Lack of Evidence for Infection of or Effect on Growth of Hematopoietic Progenitor Cells After In Vivo or In Vitro Exposure to Human Immunodeficiency Virus

By Jean-Michel Molina, David T. Scadden, Mamoru Sakaguchi, Barbara Fuller, Annie Woon, and Jerome E. Groopman

The pathogenesis of the hematologic abnormalities commonly observed in patients with acquired immunodeficiency syndrome (AIDS) is incompletely understood. We report here that in vitro growth of myeloid (CFU-GM) and erythroid (BFU-E) progenitor cells from six patients with AIDS was not significantly different from that of normal human immunodeficiency virus (HIV) seronegative donors: 25.3 ± 5 CFU-GM per 5 × 10⁴ low density marrow cells and 33.5 ± 5 BFU-E were observed in AIDS patients versus 32.7 ± 5 CFU-GM and 42.1 ± 5 BFU-E in controls. Furthermore, no HIV-DNA in individual colonies (CFU-GM and BFU-E) could be detected using the polymerase chain reaction (PCR) technique, although HIV-1 DNA was detected in peripheral blood mononuclear cells from the same patients. Similarly, normal bone marrow cells exposed in vitro to different isolates of HIV or recombinant purified HIV-1 envelope glycoprotein (gp) 120 did not exhibit any difference in growth of CFU-GM or BFU-E as compared with mock exposed bone marrow cells. HIV-1 DNA could not be detected by the PCR technique in individual colonies derived from HIV exposed marrow. This study suggests that committed myeloid and erythroid progenitors from AIDS patients are responsive to hematopoietic growth factors in vitro and do not appear to contain HIV-1 DNA. Also, HIV or its envelope gp did not alter the growth of hematopoietic progenitor cells in vitro. No evidence of HIV infection of progenitor cells could be demonstrated. Impaired hematopoiesis in patients with AIDS may not be related to direct effects of HIV on committed progenitor cells.

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HEMATOLOGIC ABNORMALITIES are commonly observed in patients infected with the human immunodeficiency virus (HIV). In addition to the low CD4 T-cell counts characteristic of patients with acquired immunodeficiency syndrome (AIDS), leukopenia, anemia, thrombocytopenia, and bone marrow changes are frequently observed. The pathophysiology of these abnormalities is incompletely understood, although impaired hematopoiesis is at least a contributory mechanism. It has been previously reported that the growth of hematopoietic progenitor cells may be inhibited in vitro by immunoglobulin (Ig) present in the sera of patients with AIDS, by a soluble 25-Kd glycoprotein (gp) produced by bone marrow cells from AIDS patients, or may be enhanced by T-cell depletion of bone marrow mononuclear cells. Recent reports also suggest, using in situ hybridization, that HIV may infect megakaryocytes or earlier blood cell progenitors in vivo. In addition, purified populations of myeloid progenitor cells have been reported to be susceptible to HIV infection in vitro. To further assess the direct role of HIV in the impairment of hematopoiesis in AIDS, we studied the in vitro growth of hematopoietic progenitor cells from AIDS patients. We assayed for the presence of HIV-DNA in single colonies using the polymerase chain reaction (PCR) amplification technique. Also, we assessed the in vitro growth of normal progenitor cells after in vitro exposure to HIV and again assayed for HIV-DNA by the PCR technique. Our results suggest that progenitor cells from AIDS patients are responsive in vitro to colony-stimulating factors, that their number is not significantly different from that of normal controls, and that they did not appear to contain HIV-DNA. Exposure to HIV or its envelope gp (gp120) did not alter the growth of normal progenitor cells in vitro, and no evidence of HIV infection of these colonies could be found. Impaired hematopoiesis in AIDS may not be related to a direct effect of HIV on bone marrow progenitor cells.

MATERIALS AND METHODS

Patients. Six patients who met the Center for Disease Control's case definition for AIDS were enrolled in this study, as well as four healthy seronegative donors who provided a total of eight normal marrows. Written informed consent was obtained from all participants and the study was approved by the Institutional Review Board of the New England Deaconess Hospital (Boston, MA).

