Inhibition of Purified CD34+ Hematopoietic Progenitor Cells by Human Immunodeficiency Virus 1 or gp120 Mediated by Endogenous Transforming Growth Factor β1

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Summary

Human CD34+ hematopoietic progenitor cells, stringently purified from the peripheral blood of 20 normal donors, showed an impaired survival and clonogenic capacity after exposure to either heat-inactivated human immunodeficiency virus (HIV) 1 (strain IIIB) or cross-linked envelope gp120. Cell cycle analysis, performed at different times in serum-free liquid culture, showed an accumulation in G0/G1 in HIV-1- or gp120-treated cells and a progressive increase of cells with subdiploid DNA content, characteristic of apoptosis. In blocking experiments with anti-transforming growth factor (TGF) β1 neutralizing serum or TGF-β1 oligonucleotides, we demonstrated that the HIV-1- or gp120-mediated suppression of CD34+ cell growth was almost entirely due to an upregulation of endogenous TGF-β1 produced by purified hematopoietic progenitors. Moreover, by using a sensitive assay on the CCL64 cell line, increased levels of bioactive TGF-β1 were recovered in the culture supernatant of HIV-1/ gp120–treated CD34+ cells. Anti-TGF-β1 neutralizing serum or TGF-β1 oligonucleotides were also effective in inducing a significant increase of the plating efficiency of CD34+ cells, purified from the peripheral blood of three HIV-1–seropositive individuals, suggesting that a similar mechanism may be also operative in vivo. The relevance of these findings to a better understanding of the pathogenesis of HIV-1–related cytopenias is discussed.

A common feature of the progression toward AIDS is that, besides the reduction in CD4+ T cell count, other peripheral blood cytopenias such as anemia, granulocytopenia, and thrombocytopenia invariably take place in up to 80% of HIV-1–seropositive subjects (1). The hematopoietic dysfunction in symptomatic HIV-1–seropositive subjects is underscored by an impaired in vitro growth capacity of either peripheral blood or bone marrow hematopoietic progenitor cells (2). Significantly, CD34+ cells purified from the bone marrow of AIDS patients also show poor colony-forming ability (3–6).  

Although a variety of mechanisms have been claimed in the pathogenesis of peripheral blood cytopenias of AIDS patients (1), the role played by HIV-1 remains initially elusive. In fact, direct HIV-1 infection of CD34+ hematopoietic progenitor cells isolated from HIV-1–seropositive carriers has been reported only in a limited subset of cases and can hardly account for the functional impairment of hematopoiesis observed in these patients (3–9). Similarly, only a minority of purified CD34+ cells are susceptible to either productive or latent infection with HIV-1 in vitro (9–15). Therefore, the direct infection of hematopoietic stem/progenitor cells does not seem to be a leading cause for the observed pathophysiology, suggesting that mechanisms other than direct infection may be responsible for the AIDS-associated hematopoietic suppression.

In this context, we have previously shown that the in vitro exposure to either lymphocytotropic strains of HIV-1 (IIIB or ICR-3) or cross-linked gp120 significantly impaired the survival and growth of the TF-1 CD34+ hematopoietic cell line as well as bone marrow CD34+ cells (16). This suppressive effect appeared to be greatly dependent on the viral load, but took place in the absence of a productive or latent infection and was likely mediated by specific interactions of envelope gp120 with the CD4 antigen, expressed at low level on the surface of a subset of human hematopoietic progenitor cells (17, 18).

It was previously shown by single-cell cultures and limiting dilution analysis that early hematopoietic progenitor cells are able to produce autocrine TGF-β1 (19, 20), which...
is thought to play an essential role in the maintenance of the quiescence state of stem cells and more immature hematopoietic progenitors. Here we explore whether endogenous production of TGF-β1 could take part in the HIV-1/gp120 inhibitory effect on CD34+ hematopoietic progenitors. To do this, we studied the effect of anti-TGF-β1 serum or TGF-β1 antisense oligomers on the survival and clonogenic capacity of CD34+ cells, purified from the peripheral blood of healthy donors and HIV-1-seropositive carriers, in both serum-free suspension and semisolid assays.

Materials and Methods

Growth Factors, Antibodies, and Oligodeoxynucleotides. rIL-3 and stem cell factor (sSCF), were purchased from Genzyme Corp. (Cambridge, MA). Erythropoietin (rEp) was kindly provided by Cilag (Milan, Italy). Purified TGF-β1 was purchased from R&D Systems, Inc. (Minneapolis, MN).

