RAPID COMMUNICATION

In Vitro Human Immunodeficiency Virus-1 Infection of Purified Hematopoietic Progenitors in Single-Cell Culture


Uni- or multi-lineage suppression of hematopoiesis is observed in the majority of acquired immunodeficiency syndrome (AIDS) patients. The mechanism(s) underlying these abnormalities is not understood: particularly, the human immunodeficiency virus (HIV) infection of hematopoietic progenitor and stem cells (HPCs/HSCs) is highly controversial.

We report that CD34+ HPCs from adult peripheral blood (PB) are in part CD4+ and susceptible to in vitro HIV infection. Primitive CD34+ HPCs were ~90% purified from PB. Double labeling for CD34 and CD4 membrane antigens was shown for 5% to 20% of the purified cells, thus suggesting their potential susceptibility to HIV-1 infection. The enriched HPC population, challenged with purified or unpurified HIV-1 strains, was cloned in unicellular methylcellulose culture. The single colonies generated by erythroid burst-forming units (BFU-E), granulocyte-macrophage colony-forming units (CFU-GM), and granulocyte-erythrocyte-megakaryocyte colony-forming units (CFU-GEMM) were analyzed for the presence of HIV, ie, for gag DNA, tat mRNA, and p24 protein by PCR, reverse transcription PCR (RT-PCR), and enzyme-linked immunosorbent assay, respectively. In the first series of experiments incubation of HPCs with HIV-1 at multiplicities of infection (MOI) ranging from 0.01 to 10 TCID50/cell consistently yielded an 11% to 17% infection efficiency of BFU-E-generated colonies, thus indicating the sensitivity of HPCs to in vitro HIV infection. An extensive series of experiments was then performed on HPCs challenged with HIV at 0.1 MOI level. In the initial studies proviral gag sequences were detected in 9.2% of 121 analyzed CFU-GM colonies. In further experiments tat mRNA was monitored in 17% and 23% of BFU-E and CFU-GM colonies, respectively, but never in CFU-GEMM clones. Finally, 12% of CFU-GM clones and rare erythroid bursts were shown to be positive for the p24 viral protein. In control studies, purified HPCs grown in liquid suspension culture were induced to terminal unilineage erythroid, monocyte, or granulocytic differentiation: monocytes were consistently HIV-infected, whereas mature-terminal erythroblasts and granulocytes were not. Our observations indicate that a minority of primitive HPCs, but not of the multipotent HPCs, may be susceptible to HIV infection, whereas mature HPCs appear resistant to HIV infection.

Patients infected with human immunodeficiency virus (HIV) exhibit not only a decrease of CD4+ peripheral blood (PB) lymphocytes, but also unilineage suppression of bone marrow (BM) hematopoiesis (ie, thrombocytopenia, granulocytopenia, anemia, lymphopenia), up to pancytopenia in the late stage of HIV infection. These hematopoietic disorders are frequently associated with impaired hematopoietic progenitor cell (HPC) growth and BM dysplasia, plasmacytosis, and lymphoid infiltrates.

Different mechanisms have been suggested to underlie these hematopoietic abnormalities. A suppressive role of anti-HIV gp120 antibodies and viral products (ie, tat, nef) has been postulated. The mechanism of the suppressive action is uncertain: although a direct effect on HPC growth cannot be excluded, several reports indicate indirect mechanisms via infection of BM T cells and/or monocytes, which leads to release of a glycoprotein inhibitor and an enhanced production of inhibitory cytokines, and an altered release of hematopoietic growth factor(s) (HGF).

A key aspect under scrutiny is the capacity of HIV to infect HPCs. In this regard, controversial studies have indicated or excluded the HIV capacity to infect HPCs in vitro. CD34+ hematopoietic cells from acquired immunodeficiency syndrome (AIDS) patients were reportedly either HIV- or HIV- of HIV-.

Our studies at single HPC level indicate that a minority of primitive HPCs purified from PB are CD34+ and susceptible to HIV-1 infection.