Blood and bone marrow collection. Bone marrow was aspirated from the posterior iliac crest into syringes containing preservative-free heparin (Sigma Chemical, St Louis, MO). The volume of the aspiration was limited to 2 to 3 mL in order to reduce blood contamination. Four normal seronegative donors gave bone marrows on multiple occasions. Peripheral blood was simultaneously obtained from the AIDS patients. Light-density mononuclear cells (MNC) were collected after density centrifugation over Ficoll-Hypaque gradients (Pharmacia, Piscataway, NJ), and then washed twice in Iscove's Modified Dulbecco's medium (IMDM) (GIBCO Laboratories, Grand Island, NY) supplemented with L-glutamine (2 mmol/L), penicillin (250 U/mL), streptomycin (250 μg/mL), and 20% fetal calf serum (FCS) (JR Scientific, Inc, Woodland, CA). Bone marrow MNC were further depleted of adherent cells by overnight incubation on tissue culture dishes (Lux Nunc, Inc, Naperville, IL) pretreated with FCS as described. The nonadherent cells were then gently removed and washed twice in IMDM.

T-cell depletion from bone marrow MNC. Nonadherent light-density bone marrow cells were depleted of T cells by rosetting with 2-amino-ethylisothiouronium bromide (AET) (Sigma) treated sheep red blood cells (SRBC) (Whittaker Bioproducts, Walkersville, MD) as previously described. Briefly, AET-treated SRBC (1% vol/vol)
were mixed with 2 x 10^6 bone marrow cells/mL and incubated for 15 minutes at 37°C. After centrifugation at 200g for 5 minutes at room temperature, the cells were incubated for 60 minutes at 4°C. The nonrosetting T-cell–depleted fraction was then separated by a second Ficoll-Hypaque gradient centrifugation. The efficiency of T-cell depletion was assessed by flow cytometry using the anti-Leu 4 (CD3) monoclonal antibody (MoAb) (Becton Dickinson, Mountain View, CA). Less than 2.6 ± 1% of T-cell–depleted bone marrow MNC were stained with the anti-Leu 4 MoAb after this T-cell depletion procedure as analyzed by flow cytometry (Becton Dickinson, Immunocytochemistry Systems), while 20% ± 5% of non–T-cell-depleted bone marrow MNC were stained with this anti-Leu 4 antibody. Also, 5% ± 1% of T-cell-depleted bone marrow MNC were stained with the anti-Leu 3A (CD4) MoAb.

**Viruses.** HIV-1 IIIB was obtained from R.C. Gallo (National Institutes of Health, Bethesda, MD) and HIV-2 ROD-1 was from L. Montagnier and F. Clavel (Institut Pasteur, Paris, France). Highly concentrated virus stocks were prepared using the method of Vujicic et al. using H9 cells. These virus preparations contained 10 x 10^6 cpm/mL of HIV reverse transcription activity and were highly infectious for H9 cells (at a dilution of virus of 10^5, 50% of H9 cell cultures were infected; referred to as tissue culture infectious dose 50, or TCID50). Mock-infected preparations were also prepared using the same protocol.

HIV-1 Ba-L, which replicates efficiently in fresh macrophages, was obtained from Drs Gartner and Popovic (National Cancer Institute). Virus stocks were made from supernatants of the third passage of this strain on peripheral blood monocytes-macrophages. The HIV-1 Ba-L virus stocks contained 250 ng/mL of HIV-I p24 antigen with an infectivity of 5 x 10^5 TCID 50 for macrophages. Supernatants from uninfected macrophages were used as controls.

Purified recombinant HIV-1 IIIB gp envelope gp 120 (rgp 120) expressed in Chinese hamster ovary cells was a gift of Genentech, Inc (San Francisco, CA). The purified material was provided at a concentration of 1.8 mg/mL.

**Infection of normal bone marrow cells.** Light-density nonadherent bone marrow cells from normal donors were resuspended in IMDM at a final concentration of 4 x 10^6 cells/mL and incubated for 2 hours at 37°C with equal volume of HIV-1 IIIB, HIV-2 ROD, HIV-1 Ba-L virus stock, mock preparations, or with rgp 120 (1 µg/mL final concentration). Five independent experiments were performed with each virus strain and four with rgp 120. T-cell–depleted nonadherent bone marrow cells were also exposed to HIV-1 IIIB according to the same protocol. Three independent experiments were performed. In each experiment, the bone marrow cells were plated for colony assay without further washings.