In neutralizing experiments, rabbit anti-TGF-β1 (R&D Systems, Inc.), rabbit anti-IFN-α (104 neutralizing units/ml; Bio-source, Camarillo, CA) and rabbit anti-TNF-α (Genzyme Corp.) polyclonal sera were used. In preliminary experiments, 20 µl of anti-TGF-β1 serum could completely neutralize 100 ng of TGF-β1.

21mers corresponding to the antisense, sense, or missense sequences flanking the translation initiation regions of the mRNA for TGF-β1 were prepared as described by Hatzfeld et al. (19). The sequence of the phosphorothioate oligonucleotides are as follows: TGF-β1 antisense, 5'-CCCCGGAGGCGGATGGGGAG-3'; TGF-β1 sense, 5'-TCCCCCATGGCCCTCCTGGG-3'; TGF-β1 missense, 5'-GGCGAGCGAG TGAGCGGCGG-3'.

Isolation of CD34+ Progenitor Cells from Peripheral Blood. Informed consent for the study was obtained according to the Helsinki declaration of 1975 from 20 healthy donors and 3 HIV-1-seropositive subjects. Mononuclear cells were isolated from leukopheresis units (healthy donors) or 60 ml of peripheral blood (HIV-1-seropositive donors) by Ficoll-Paque (d = 1.077 g/ml; Pharmacia, Uppsala, Sweden), rinsed, and adherence-depleted overnight. Nonadherent cells were collected and aliquoted at a concentration of 25 × 106 cells/tube. 50 µl of the following mAbs were added to each tube: anti-CD2, anti-CD3, anti-CD8, anti-CD11, anti-CD14, anti-CD19, anti-CD20 (Becton Dickinson & Co., San Jose, CA) in the presence of 0.5% BSA (fraction V; Sigma Chemical Co., St. Louis, MO). After two washings, 100 × 106 immunomagnetic beads, coated with anti-mouse IgG (MPC 450 Dynabeads; Dynal, Oslo, Norway) were then added to each tube to obtain an immunomagnetic bead/cell ratio of 1:1 in a final volume of 0.4 ml for 30 min in ice, under continuous agitation. Lineage-positive cells were removed by a magnet (MPC1 Dynabeads; Dynal) and the remaining cells were pelleted at a concentration of 5 × 106 cells/tube. After these negative selections, CD34+ cells were isolated using a magnetic cell sorting program (Mini-MACS; Miltenyi Biotech, Auburn, CA) and the CD34 isolation kit in accordance with the manufacturer's recommendations.

The purity of CD34-selected cells was determined for each isolation by flow cytometry using a mAb that recognizes a separate epitope of the CD34 molecule (HPCA-2; Becton Dickinson & Co.) followed by a goat anti-mouse IgG directly conjugated to fluorescein (GAM-FITC). CD34+ cells averaged 95–98%. No differences in CD34 purity were observed in HIV-1-seronegative and -seropositive donors.

The presence of proviral DNA in CD34+ cells purified from HIV-1-seropositive subjects was examined by PCR, following a previously described procedure (7), with a sensitivity of 10 proviral copies in a background of 106 cells. Aliquots of 20,000 CD34+ cells were amplified with the HIV-1 gag-specific primers SK38-SK39. PCR runs included several reactions containing all reagents except DNA as negative controls, as well as HIV-1+ controls represented by H9 and Jurkat T cells chronically infected with HIV-1. At the end of the amplification reaction, 25-µl aliquots of the amplified products were resolved in a 3% agarose gel.

Viruses and Recombinant Viral Proteins. Virus stock was represented by the supernatant of H9 lymphoblastoid T cell cultures of optimal cell density (0.5–1.5 × 106 cells/ml) and harvested 14 d after infection with HIV-1 (strain IIIB). It contained a reverse transcriptase (RT) activity of 1.5 × 106 cpm/ml with an infectivity of 3 × 106 TCID50 (tissue culture infectious dose 50) equivalents for lymphocytes, determined as previously described (14). 1 ml of purified, high-titer stock of HIV-1 was first heat-inactivated at 59°C for 45 min, and then added to CD34+ cells for 2 h at 37°C. Control (mock-treated) cultures were run in parallel by challenging CD34+ cells with 1 ml of the supernatant of uninfected H9 lymphoblastoid T cells cultured under optimal conditions. After virus adsorption, the cells were plated in liquid or semisolid cultures. The absence of infectious virus after heat inactivation was checked by adding HIV-1 IIIB to permissive T lymphoblastoid H9 and Jurkat T cell lines or PHA-stimulated PBMC. In some experiments, CD34+ cells were treated with heat-inactivated HIV-1 plus increasing concentrations (1–100 ng/ml) of purified TGF-β1.