MATERIALS AND METHODS

HGFs and Culture Medium

Recombinant human interleukin-3 (rhIL-3; 1.7 × 10^7 U/mg) and rh granulocyte-macrophage colony-stimulating factor (rhGM-CSF; 1.7 to 2.5 × 10^7 U/mg) were supplied by the Genetics Institute (Cambridge, MA), erythropoietin (rhEpo) and kit-ligand (rhKL) by Amgen (Thousand Oaks, CA), and Immunex (Seattle, WA), respectively. rhG-CSF and rhM-CSF were purchased from R and D Systems (Minneapolis, MN), Iscove’s modified Dulbecco’s medium (IMDM; GIBCO, Grand Island, NY) was freshly prepared weekly.

Adult PB

Adult PB was obtained from 20- to 40-year-old healthy male donors after informed consent. Blood, 450 mL ± 10%, was collected in preservative-free CPDA-1 anticoagulant (citrate-phosphate-dextrose-adrenaline). A buffy coat was obtained by centrifugation (Beckman [Palo Alto, CA] 16M/E; 1,400 rpm/20 minutes at room temperature).

HPC Purification

Adult PB HPCs were purified according to a modification of the method reported by Gabbianelli et al: (IA) PB samples were separated by density gradient centrifugation a...
rated over a Ficoll-Hypaque density gradient (d, 1.077) (Pharmacia, Piscataway, NJ). (IB) PB mononuclear cells (PBMCs) resuspended in IMDM containing 20% heat-inactivated fetal calf serum (FCS; Gibco) were treated with three cycles of plastic adherence. (II) Cells were separated by centrifugation on a discontinuous Percoll (Biochrom KG, Berlin, Germany) four-step gradient (d, 1.052, 1.056, 1.060, 1.065). (III) Step III purification was potentiated (step IIIIP) as follows: low-density cells (1.052 and 1.056 fractions) were collected and incubated with appropriate amounts of an antibody to anti-CD14 and CD36) and -granulocyte (anti-CD11b and CD15) monoclonal antibodies (MoAbs) as described supplemented with anti-CD45, -CD11a, and -CD71 MoAbs (Becton Dickinson, Mountain View, CA). Cells were resuspended in IMDM containing 1 mg/mL bovine serum albumin (BSA; 96% to 99% purified, Fraction V, No. A4503; Sigma, St Louis, MO), incubated (1 hour at 4°C) with immunomagnetic monodisperse microspheres coated with sheep antibody to mouse IgG (different subclasses) and IgM (Dynabeads M450; Dynal, Oslo, Norway) at a 4 beads/cell ratio and then separated with a magnet. Residual cells were then counted and incubated again (1 hour at 4°C) with the same type of magnetic beads at a ratio of 10 beads/cell. Finally, cells were counted again and incubated (30 minutes at 4°C) with magnetic beads coated with affini purified antihuman IgG1 (Fc specific, Dynabeads 110.04; Dynal) at a 10 beads/cell ratio. Throughout this procedure the concentration of magnetic beads never exceeded 50 x 10^6 beads/mL.

**HPC Immunofluorescence Analysis**

The following MoAbs directly conjugated with fluorochrome (fluorescein isothiocyanate [FITC] or phycoerythrin [PE]) were used to characterize the membrane phenotype of step IIIP HPCs: anti-CD34 (HPCA-2 clone; Becton Dickinson), -HLA-DR, -CD4, -CD8, -NK lymphocyte (anti-CD16, CD56 and CD57), monocyte (anti-CD14 and CD36) and -granulocyte (anti-CD11b and CD15) monoclonal antibodies (MoAbs) as described supplemented with anti-CD45, -CD11a, and -CD71 MoAbs (Becton Dickinson, Mountain View, CA). Cells were resuspended in IMDM containing 1 mg/mL bovine serum albumin (BSA; 96% to 99% purified, Fraction V, No. A4503; Sigma, St Louis, MO), incubated (1 hour at 4°C) with immunomagnetic monodisperse microspheres coated with sheep antibody to mouse IgG (different subclasses) and IgM (Dynabeads M450; Dynal, Oslo, Norway) at a 4 beads/cell ratio and then separated with a magnet. Residual cells were then counted and incubated again (1 hour at 4°C) with the same type of magnetic beads at a ratio of 10 beads/cell. Finally, cells were counted again and incubated (30 minutes at 4°C) with magnetic beads coated with affinity purified antihuman IgG1 (Fc specific, Dynabeads 110.04; Dynal) at a 10 beads/cell ratio. Throughout this procedure the concentration of magnetic beads never exceeded 50 x 10^6 beads/mL.