**Bone marrow colony assays.** Light-density nonadherent bone marrow cells were cultured at a concentration of 5 x 10^4 cells/mL in 1 mL of a semisolid matrix in 35-mm tissue culture dishes (Lux Nunc) as previously described. For erythroid progenitor cultures (BFU-E), the semisolid matrix consisted of a mixture of 0.9% methylocellulose (Genetics Institute, Cambridge, MA) in IMDM with 30% FCS (JR Scientific Inc), 0.9% deionized bovine serum albumin (Sigma; fraction V), and 10^{-4} mol/L L-2-mercaptoethanol (Sigma). For culture of granulocyte-macrophage progenitors (CFU-GM) the semisolid culture consisted of 0.3% Agar (Bacto Agar; Difco, Detroit, MI), 40% IMDM, 20% FCS, and 10^{-4} mol/L a-thio glycerol (Calbiochem Behring, La Jolla, CA). Purified recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF) (Genetics Institute) was added to the agar and methylocellulose cultures at a concentration of 1.8 mmol/L to support colony growth. Similarly, purified recombinant human erythropoietin (EPO) (Genetics Institute) was added to methylocellulose cultures at a final concentration of 2 U/mL. T-cell–depleted light-density nonadherent bone marrow cells were also plated, but at a concentration of only 10^6 cells/mL so that only zero to three colonies could be scored in a single dish with a low background of non–colony-forming cells. The colonies were then individually plucked from either agar or methylcellulose under direct microscopic visualization using microcapillary tips (Marsh Biomedical Products, Rochester, NY) and then processed for PCR analysis. These T-cell–depleted bone marrow cells were also cultured in liquid culture with IMDM supplemented with 20% FCS and rG-CSF (1.8 nmol/L) for 14 days to determine the number of T cells at day 14 by flow cytometry. There were no detectable T cells at that time using staining with the anti-Leu-4 MoAb, whereas 50% ± 5% of these cells were stained with the monocyte marker anti-Leu M3.

**Virus isolation from peripheral blood MNC of AIDS patients.** HIV-1 was isolated from lymphocytes of AIDS patients, using previously described methods. Briefly, peripheral blood MNC from AIDS patients obtained after Ficoll-Hypaque density gradient centrifugation were cocultured in the presence of interleukin-2 (Biogen, Cambridge, MA) and phytohemagglutinin (PHA)-stimulated peripheral blood lymphocytes from seronegative donors. Cultures were monitored by measurement of HIV-I p24 antigen in the supernatant (Abbott Laboratories, North Chicago, IL).

**PCR analysis.** DNA was extracted by a method previously described. Briefly, peripheral blood MNC were suspended at a concentration of 10^{6} cells/mL in a solution containing 100 mmol/L KCl, 10 mmol/L Tris- HCl (pH 8.3), and 2.5 mmol/L MgCl2. An equal volume of a solution containing 10 mmol/L Tris- HCl (pH 8.3), 2.5 mmol/L MgCl2, 1% Tween, and 100 µg/mL of Proteinase K (Boehringer-Mannheim, Indianapolis, IN) was added. CFU-GM and BFU-E (each containing 800 to 2,000 cells/colony) were aspirated by a microcapillary pipette and suspended in 50 µL of each of the above solutions. Samples were digested with Proteinase K for 1 hour at 65°C, followed by a 10-minute inactivation at 95°C.

PCR amplification was conducted by a method previously described. For amplification of HIV sequences, gag-specific primers, SK100 and SK104, were used in a reaction cycle consisting of denaturation at 90°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute repeated 15 times. One tenth of the reaction volume was then removed and used as a template for a subsequent amplification with gag-specific primers, SK38 and SK39; SK38 is located interior to the SK100 primer on the gag region. The second amplification was denatured at 95°C for 30 seconds, annealed for 30 seconds at 65°C, and extended at 72°C for 1 minute repeated 15 times. The product of this reaction was then denatured by boiling and mixed with 30 µL end-labeled SK19 in 100 mm NaCl, 15 mm EDTA, and incubated at 56°C for 30 minutes. The product of the liquid hybridization was electrophoresed through a 10% polyacrylamide gel and autoradiographed for 2 hours and overnight. Amplification of the β-globin gene using primers PC03 and PC04 were independently performed to control for DNA content and quality. The sensitivity of PCR detection of HIV was assayed by dilution of chronically HIV-infected THP-1 cells in uninfected THP-1 cells. THP-1 is a human monocyte cell line (ATCC TIB 202). In addition, the T-lymphocyte cell line, ACH-2 (AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH, Reagent No. 349 from Contributor, Dr Thomas Folks, Centers for Disease Control, Atlanta, GA), which was reported to contain one HIV proviral copy per cellular genome, was used to assess PCR sensitivity. Methylocellulose and soft agar were added to independent titrations of ACH-2 cells to control for the effect of these contaminants on the PCR reaction. The quantities used duplicated those of the bone marrow colony samples by plucking acellular plugs of mock bone marrow cultures and mixing them with the ACH-2 cell dilutions.