In experiments with recombinant env proteins, several doses (10 ng–10 µg) of baculovirus-derived HIV-1 gp120 (ABT, Cambridge, MA) were added to cells for 1 h at 4°C followed by 30 min at 4°C with 20 µl rabbit anti-gp120 (ABT) serum before plating. To control for nonspecific protein effects, we performed experiments with baculovirus-derived recombinant p24 (ABT) murine IgG, human IgG followed by 20 µl of rabbit anti-p24 (ABT), rabbit anti-mouse IgG, or rabbit anti-human IgG antisera, respectively. Normal rabbit serum was also included as additional control.

Serum-Free Suspension Cultures. To eliminate the influence of TGF-β1 contained in serum or plasma (21), purified CD34+ cells were resuspended in serum-free medium (IMDM containing 10−4 M BSA-adsorbed cholesterol and nucleosides, 10 µg/ml each, 0.5% BSA, 10 µg/ml insulin, 2% 200 µg/ml iron-saturated transferrin, 5 × 10−3 M 2-B-ME) containing IL-3 (0.4 ng/ml) and SCF (40 ng/ml). 50,000 cells/well were incubated in 48-well flat-bottom tissue culture plates (Nunc, Roskilde, Denmark) in 0.2 ml of medium at 37°C in a water-saturated atmosphere of 5% CO2 for the next 15 d. Using Trypan blue dye exclusion, the number of viable cells was determined over this 15-d period.

To minimize the influence of possible endotoxin contaminations, all the experimental procedures were performed in endotoxin-free plastic ware. According to the manufacturer's information, the levels of endotoxin contamination in the cytokine preparations were <0.13 endotoxin U/ml by the Limulus assay (E-Toxate; Sigma Chemical Co.; limit of detection, 0.06 EU/ml).

Cell Cycle Analysis and [3H]Thymidine Incorporation Assay. At different time points, cells were harvested from liquid culture,
fixed in 70% ethanol for 1 h at 4°C, and then incubated with 20 μg/ml of RNase for 30 min at 37°C. Nucleic DNA was stained with 50 μg/ml propidium iodide (PI; Sigma Chemical Co.) and allowed to equilibrate for 10 min in the dark before being analyzed as described (22). Fluorescence analysis of individual nuclei was performed by the use of a FACScan® flow cytometer equipped with an argon–ion laser (488-nm wavelength; 100-mW light output) and lysil II software (Becton Dickinson & Co.). The fluorescence intensity from cell nuclei stained with PI is proportional to the cellular DNA content.

For the [3H]thymidine incorporation assay, cells were counted at different time points of liquid culture, and then seeded at 50,000/100 μl in 96-well flat bottom tissue culture plates (Nunc). 1 μCi [3H]thymidine (6.7 Ci/mmol; DuPont New England Nuclear, Boston, MA) was added to each well for 4 h of incubation. Radioactivity incorporated into DNA was measured by liquid scintillation counting.

**Detection of TGF-β1 mRNA.** Total RNA was isolated from ~1 × 10^6 enriched CD34+ cells by using RNAzol B (Biotex Texas, Houston, TX), according to the manufacturer’s instructions, and resuspended in 10 μl of diethylpyrocarbonate-treated water. Reverse transcription was performed for 10 min at room temperature and for 60 min at 42°C on 1 μg of RNA (equivalent to 5 × 10^5 cells) in 20 μl of a reaction mixture containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 5 mM MgCl2, 0.1% gelatin, 100 μM random hexamer primers, 20 μg plasmid RNAse inhibitor (Boehringer Mannheim, Postfach, Germany), 100 U of reverse transcriptase (Perkin–Elmer Cetus Instruments). The TGF-β1 primers 5'-AGTGTGTTATCCGTGCTGTC-3' were prepared according to Keckow et al. (23) and define a 186-bp fragment extending from +1358 to +1544 in the TGF-β1 transcript (24). PCR reaction was performed in a 100-μl vol for 35 cycles (denaturing 1' at 94°C, annealing 1' at 55°C and extension 1'30" at 72°C). 20 μl of the PCR products was then resolved in a 3% agarose gel. Positive control was a TGF-β1 cDNA digested with PstI (24), whereas the negative control was a TGF-β2 cDNA digested with BamH1 (25), each at 1,200 template copies.

