoietic progenitors expressing the CD34 antigen are not a significant reservoir of HIV-1 infection in the majority of asymptomatic seropositive patients. In addition, the functional capacity of CD34+ cells from HIV-1+ patients in vitro is comparable with that observed in CD34+ cells isolated from normal uninfected controls. The low proviral copy numbers detected in samples of 250,000 highly enriched CD34+ cells suggests that these cells are either not infected in vivo or the frequency of infection is less than 1 in 50,000 to 1 in 250,000 cells. Because it is not technically feasible to obtain a 100% pure population of sorted cells, the possibility of contamination cannot be ruled out. However, the absence of HIV-1 in the long-term cultures indicates that these early progenitor cells are not infected.

To directly assess hematopoietic progenitors for evidence of HIV-1 infection we used PCR to amplify integrated HIV-1 DNA (ie, proviral DNA) in a variety of cell populations. When we examined highly purified CD34+ cells directly for the presence of HIV-1 proviral DNA, we found that samples from only two of 10 patients were PCR+. Although each of these patients gave positive PCR results in two of three

<table>
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<th>Table 2. Methylcellulose Colony Assay</th>
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<td>HIV</td>
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<td>Controls</td>
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Colony counts are expressed as the mean ± SD of the number of colonies scored per 1 × 10^5 cells cultured.

**Table 3. Detection of HIV-1 pol and gag Gene Sequences**

<table>
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<tr>
<th>Patient No.</th>
<th>CD34-</th>
<th>BMMC</th>
<th>CD34-</th>
<th>BFU-E/CFU-E</th>
<th>CFU-GM</th>
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A total of 2.5 × 10^5 cells were assayed in each PCR reaction with a sensitivity of detecting one HIV-1 proviral DNA in a background of 2.5 × 10^5 cells. The two positive CD34+ assays were each repeated on two separate occasions and were positive in two of three samples.

Abbreviations: ND, not done; +, positive PCR reaction; -, negative PCR reaction.
CD34 CELLS ARE NOT A RESERVOIR FOR HIV

Nonetheless, this approach can provide insight into whether the CD34+ cells that give rise to colonies are infected by HIV-1. When CD34+ cells plated into long-term stromal cultures were examined, we found all colonies tested were PCR+ for HIV-1 proviral DNA. This includes colonies from the two patients whose sorted CD34+ cells were PCR+. In addition, all tissue culture wells in which CD34- cells were incubated were HIV-1+. Although lending credence to the supposition that hematopoietic progenitors are not infected by HIV-1, this result could also be explained by a lytic infection of infected progenitors. However, a recent study has shown that the number of cells surviving in long-term stromal cultures was not reduced in HIV-1+ patients compared with controls.24 When methylcellulose colonies from...
sorted CD34+ cells were analyzed, we found that only one of 10 pooled samples was PCR+. The number of copies of HIV-1 proviral DNA in the one positive pooled colony was less than 2, which is most consistent with contamination by a T cell or monocyte/macrophage cell within the culture system. Although a lytic infection could account for these findings, in vitro data has not provided evidence of a cytopathic effect when CD34+ cells are incubated with HIV-1 up to 40 to 60 days postinfection.16

The finding that HIV-1 is not detected by PCR in the majority of CD34+ hematopoietic progenitors corroborates the reports by other investigators.13-15,25 It is important to note that these studies focused on patients at a more advanced stage of HIV-1 infection and included many patients with an AIDS-defining illness and peripheral blood cytopenias. Additionally, the PCR assays had variable sensitivities ranging from detection of one HIV-1 proviral DNA in a background of 1,000 to 10,000 cells. The current study examined asymptomatic patients and used a PCR assay with a much higher sensitivity, detecting one HIV-1 proviral DNA in a background of 250,000 cells.

Although these data suggest that CD34+ cells are not a reservoir of infection in HIV-1+ patients, there is evidence to suggest that a subset of patients with more advanced infection may harbor HIV-1 in the CD34+ progenitor cell compartment. Stanley et al.16 examined CD34+ progenitors from 22 Americans and 62 Zairian HIV-1+ patients and found that three and 19 patients, respectively, were PCR+ for HIV-1 proviral DNA. The mean CD4 count of the Americans who were PCR+ and PCR- were three and 399 cells/mm3, respectively. Of the Zairian patients tested, all of whom were hospitalized and symptomatic, the mean CD4 count was 99 cells/mm3 in both PCR+ and PCR- patients. The sensitivity of detection was one HIV-1 proviral DNA in a background of 100,000 cells and the criteria for calling a patient PCR+ required a stronger PCR signal than the CD34- cells. Although our findings are consistent with the results described in the PCR+ American patients with well preserved CD4+ T-cell counts, it raises a question as to whether CD34+ cells are infected in a subset of patients with more advanced HIV-1 disease. The higher percentage of PCR+ patients with advanced CD4+ T-cell counts less than 100 cells/mm3 was not found in a report by Von Laer et al.15 They examined CD34+ cells in 14 HIV-1 patients with a median CD4 count of 60 cells/mm3. Only one of 14 patients had a positive HIV-1 PCR signal and the one positive patient had a CD4+ T-cell count of 30 cells/mm3. None of the patients in that study had a CD4+ T-cell count less than 20 cells/mm3. It is important to note that the sensitivity of the PCR assay was not given. Taken together, these results suggest that significant infection of CD34+ cells in American patients does not occur until CD4+ T-cell counts decrease below 10 cells/mm3. Because of the small number of advanced HIV-1+ patients who have been studied, a larger series is needed to confirm these results.