**HPC Infection and Growth in Unicellular Culture**

CD34+ HPCs, 1 x 10^6, maintained overnight in liquid culture (5 x 10^6 cells/mL) supplemented with KL, IL-3, GM-CSF ± Ep at saturating dosage (see below), were then pelleted and resuspended in a maximum volume of 50 mL in the presence of the appropriate HIV MOI. As a negative control, cells were mock-treated or incubated with either heat-inactivated virus (65°C for 1 hour) or supernatant from the 8E.5 HIV-1 chronically infected cell line releasing noninfectious viral particles. After a 2-hour adsorption at 37°C under shaking, complete medium was added to reach a volume of 0.2 mL. Cells were incubated overnight at 37°C in a 5% CO₂, 90% N₂ humidified atmosphere. Cells were then extensively washed, diluted (5 cells/mL) in semisolid medium containing 0.9% methylcellulose, 40% FCS in IMDM supplemented with α-thioglycerol (10^-4 mol/L) (Sigma), KL (100 ng/mL), IL-3 (100 U/mL), GM-CSF (10 ng), and Ep (3 U) (in some experiments Ep was not added). After gentle mixing 100 μL of the cell suspension was seeded in single wells (96 flat-bottom wells/plate; Falcon) and incubated as indicated above. Colonies were scored after 14 to 17 days under an inverted microscope (Zeiss, Oberkochen, Germany) and picked up for morphologic or polymerase chain reaction (PCR)/reverse transcriptase-PCR (RT-PCR) analysis.

**Infection of HPC-Generated Erythroid, Granulocytic, and Monocytic Cells**

Step III HPCs were grown in liquid suspension cultures (5 x 10^6 cells/mL) for selective erythroid, granulocytic, or monocytic differentiation. In erythroid differentiation FCS+ culture, very low doses of IL-3 (0.01 U/mL) and GM-CSF (0.001 ng/mL) and plateau level of Ep (3 U/mL) were added. Granulopoietic FCS+ culture medium was supplemented with low doses of IL-3 (1 U/mL) and GM-CSF (0.1 ng or 2.5 U) and plateau-level G-CSF (500 U). Monocytic differentiation culture medium contained IMDM, 40% FCS, and M-CSF (500 U/mL). Cells were cultured for 12 (FCS+) or 15 (FCS+) days and periodically diluted to 5 x 10^6 cells/mL; thereafter, they were HIV-infected as described above, cultured until day 14 (FCS+) or 17 (FCS), and analyzed by RT-PCR.

**PCR Analysis**

RT-PCR. Single colonies were plucked, washed twice with PBS (100 μL of 4 mol/L guanidine thiocyanate in the presence of 12 μg RNA of Escherichia coli, and extracted by the CsCl gradient technique). RNA was reverse transcribed according to the manufacturer's instructions (Boehringer, Mannheim, Germany). PCR was performed in a final volume of 50 μL in the presence of 2.5 U of Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT). The samples were amplified through 40 cycles which included the follow-
ing parameters: denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds and primer extension at 72°C for 30 seconds (Gene Amp PCR System 9600; Perkin-Elmer).

PCR. Single colonies were washed in PBS and digested with proteinase K (60 μg/mL) in 0.3% NP-40, 10 mmol/L NaCl, 3 mmol/L MgCl₂, 20 mmol/L TRIS, 0.4% Tween for 60 minutes at 56°C. The proteinase was then inactivated at 95°C for 5 to 10 minutes. After digestion the samples were spun down, and the supernatant stored at 4°C until amplified. PCR parameters were as described for RT-PCR.

PCR products were run on 1.8% agarose gel and transferred onto nitrocellulose membrane. Filters were hybridized using an end-labeled probe and then autoradiographed at -80°C.

To inhibit a high background and then autoradiographed at -80°C.