**TGF-β1 Protein Determination.** Supernatants were collected from serum-free 3d CD34+ cell suspension cultures. These samples were tested for TGF-β1 activity after transient acidification: pH 4. In the supernatants was reduced to pH 2 by the addition of 5 mol/liter HCl for 2 h and then neutralized to pH 7 with 1.4 mol/liter NaOH in 0.7 mol/liter Hepes. Titers of TGF-β1 were expressed in nanograms per milliliter based on a standard curve that was generated with each set of assays by using purified human TGF-β1 (R&D Systems, Inc.). The total amount of TGF-β1 in CD34+ cell culture supernatant was determined by antibody neutralization. The bioassay on CCL64 mink lung epithelial cells (26) was performed as described previously (27). Briefly, 10^6 CCL64 cells/well were seeded in 0.2 ml of serum-free medium in 96-well flat-bottom tissue culture plates (Nunc). Serial concentrations of purified TGF-β1 or CD34+ cell culture supernatants were added to CCL64 cells in appropriate dilutions in the absence or presence of 20 μl of anti-TGF-β1 serum. Cultures were incubated at 37°C for 24 h, and 1 μCi [3H]thymidine (DuPont) was added to each well during the final 4 h of incubation. Radioactivity was measured by liquid scintillation counting.
lying the inhibitory effect of HIV-1 virions or envelope gp120 on PB hematopoietic progenitors, a serum-free liquid suspension assay was devised to monitor the survival of CD34+ cells. Typically, 50,000 cells, in a final volume of 0.5 ml, were added per well to 48-well flat-bottom tissue culture plates containing SCF (40 ng/ml) plus IL-3 (0.4 ng/ml). As can be seen in Fig. 2 A, these culture conditions allowed for the survival and minimal proliferation of CD34+ cells during the next 15 d at 37°C, at which time they plateaued at only a three- to fourfold amplification of starting cells. Of note, >60% of the 15-d cell population was still CD34+. At this time point, no significant differences in the phenotypic expression of CD34+ antigen were observed between HIV-1-mock-treated and treated groups (data not shown). In the presence of HIV-1 or cross-linked gp120, a significant (p < 0.01) inhibition of the total number of viable cells was observed from days 6–9 onward. Consistently, a progressive inhibition of DNA synthesis with lower levels of [3H]thymidine incorporation was seen in HIV-1/gp120–treated cultures from day 3 onward (Fig. 2 B). Analysis of the cell cycle performed by flow cytometry after PI staining showed that >60% of control cells were in G0/G1 at any time point considered with a background (≤2%) of subdiploid DNA (Table 1). HIV-1 or cross-linked gp120 induced a significant accumulation of cells in G0/G1 phases (80–90%) with a progressive increase of subdiploid DNA (up to 10–11%), which is considered characteristic of apoptosis. An example of such experiments is reported in Fig. 3.

We then set out to determine whether the inhibitory effect of HIV-1 glycoproteins on the survival and clonal growth of hematopoietic progenitors could be mediated by TGF-β1, a pleiotropic cytokine that plays an important physiological role in the negative regulation of hematopoiesis (21, 29). Several lines of evidence suggested that TGF-β1 could be the mediator of the HIV-1/gp120–induced inhibition. In fact, (a) an autocrine production of TGF-β1 by hematopoietic progenitor cells has been dem-
Table 1. Cell Cycle Analysis of PB CD34+ Cells Performed at Different Time Points of Suspension Culture