**Statistical analysis.** Two-tailed Student's tests were used for statistical comparisons of the number of colonies between AIDS and

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normal patients, or between HIV and mock-exposed bone marrow cells. Data are presented as mean ± SEM.

RESULTS

Clinical characterization of AIDS patients. The clinical and biologic features of the six AIDS patients are reviewed in Table 1. All patients were treated with zidovudine AZT (1,200 mg/d) and monthly prophylactic aerosolized pentamidine (300 mg) at the time of the study. No opportunistic infection was present in any of these patients at the time of the study, but all six had Kaposi’s sarcoma.

Growth of hematopoietic progenitor cells from AIDS patients. To address the ability of hematopoietic progenitor cells from AIDS patients to form myeloid or erythroid colonies in vitro after stimulation with rGM-CSF and rEPO, light-density nonadherent bone marrow cells from six patients with AIDS were grown in semi-solid culture, and the number of granulocyte-macrophage colonies (CFU-GM) and early erythroid colonies (BFU-E) was scored after 14 days in culture. Mean colony counts were 25.3 ± 5 CFU-GM in the agar system and 33.5 ± 5 BFU-E in the methylcellulose culture per 5 × 10⁶ cells plated (Fig 1). These numbers of colonies were about 20% lower than those observed in cultures of eight normal specimens (mean of 32.7 ± 5 CFU-GM and 42.1 ± 5 BFU-E) (Fig 1). However, these differences were not significantly different (P = .2), and the morphology of the colonies was similar in both groups of patients.

Detection of HIV-1 DNA by PCR in CFU-GM or BFU-E from AIDS patients. To determine if bone marrow progenitor cells were infected with HIV, we used the PCR technique to assay HIV DNA. Because in each colony all cells are derived from a single progenitor, a copy of HIV-DNA should be present in all daughter cells if it were present in the progenitor cell. The number of cells present in individual colonies, as determined by counting aspirated colonies resuspended in isotonic buffer, was 0.8 to 2.0 × 10⁵. Therefore, we set the sensitivity of PCR detection of HIV such that 10³ or greater infected cells would be detected, but contaminating monocytes or T lymphocytes would not. The sensitivity of our PCR analysis was determined by dilution of chronically HIV-1 infected THP-1 monocytes with uninfected THP-1 cells. The assay was sensitive to 10⁻⁴ dilution of infected cells corresponding to 100 HIV infected cells in a sample of 10⁶ cells/mL. The assay was repeated using the T-lymphocyte-derived, HIV-infected ACH-2 cell line with and without methylcellulose or soft agar. The sensitivity of HIV DNA detection was unaffected by the presence of these materials used in bone marrow culture. This level of sensitivity assured us that DNA from the bone marrow colonies would be PCR positive if HIV infected, but that small numbers (ie, <10²) of contaminating T cells or macrophages would not result in a misleading positive PCR result. All BFU-E and CFU-GM derived from the five AIDS patients were negative for HIV by PCR (Table 2). PCR for globin DNA was performed on the same DNA samples to control for DNA quality and was positive (data not shown). HIV-DNA was detected in peripheral blood from three of three of these AIDS patients by PCR (Table 2), and virus was recovered from peripheral blood MNC by cocultivation in all cases.

Effect of different HIV strains on the in vitro growth of normal hematopoietic progenitor cells. Normal bone marrow cells were exposed to a lymphotropic strain of HIV-1 (HIV-1 IIIb), a monocytotropic strain of HIV-1 (HIV-1 Ba-L), HIV-2 ROD, or purified recombinant HIV-1 envelope (rgp120) for 2 hours at 37°C and then plated in a semi-solid matrix. The number of colonies was scored 14 days later. As shown in Fig 2, no significant differences in the number or morphology of CFU-GM or BFU-E were observed between HIV or rgp120 exposed cultures or mock exposed cultures.