The PCR primers recognizing a conserved region of the gag sequence were: sense 5’AGAGATGGTGCGAGGCGTG3’, antisense 5’GTCTAAAGCTTCCTTGGTGTC3’, and the specific probe 5’GGGCAAGCAGGGAGCTAG3’.28 The RT-PCR primers recognizing a conserved tat sequence were: sense 5’CCCAAGGCTCGGCTTGCCTGAA3’, antisense 5’CTATTCTCTGGGGCTGTCG3’, and the specific probe 5’GAGCCAGTAGATCTAGACAGC3’.29 The primers used for β₂-microglobulin cDNA were: sense 5’AACCAACGATCTTGTACACGC3’, antisense 5’CTGCTCAGATACATCAAACTG3’, and the probe was a 35-bp oligonucleotide comprised in the amplified 251-bp fragment.30

CD34⁺ ELISA

Evaluation of intracellular p24 antigen was performed by lysing single colonies in 200 μL of TNE buffer (10 mmol/L TRIS, 100 mmol/L NaCl, 1 mmol/L EDTA, pH 7.4) containing 0.1% of Triton X-100. After 5 minutes of incubation on ice, cell lysates were briefly spun and supernatants were tested by ELISA (Antigen Capture Assay; Abbott, North Chicago, IL).

RESULTS

HPCs were stringently purified from normal adult PB using a procedure32 recently modified by a potentiated negative selection (step IIIP) to improve HPC recovery;22 in five separate experiments the frequency of step IIIP HPCs (CFU-GEMM + BFU-E + CFU-GM) in clonogenetic assay was 80.0 ± 2.7 (mean ± SEM values) (Fig 1).

The membrane phenotype of step IIIP cells was evaluated by double immunofluorescence for the expression of CD34 and CD4 antigens (representative results in Fig 2). This analysis showed that (1) greater than 90% of step IIIP cells are CD34⁺ (see also Fig 1); (2) a low proportion of CD34⁺ cells displays a dim but significant reactivity with PE-labeled anti-CD4 MoAb; in five different experiments the range of CD34⁺/CD4⁺ cells was between 5% and 20% (two representative experiments are shown in Fig 2); (3) this CD34⁺/CD4⁺ cell population was monitored when anti-CD4 MoAb was included (as routinely performed, see Fig 2, top and middle panels) or excluded (Fig 2, bottom panel) in the MoAb cocktail used for step IIIP negative selection. In the latter case (bottom panel), a small population of CD34⁺/CD4⁺ T lymphocyte was observed (the majority of T cells was presumably eliminated by the anti-CD3, -CD7 MoAbs always included in the cocktail). These results indicate that anti-CD4 MoAb removes CD4⁺ lymphocytes which exhibit a high CD4 density, while it spares CD34⁺/CD4⁺ HPC showing low CD4 density.

Initially, control studies were performed to validate the HIV-1 infection procedure. Step IIIP HPCs were grown in liquid suspension culture to induce unilineage differentiation up to terminal monocytic, granulocytic, or erythroid maturat

and then infected with NL4-3 or HTLVIIIB HIV-1 strain (results with NL4-3 are presented in Fig 3; identical results were obtained with the HTLVIIIB strain, not shown): (1) mononuclear cultures, composed by a majority of monocytes/macrophages exhibiting typical monocyty markers (ie, CD14 antigen), were consistently HIV-infected as evaluated by RT-PCR analysis; (2) granulopoietic cultures, containing a large majority of neutrophils (>90%) and displaying typical granulocytic markers (eg, CD11b) were not infected by HIV-1; (3) erythroid cultures, mainly composed by mature-terminally erythroblasts (98%) expressing the erythroid marker glycophorin A, were HIV-1 negative by RT-PCR.

Purified step IIIP cells were incubated with a graded HIV/ cell ratio (HTLVIIIB strain, TCID₅₀/cell) from 0.01:1 to 10:1 (Fig 4). Control cells were incubated with (1) the supernatant obtained from W-3, a chronically infected cell line releasing noninfectious viral particles, (2) heat-inactivated virus, or (3) were mock-treated. The cells were then grown at 0.5 cell/well in semisolid culture in the presence of KL, IL-3, GM-CSF, and Ep. BFU-E colonies were analyzed after 14 days. At all MOI levels, the presence of tat mRNA was detected in 11% to 17% of the BFU-E colonies, but not in the three control groups.