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>Cell cycle composition</th>
<th>Cells with subdiploid DNA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>S G2/M G0/G1</td>
<td>% of cells</td>
</tr>
<tr>
<td>3d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>23 8 69</td>
<td>2</td>
</tr>
<tr>
<td>HIV-1</td>
<td>11 4 85</td>
<td>6</td>
</tr>
<tr>
<td>HIV-1 + CD4</td>
<td>21 9 70</td>
<td>2</td>
</tr>
<tr>
<td>Cross-linked gp120</td>
<td>9 3 88</td>
<td>7</td>
</tr>
<tr>
<td>Cross-linked p24</td>
<td>25 9 66</td>
<td>1</td>
</tr>
<tr>
<td>6d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26 9 65</td>
<td>1</td>
</tr>
<tr>
<td>HIV-1</td>
<td>7 3 91</td>
<td>9</td>
</tr>
<tr>
<td>HIV-1 + CD4</td>
<td>24 10 66</td>
<td>3</td>
</tr>
<tr>
<td>Cross-linked gp120</td>
<td>7 3 90</td>
<td>10</td>
</tr>
<tr>
<td>Cross-linked p24</td>
<td>28 10 62</td>
<td>2</td>
</tr>
<tr>
<td>12d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29 9 62</td>
<td>2</td>
</tr>
<tr>
<td>HIV-1</td>
<td>3 2 95</td>
<td>11</td>
</tr>
<tr>
<td>HIV-1 + CD4</td>
<td>26 8 66</td>
<td>1</td>
</tr>
<tr>
<td>Cross-linked gp120</td>
<td>4 3 93</td>
<td>12</td>
</tr>
<tr>
<td>Cross-linked p24</td>
<td>26 10 64</td>
<td>3</td>
</tr>
</tbody>
</table>

Data represent the means of three separate experiments performed in duplicate. Standard deviations were within 9% of the mean.

...continuing text...

Figure 3. Cell cycle analysis of CD34+ cells treated with H9 uninfected cell culture supernatant (A; control) and heat-inactivated HIV-1 or gp120 (B) after 12 d of suspension cultures as described in Materials and Methods. A representative of three separate experiments is shown. x axis, PI fluorescence. y axis, relative number of cells.
or cross-linked p24. The addition of soluble CD4 completely blocked the biological activity of TGF-β1 recovered in HIV-1-treated CD34+ cells. Interestingly, a suppressive effect on the colony formation, similar to that previously found in the presence of either HIV-1 virions or cross-linked gp120 (Fig. 1 B), was also observed in CD34+ cell cultures supplemented with low concentrations (1-10 ng/ml) of purified TGF-β1 (Fig. 7 A). These concentrations were comparable to those found in the culture supernatant of HIV-1/gp120-treated CD34+ cells. Together, these data further suggest that minimal amounts of endogenous TGF-β1 released by CD34+ cells can account for the inhibitory activity of HIV-1/gp120.

Since elevated levels of circulating TGF-β1 have been documented in HIV-1-seropositive subjects (34), and TGF-β1 is able to trigger its own production (35), we next evaluated the effect of purified TGF-β1 in combination with
Figure 6. (A) TGF-β1 mRNA expression in CD34+ cells. (A) Lane M, molecular weight markers; lane 1, TGF-β2 cDNA (negative control); lane 2, TGF-β1 cDNA (positive control); lane 3, CD34+ cells treated with H9 uninfected cell culture supernatant; lane 4, H2O (negative control); lane 5, CD34+ cells treated with heat-inactivated HIV-1; lane 6, CD34+ cells treated with heat-inactivated HIV-1 plus soluble CD4. (B) Quantitative evaluation of the amount of TGF-β1 released in culture supernatants by CD34+ cells 3 d after exposure to H9 uninfected cell culture supernatant (control), heat-inactivated HIV-1, cross-linked gp120, heat-inactivated HIV-1 plus soluble CD4, and cross-linked p24 used at the same concentrations reported in the legend to Fig. 1. A representative of three separate experiments is shown.

heat-inactivated HIV-1 (Fig. 7 B). Exposure to high concentrations of TGF-β1 (100 ng/ml) plus HIV-1 resulted in an additive inhibitory effect on CD34+ cell colony formation, suggesting that exogenous TGF-β1 may also participate in the upregulation of endogenous TGF-β1 in hematopoietic progenitors.

Figure 7. Suppressive effect of increasing concentrations of TGF-β1 alone (A) or in combination with heat-inactivated HIV-1 (B) on the colony size of different types of progenitors in semisolid cultures. Data are reported as mean ± SD of three to five separate experiments performed in duplicate.