If CD34+ cells are infected in vivo in patients with advanced HIV-1 disease then it would be worthwhile examining CD34+ subsets to determine if a committed progenitor cell or a more primitive stem cell candidate is infected (ie, CD34+Thy-1+ or CD34+CD38+).19,20 It has been previously shown that viral DNA synthesis is defective in resting cells27 and that a cellular cofactor, present in activated cells, is necessary to complete reverse transcription.22 From these findings, one would predict that quiescent stem cells should be resistant to HIV-1 infection. The positive PCR signal seen in some patients with advanced disease may be due to the infection of an early myelomonocytic precursor that still expresses CD34 antigen or a cycling hematopoietic stem cell. If stem cells were infected, then one would expect all of their progeny to contain proviral DNA including granulocytes and CD8 cells for which there is no evidence of infection. These data suggest that if CD34+ cells are infected in advanced disease, the host cell is likely a committed progenitor, perhaps restricted to the CD4+ T-cell pathway of development.

With regard to the functional capacity of CD34+ progenitors in asymptomatic HIV-1+ patients, we found no differences in the number of methylcellulose colonies generated as compared with values obtained in similar samples from normal controls. Essentially, all of the HIV-1 in vitro colony assay data reported in the literature has been done in AIDS/ AIDS-related complex (ARC) individuals with the majority showing a reduction in the number of in vitro colonies.12,15,24 In one report, this diminution in the number of colonies was reversed with T-cell depletion, suggesting a suppressive effect of autologous T cells.12 In another study examining a simian immunodeficiency virus (SIV) rhesus macaque model, the decrease in the number of in vitro colonies was almost completely reversed by the addition of high-dose interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor.20 This correlates well with the in vivo data from HIV-1+ patients with peripheral blood cytopenias, many of whom respond very well to exogenous growth factors. In contrast to the above data, which show a suppression of in vitro colony formation in AIDS/ARC patients, other studies have shown no difference when compared with seronegative controls.26,27 Molina et al.28 found no statistically significant difference in the number of BFU-E and CFU-GM colonies cultured in vitro from AIDS patients (median CD4 count of 21 cells/mm3). Taken together, these data suggest that in vitro methylcellulose colony formation may be suppressed in a subset of HIV-1+ patients.

The second method we used to examine the functional competence of CD34+ progenitors in HIV-1+ patients is the long-term stromal culture assay. Although the methylcellulose system targets lineage committed progenitors, the long-term stromal culture system with limit-dilution analysis quantitates the frequency of more primitive cells able to give rise to sustained hematopoiesis for several weeks to months.29 We observed a 15-fold enrichment in the number of growth-positive wells when CD34+ cells were plated in limiting dilution cultures as compared with bone marrow mononuclear cells. This is similar to the results obtained from our uninfected controls, further attesting to the normal functional capacity of these cells in vitro.

We have shown by direct PCR analysis of CD34+ cells and PCR analysis of their progeny generated in vitro that CD34+ cells are not a major reservoir of HIV-1 infection.
in the asymptomatic seropositive patient. Furthermore, the functional capacity of these cells in vitro is intact as measured by the methylcellulose colony assay and long-term culture limit-dilution analysis. Because CD34+ cells do not appear to be infected in the asymptomatic HIV-1+ patient, it could potentially serve as a useful target cell in gene therapy trials designed to protect developing CD4+ T cells from infection by HIV-1. One such strategy involves the intracellular expression of hammerhead-type ribozymes directed against HIV-1 RNA.35,36 Transduction of T-cell lines with the hairpin ribozyme has been shown to confer resistance to HIV-1 infection by interfering with both early and late events in the HIV-1 replicative cycle. Using a similar strategy, it may be possible to transfec CD34+ cells and protect its progeny from HIV-1 infection. This may be particularly useful in CD34+ BM transplantation in a subset of HIV-1-associated lymphoma patients with well-preserved CD4+ T-cell counts and no other AIDS-defining illness. It has previously been reported that allogeneic BM transplant, in combination with high-dose antiretroviral therapy, markedly decreased the viral burden in an HIV-1 lymphoma patient.37 However, a major concern is that the cellular reservoir of HIV-1 is not completely eradicated and will infect donor-derived cells. A protective mechanism, such as transfection of CD34+ cells with a hairpin ribozyme, would, in theory, prevent donor cells from being subsequently infected by HIV-1. Further studies to assess the feasibility of this approach in primitive hematopoietic progenitor cells are warranted.

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REFERENCES

CD34+ progenitor cells from asymptomatic patients are not a major reservoir for human immunodeficiency virus-1

TF Neal, HK Holland, CM Baum, F Villinger, AA Ansari, R Saral, JR Wingard and WH Fleming