In a subsequent series of experiments, step IIIP cells were HIV-challenged (HTLVIIIB strain, 0.1 TCID₅₀/cell) and then cultured in the presence of KL, IL-3, GM-CSF, and Ep. A limiting dilution cell cloning was performed (0.5 cell/well). Single colonies plucked after 14 to 17 days of incubation were analyzed for the presence of specific tat mRNA sequences with β₂-microglobulin mRNA as internal control: interestingly,
I) Standard negative selection (MoAbs comprise anti-CD4 MoAb)

II) Modified negative selection (MoAbs do not comprise anti-CD4 MoAb)

Fig 2. Flow cytometry of HPCs purified by the standard step III P MoAb cocktail (top [experiment 575] and middle [experiment 582]) or the same cocktail without anti-CD4 MoAb (bottom, experiment 582) [two representative experiments are shown]. For each cell population the scatter of the propidium iodide negative cells and the negative control (labeling with irrelevant PE- and FITC-conjugated MoAb) are shown from left to right. Cells were double labeled with PE-labeled anti-CD4 and FITC-conjugated anti-CD34 or PE-labeled anti-CD34 and FITC-conjugated anti-HLA-DR.

17.3% ± 1.8% and 22.6% ± 3.7% (mean ± SEM values) of BFU-E and CFU-GM clones, respectively, were tar+, whereas CFU-GEMM colonies were always negative, as observed for mock- or 8E.5-treated controls (Fig 5A and B, middle panels).

In other experiments step III P cells were infected with purified or unpurified HIV (NL4-3 or HTLVIIIB strain, respectively, 0.1 TCID50/cell) and then grown in the presence of KL, IL-3, and GM-CSF (Ep was either added or not). Individual GM and BFU-E colonies were analyzed by PCR for the presence of proviral gag sequence. In a representative experiment with the NL4-3 strain, 9.2% ± 1.3% of CFU-GM colonies were HIV+ (Fig 5B, left panel); similar results were obtained on BFU-E colonies (results not shown). In experiments with the same NL4-3 challenge, we assayed single colonies obtained in single-cell culture for viral p24: p24 was detected in only 1 of 37 BFU-E colonies, whereas 4 of 33 CFU-GM colonies were positive (Fig 5B, right panel).

DISCUSSION

The significance of previous reports on in vitro HIV infection of HPCs, yielding either positive or negative results, is uncertain. Positive studies have been performed on CD34+ partially purified HPCs, which were then grown in bulk cultures: because the presence of monocytic cells in the infected and cultured cell population was not excluded, the HIV capacity to infect HPCs rather than the contaminating monocytic cells was not established. Negative studies were performed on unpurified BM HPCs infected and then grown in clonogenetic culture: HIV sequences in the individual colonies were not demonstrated by PCR analysis.

In view of these ambiguities, we have developed methodology for HIV infection of single HPCs and HIV detection in their clonal progeny. Thus, early HPCs were ~80% purified from PB, their membrane phenotype (CD34+/33-45RA-/71-) indicates their early stage of differentiation. The purified HPCs were HIV-infected and then cloned in methylcellulose culture at a seeding density of 0.5 cell/well, thus excluding presence of two or more cells in a single well. In an extensive series of experiments, this procedure consistently caused HIV-1 infection of 9% to 23% of BFU-E and CFU-GM, as evaluated in their clonal progeny by PCR, RT-PCR, and ELISA (except for ELISA of erythroid bursts, see below). It is noteworthy that (1) we have used both unpurified and purified HIV isolates, namely the HTLVIIIB and NL4-3 strains, respectively: both strains yielded equivalent results for HPC and monocyte infections (the NFL4-3 strain showed a more expanded tropism compared with the preferentially lymphotropic HTLVIIIB strain; but Folks et al showed the ability of HTLVIIIB to infect human hematopoietic precursors maturing to monocytes); (2) both inactivated HIV and an HIV mutant with loss of infectivity were included in the controls; (3) a large dosage range was used: interestingly, the HPCs were effectively infected even at low MOI level; (4) the HIV infection of the single HPC progeny was evaluated at both DNA and RNA level by gag PCR and tar RT-PCR, respectively (the latter was performed with primers through large spliced sequences to exclude PCR of viral RNA or DNA sequences absorbed onto the analyzed cells); (5) 12% of the CFU-GM clones were positive for p24, as evaluated by ELISA. (6) The efficiency of the HIV infection system was further validated by infection of HPC-generated terminal monocytic cells versus lack of infection of HPC-generated terminal erythroid and granulocytic cells.

A minority of CD34+ HPCs coexpress the CD4 antigen, thus in line with a previous report on the presence of CD4...
on murine stem cells. The possibility exists that CD4 on HPC membrane functions as the portal entry for HIV, although other mechanisms cannot be excluded. Ongoing studies aim to verify the HIV infection susceptibility of sorted CD34+/CD4+ versus CD34-/CD4- HPCs.