In a last group of experiments, we investigated the effect of the addition of antisense TGF-β1 oligonucleotides or anti-TGF-β1 serum to CD34+ cells purified from the PB of three HIV-1-seropositive individuals, whose hematologic parameters are shown in Table 2. Of note, the presence of HIV-1 infection in enriched CD34+ cells was preliminarily excluded by gag DNA PCR, performed as described previously (7). A substantial increase in the num-

Table 2. Hematological Features of HIV-1-seropositive Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Plt × 10^5/liter</th>
<th>Hb g/dl</th>
<th>WBC × 10^9/liter</th>
<th>CD4 × 10^4/liter</th>
<th>CD8 × 10^4/liter</th>
<th>gag DNA in CD34+ cells</th>
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<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>76</td>
<td>13.0</td>
<td>4,250</td>
<td>128</td>
<td>497</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>96</td>
<td>11.5</td>
<td>5,100</td>
<td>89</td>
<td>715</td>
<td>negative</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>107</td>
<td>10.6</td>
<td>3,900</td>
<td>113</td>
<td>863</td>
<td>negative</td>
</tr>
</tbody>
</table>

Plt, platelets; Hb, hemoglobin; WBC, white blood cells; CD4, CD4+ T cells; CD8, CD8+ T cells.
Figure 8. Enhancement of colony number (A) and size (B) by different types of progenitors in semisolid cultures, and total number of CD34+ cells (C) purified from three HIV-1-seropositive subjects in suspension cultures in the presence of TGF-β1 antisense oligomers or anti-TGF-β1 serum. Data are reported as means of experiments performed in duplicate.

An HIV-1/gp120 suppression of hematopoiesis based on an indirect mechanism, such as an increased release of endogenous TGF-β1, may explain several findings reported in the literature: (a) the frequency of CD34+ cells (number of CD34+/bone marrow mononuclear cells) is not significantly reduced until very late stages of HIV-1 disease (3-9); (b) nevertheless, CD34+ cells challenged in vivo or in vitro with HIV-1 or viral products show, at least in some studies, an impaired colony-forming ability (3-7, 12-16); and (c) no correlations are found between presence of viral infection, either productive or latent, and impaired colony growth of CD34+ hematopoietic progenitors purified from HIV-1-seropositive subjects (3-7, 9).

Experimental evidence suggests that TGF-β1 acts on target cells by arresting or delaying cells in G0/G1 of the cell cycle (30, 31), as well as by inducing apoptosis (32). Both of these effects were observed in HIV-1/gp120–treated CD34+ cells. The activity of TGF-β1 is mediated, at least in part, by maintaining the retinoblastoma growth suppressor protein in an active underphosphorylated state (37, 38) through an inhibition of the synthesis of the cyclin–dependent kinase (and/or its associated cyclin) that phosphorylates the retinoblastoma or a block of the formation of this cyclin–kinase complex (39). TGF-β1 may also inhibit cell cycling by downmodulating the expression of growth factor receptors (40).

Remarkably, neither a productive nor latent infection was required to upregulate endogenous TGF-β1. Therefore, defective virions and/or free glycoprotein gp120, which are produced in abundance by infected cells (41), may be effective as infective virions in the induction of this TGF-β1–mediated inhibitory effect. The mechanism(s) by which HIV-1 or free gp120 upregulates the production and release of TGF-β1 remains to be fully elucidated. Analogous to mature myeloid cells (42, 43), purified CD34+ hematopoietic progenitor cells also constitutively express TGF-β1 mRNA. However, due to the low number of CD34+ cells achievable from each sample, the presence of TGF-β1 mRNA could only be evaluated by RT-PCR. This analysis did not show any significant quantitative difference in the levels of TGF-β1 mRNA between cells treated with the supernatant of H9 uninfected cultures and cells treated with heat-inactivated HIV-1. Clearly, it will be important to determine whether gp120 affects the stability of mRNAs or the translation of TGF-β1 in CD34+ cells, as proposed for mature monocytes/macrophages.

Finally, our data indicate that the influence of endoge-
nous TGF-β1 on hematopoietic progenitors could be potentiated by combination with inhibitory cytokines, including exogenous TGF-β1, produced by infected bone marrow and PB accessory cells in response to HIV-1 infection or exposure to recombinant proteins (15, 23, 27, 42-44).

References