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (y)</th>
<th>Prior OI</th>
<th>CD4</th>
<th>p24</th>
<th>AZT</th>
<th>IFN</th>
<th>ANC</th>
<th>HGB</th>
<th>PLTS</th>
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<tr>
<td>1</td>
<td>39</td>
<td>−</td>
<td>134</td>
<td></td>
<td>5 wk</td>
<td>2 wk</td>
<td>528</td>
<td>10.9</td>
<td>105</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>+</td>
<td>10</td>
<td></td>
<td>16</td>
<td>10</td>
<td>572</td>
<td>13.9</td>
<td>257</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>+</td>
<td>21</td>
<td>298</td>
<td>16</td>
<td>−</td>
<td>459</td>
<td>14.0</td>
<td>247</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>+</td>
<td>10</td>
<td>45</td>
<td>1</td>
<td>−</td>
<td>900</td>
<td>11.6</td>
<td>258</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>−</td>
<td>125</td>
<td></td>
<td>17</td>
<td>4</td>
<td>705</td>
<td>12.3</td>
<td>97</td>
</tr>
<tr>
<td>6</td>
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<td>20</td>
<td>479</td>
<td>&gt;52</td>
<td>760</td>
<td>11.1</td>
<td>118</td>
<td></td>
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Abbreviations: OI, opportunistic infection; CD4, cells/mm³; p24, serum p24 antigen pg/mL; AZT, length of treatment with AZT; IFN, length of treatment with interferon-α; ANC, absolute neutrophil count cells/mm³; HGB, hemoglobin g/dl; PLTS, platelet count × 10⁹ cells/mm³.
Detection of HIV-1 DNA in hematopoietic progenitor cells in vitro exposed to HIV-1. Colonies derived from the in vitro exposed bone marrow progenitors were evaluated for the presence of HIV-DNA. To avoid T-cell or monocyte contamination when plucking the colonies, light-density nonadherent bone marrow cells were depleted of T cells by rosetting with AET treated SRBC, exposed to HIV-1 IIIB or HIV-1 Ba-L for 2 hours and plated at very low concentrations (10³ cells/mL and per dish). At this concentration there were no more than three colonies per dish at day 14 and almost no extraneous cells in the culture matrix. Individual colonies were then harvested and assayed for HIV by PCR. As shown in Fig 3, no HIV-1 DNA was detected by PCR in

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Bone Marrow BFU-E HIV+/No. Tested</th>
<th>Bone Marrow CFU-GM HIV+/No. Tested</th>
<th>PBMC</th>
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<tr>
<td>1</td>
<td>0/11</td>
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<td>0/14</td>
<td>0/15</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>0/10</td>
<td>0/15</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>0/10</td>
<td>0/15</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>0/14</td>
<td>0/15</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>0/59</td>
<td>0/75</td>
<td>3/3</td>
</tr>
</tbody>
</table>

Abbreviations: BFU-E, burst forming unit-erythroid; CFU-GM, colony-forming unit granulocyte-macrophage; PBMC, peripheral blood mononuclear cells.

Fig 2. Numbers of CFU-GM and BFU-E colonies obtained after in vitro growth of 5 x 10⁴ bone marrow cells from healthy donors after in vitro exposure to HIV-1 IIIB (A), HIV-1 Ba-L (B), HIV-2 ROD (C), or rgp 120 (1 μg/mL) (D). Data as presented are mean ± SEM and are the results of five independent experiments with each strain of virus and of four independent experiments with rgp 120.
Fig 3. Representative PCR analysis of bone marrow colonies. Colonies exposed to HIV-1 IIIB in vitro were harvested, DNA processed, and PCR performed for HIV-1 DNA as described in the text. Lanes 1 through 14 are CFU-GM, lanes 16 through 28 are BFU-E, and lanes 15, 29, and 30 are HIV-1 IIIB infected H9 cells as positive controls. The arrow indicates the expected 87-bp band; lower molecular weight bands are the unhybridized probe.

BFU-E or CFU-GM colonies. Control PCR for the globin gene was readily detectable using the same DNA preparations.