HIV infection has been observed for both BFU-E and CFU-GM colonies, but not for the mixed clones: the latter finding suggests that HIV susceptibility is dependent on the stage of HPC differentiation.

The 12% frequency of p24+ CFU-GM clones is close to the 23% incidence of tat-mRNA+ CFU-GM colonies. Conversely, the rarity of p24+ BFU-E clones (1 of 37) compares with the 17% frequency of tat-mRNA+ BFU-E colonies: the very low incidence of p24+ BFU-E clones may be attributed at least in part to hampered HIV expression in erythroid cells at translational level.

One interpretation of our results might be that HIV is first absorbed on the HPC membrane, possibly on the CD4 antigen, and then infects the HPC-derived monocytic progeny. This remote possibility is in contrast with several aspects: (1) the infected HPC population is extensively washed before clonogenetic culture; (2) HIV sequences are present in BFU-E colonies, which comprise less than 0.5% to 1% monocytic cells (results not shown); (3) the frequency of BFU-E colony infection is similar to that of CFU-GM colonies, which contain a high number of monocytic cells; (4) mixed colonies, while comprising a large monocytic component, are always HIV-.

The discrepancy between our positive results and the negative ones by Molina et al may be reconciled by the sharp difference of the HIV-infected cell populations (i.e., 80% purified early HPCs [our studies] or light density, T-cell-depleted BM cells comprising <0.5% early HPCs) and/or the culture conditions (i.e., presence [our studies] or absence of IL-3 and KL stimulus, which favors HPC survival and proliferation). It is possible that in the negative studies the HIV challenge was preferentially absorbed on the vast majority of non-HPC cells, thus preventing the HIV particles to effectively interact with HPCs and hence to infect them and their clonal progeny. Alternatively or additionally, HPC infection in absence of IL-3 and KL may have caused HPC necrobiosis or impaired clonogenesis (see also below).

The present observations reflect on the in vivo impairment of hematopoiesis in HIV-infected patients. Our findings on in vitro HIV infection of 10% to 20% of early HPCs are not necessarily in contrast with reports indicating that in AIDS patients CD34+ cells are HIV-. Indeed, (1) the in vivo HIV infected HPCs may represent a small subfraction of the early HPC pool and a much smaller subset of the CD34+ population, thus rendering difficult the detection of very few HIV+ early HPCs in CD34+ cells; furthermore, (2) it cannot be excluded that the HIV+ HPCs undergo necrobiosis or impaired clonogenesis (see also below).
sis: in line with the latter postulate, we observed that the purified HPCs challenged with HIV-1 at the routinely used MOI level (0.1 TCID$_{50}$) and grown in unicellular culture generate on the average a 39% lower number of colonies than mock controls. On the other hand, the number of HIV$^+$ HPCs may sharply increase in advanced AIDS because of widespread HIV infection, thus explaining the reports on in vivo HIV$^+$ CD34$^+$ cells in the advanced disease.$^{14,16}$

In conclusion, the present studies may shed light on the mechanism(s) of hematopoietic impairment in AIDS patients. More importantly, they provide a novel experimental tool to (1) explain the interaction of HIV with hematopoietic cells, particularly in the early stages of differentiation, and to (2) test anti-HIV HSC gene therapy.

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**Fig 5.** RT-PCR analysis of single colonies derived from step IIIP cells infected with HIV (0.1 TCID$_{50}$/cell) and cloned in unicellular culture. (A) Representative results on single BFU-E, CFU-GM, and CFU-GEMM colonies analyzed by RT-PCR for tat sequence. U937 and chronically infected CEMss cell lines were the negative and positive controls, respectively. $\beta_2$-microglobulin ($\beta_2$m) was the internal control. (B) Percentage of HIV$^+$ colonies, as evaluated by PCR (left), RT-PCR (middle), and ELISA (right). The mock controls comprise BFU-E-, CFU-GM-, and CFU-GEMM-derived clones. Mean ± SEM values from three (left and middle) and two (right) experiments. The number of analyzed colonies is indicated in parentheses. For further details see Results.


In vitro human immunodeficiency virus-1 infection of purified hematopoietic progenitors in single-cell culture

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