DISCUSSION

The pathophysiology of impaired hematopoiesis in AIDS is incompletely understood. Hematopoietic dysfunction can result from bone marrow involvement by various opportunistic infectious agents, by infiltration of marrow with tumor (disseminated lymphoma), or the toxic effect of therapy. In addition, impairment of progenitor cell growth in AIDS has been suggested by Stella et al., who found that the growth of bone marrow colonies was altered in vitro. This altered growth could be partially corrected by T-cell depletion of the bone marrow cells. Leiderman et al. reported that bone marrow cells from AIDS patients, when cocultivated with bone marrow cells from normal individuals, inhibited CFU-GM growth in vitro and that this was related to the release of a gp inhibitor by AIDS bone marrow MNC. We also previously reported that serum from AIDS patients could inhibit myeloid and erythroid progenitor growth from bone marrow of HIV-infected people but not normal individuals, suggesting an immune mediated inhibition of colony growth in vitro. A component of this immune mediated inhibition appeared to be antibodies in the IgG fraction, possibly directed against the envelope gp 120 of HIV.

Other reports have suggested that the hematopoietic abnormality in AIDS may be related to a direct toxic effect of HIV on bone marrow progenitors. Megakaryocytes or their progenitors may be infected with HIV in vivo as assessed by in situ hybridization, and Folks et al. have reported that bone marrow progenitor cells exposed to HIV in vitro expressed reverse transcriptase after prolonged culture. Our studies were designed to address the issue of HIV infection of progenitor cells and the effect of HIV infection on the colony-forming capacity of progenitor cells. We first assessed the in vitro growth potential of bone marrow cells from six AIDS patients using human recombinant growth factors. Our results show that the number of CFU-GM or BFU-E colonies obtained after a 14-day incubation period was not significantly different between AIDS patients and normal controls (Fig 1). It is noteworthy that all our patients were treated with AZT, an agent known to inhibit the growth of hematopoietic progenitor cells in vitro and to induce granulocytopenia and anemia in vivo. The 20% difference in colony number, though not statistically significant, could represent effects of AZT on marrow progenitors. In vitro, there is a similar degree of inhibition by AZT of BFU-E and CFU-GM growth using bone marrow from HIV infected or healthy individuals.

The ability of bone marrow cells from AIDS patients to respond to CSFs in vitro is in agreement with our previous report in AIDS-related complex and AIDS patients, and with in vivo bone marrow response of AIDS patients to administration of GM-CSF, G-CSF, or EPO. However, these data stand in contrast with other reports where a 53% to 68% reduction in the number of CFU-GM and an 80% reduction in the number of BFU-E were observed in AIDS patients as compared with healthy controls. These discrepant data remain to be explained, but might be related to the population of patients studied and to the treatments they received. All our patients had Kaposi's sarcoma and were treated with AZT, which despite its direct in vitro toxicity on progenitor cells, might, because of its anti-HIV effect, eventually lead to enhancement of the growth of these progenitor cells.

The issue of direct HIV infection of marrow progenitors was addressed by culturing CFU-GM and BFU-E from AIDS patients. We first attempted to detect HIV antigens on individual colonies using indirect immunofluorescence and were unable to do so (data not shown). We also cocultivated individual colonies with H9 cells or PHA-stimulated lymphocytes and could not recover virus. However, these methods are of low sensitivity. Therefore, we used the highly sensitive PCR amplification technique to detect HIV-DNA in single colonies. Because each colony is derived from a single progenitor cell, all cells of the colony would be expected to
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shown in Table 2, no HIV-1 DNA could be recovered from BFU-E or CFU-GM colonies from the six AIDS patients studied, although HIV-1 DNA was detected in three of three samples of peripheral blood MNC by PCR and virus could be isolated from all six patients' peripheral blood by coculture. Therefore, we did not find evidence of in vivo HIV infection of progenitor cells. However, it is possible that the 20% difference in the number of myeloid and erythroid colonies grown from AIDS patients' marrow as compared with controls was due to a specific growth disadvantage of HIV-infected progenitors. If infected cells did not generate colonies they would have been excluded from our PCR analysis. Alternatively, AZT treatment may reduce the infection of colony-forming cells and thereby diminish our ability to detect this event. It is also theoretically possible that infected progenitor cells contain HIV in forms that do not replicate coordinately with the cells, and therefore the bone marrow colonies may have HIV-DNA present in quantities below the sensitivity of our assay. In addition, the number of colonies assayed does not allow us to exclude in vivo bone marrow progenitor infection present at low prevalence. Our data suggest that if such an infection occurs, it occurs with a frequency of less than 1:134, which is consistent with the frequency of HIV infection of peripheral CD4 lymphocytes reported by others.\(^{33}\)

In an effort to overcome these limitations of small size we exposed bone marrow to HIV in vitro using high concentrations of virus to increase the likelihood of infecting susceptible cells. Normal human bone marrow progenitor cells were incubated with high inocula of HIV-1 IIIB, HIV-1 Ba-L, or HIV-2 or with rgp 120 (1 µg/mL). As shown in Fig 2, no significant difference in the number of CFU-GM or BFU-E colonies was observed between HIV-exposed or mock-exposed bone marrow cells. We then assayed for HIV-DNA in the bone marrow colonies using the PCR technique. We incubated T-cell-depleted nonadherent bone marrow cells with HIV-1 at low density so that three colonies at most could be scored per dish. In doing so, we could pluck individual colonies and avoid contaminating cells. The PCR analysis of BFU-E and CFU-GM in three different experiments did not yield any evidence of viral DNA (Table 3 and Fig 3). Therefore, we could not successfully infect myeloid or erythroid human progenitor cells in vitro with HIV using viral isolates with either lymphocyte or monocyte tropism. This observation correlates with the finding by Schnittman et al\(^{19}\) that only the circulating cells harboring HIV-DNA in HIV-infected people were lymphocytes. However, others have reported infection of bone marrow progenitor cells in vitro using a monocyte tropic strain of HIV-1.\(^{17}\) In that study, progenitor cells were selected on the basis of positivity for the surface antigen CD34 (My 10). However, only 18% of CD34 positive cells meet the biologic definition of a progenitor cell by capacity to form colonies in vitro. When CD34-bearing marrow cells were exposed to HIV, the CD34 positive cells produced viral particles in the supernatant only after 30 days of cultivation, a time when the cells were no longer CD34 positive and had undergone monocytic differentiation. It is possible that initially a very small population of CD34 positive nonprogenitor cells was infected, and on differentiation of progenitor cells into macrophages in prolonged culture, the virus was able to propagate and become detectable in the culture. The use of the PCR technique to directly analyze bone marrow colonies avoids these ambiguities.

The issue of HIV infection of human hematopoietic progenitor cells is important in understanding the mechanism of depressed blood cell counts in AIDS. It may also be important in assessing the efficacy of treatment strategies in these patients, and is of particular relevance to bone marrow transplantation, gene insertion therapy, and the clinical use of hematopoietic growth factors. Our studies indicate that hematopoietic progenitor cells from AIDS patients are responsive in vitro to GM-CSF and EPO and do not appear to contain HIV-1 DNA as detected by PCR analysis. Exposure of normal bone marrow cells to concentrated HIV of different viral strains and to recombinant gp120 did not significantly alter colony growth. In addition, no evidence of in vitro infection of BFU-E or CFU-GM by HIV was obtained despite analysis of over 300 colonies. Although our studies do not exclude a low-frequency infectious event and the loss of infected progenitors by HIV cytoxicity, we were unable to demonstrate evidence of in vivo or in vitro infection of hematopoietic progenitor cells with HIV.

Dysregulated hematopoiesis in AIDS might not be related to impairment of the progenitor cell itself, but rather to an alteration of the bone marrow microenvironment. Studies of the effects of HIV on other cellular components of the bone marrow and on the release of regulatory cytokines by these cells may provide insights into mechanisms of impairment of hematopoiesis by this retrovirus.

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REFERENCES


Table 3. PCR Analysis of Bone Marrow Colonies Exposed to HIV In Vitro

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<tr>
<th>Virus</th>
<th>BFU-E</th>
<th>CFU-GM</th>
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<tr>
<td>HIV-1IIIB</td>
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<td>0/98</td>
</tr>
<tr>
<td>HIV-1Ba-L</td>
<td>0/36</td>
<td>0/56</td>
</tr>
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<td>Total</td>
<td>0/161</td>
<td>0/144</td>
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</tbody>
</table>

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