Monocytotropic human immunodeficiency virus type 1 (HIV-1) isolates from patients with acquired immunodeficiency syndrome (AIDS) infect mononuclear phagocytes as well as activated T cells, but do not usually infect immature human mononuclear cell lines in vitro. The HL-60 promyelocytic/myeloblastic cell line and the promonocytic line, U937, were susceptible to productive infection by monocytotropic HIV-1 isolates (HIV-I,FL and HTLV-III,FL) after treatment with retinoic acid, dimethyl sulfoxide, dibutyryl cAMP, 1,25-dihydroxyvitamin D, (1,25(OH),D3), or 12-O-tetradecanoyl-phorbol-13-acetate (TPA). Virus production was only detected when these compounds were added before virus infection. Virus replication did not correlate with CD4 receptor expression because undifferentiated HL-60 cells express CD4 and the level of CD4 expression did not increase after differentiation in the presence of retinoic acid, 1,25(OH),D3, or TPA. A mature monocytic cell line (THP-1) was capable of infection without pretreatment, and treatment with differentiating agents enhanced virus production. A chronically infected cell line (J-HL-60) was isolated after HIV-I,FL infection of HL-60 cells treated with retinoic acid. Virus production in this cell line was enhanced more than 10-fold after differentiation in the presence of 1,25(OH),D3 or TPA. The majority of virus production by 1,25(OH),D3-treated J-HL-60 cells was associated with the mature, adherent population. Molecular analysis of a cloned line of J-HL-60 showed integration of a single DNA provirus. These results suggest that cellular factors associated with precursor cell differentiation along the myelomonocytic pathway are required for optimal replication of monocytotropic HIV-1 strains in vitro.

The primary cellular target for human immunodeficiency virus type 1 (HIV-1) infection is the helper T lymphocyte.1,2 Cells of the mononuclear phagocyte series may also be infected by HIV-1.3-5 Recent evidence suggests that purified CD34-positive populations of myeloid progenitor cells are susceptible to HIV-1 infection as well.6 Cellular infection is believed to occur primarily via the CD4 molecule on the surface of certain blood cells.2,4,8 Productive HIV-1 infection after virus entry appears to relate to the particular strain of HIV-1 as well as to intracellular factors which coincide with cellular differentiation and maturation.6

The precise incidence and significance of mononuclear phagocyte infection in HIV-1–positive individuals is uncertain.10,11 It is not known whether macrophages are usually infected directly or whether they carry virus as a consequence of stem cell infection.6,12 We have used human myeloid leukemic cell lines as models to examine the relationship between cellular differentiation and productive infection by monocytotropic strains of HIV-1. Our results indicate that myeloid leukemic cell lines at the myeloblast and promyelocyte stage are not productively infected by monocytotropic strains of HIV-1 unless induced to differentiate along the granulocytic series with retinoic acid, dimethyl sulfoxide (DMSO), or dibutyryl cAMP (dbcAMP), or along the monocytic series with 1,25-dihydroxyvitamin D3 (1,25(OH),D3), or 12-O-tetradecanoyl-phorbol-13-acetate (TPA). These findings suggest that induction of differentiation along the myelomonocytic pathway renders cells susceptible to productive infection with monocytotropic HIV-1 strains, but that earlier precursors may not be appropriate hosts for virus production.

**MATERIALS AND METHODS**

**HIV-1 strains, virus propagation, and measurement.** A primary isolate of HIV-1 (HIV-I,FL) and a laboratory-maintained strain of HTLV-III,FL were provided by Y. Koyanagi and I.S.Y. Chen (UCLA, Los Angeles, CA).13 An HTLV-III,FL isolate was a gift of M. Popovic and R. Gallo (National Cancer Institute, Bethesda, MD).10 The HIV-I,FL and HTLV-III,FL viruses were propagated in phytohemagglutinin-stimulated peripheral blood lymphocytes. The HTLV-III,FL virus was propagated in granulocyte-macrophage colony-stimulating factor (GM-CSF)-stimulated peripheral blood mononuclear phagocytes. HIV-1 infection was initiated by incubating 1 × 10⁵ cells with either 0.25 mL filtered cell-free supernatant (estimated at 250 ng of viral p24 protein). Infection was performed in the presence of 10 μg/mL 1,5-dimethyl-1,5-diazaundecamethylene polymethobromide (Polybrene; Sigma, St Louis, MO) for 2 hours at 37°C. As a negative control, cells were incubated with a heat-inactivated virus isolate. After washing three times with Iscove's modified Dulbecco's medium (IMDM; Irvine Scientific, Santa Ana, CA), the cells were resuspended in fresh medium to a concentration of 5 × 10⁶ cells/mL.

To assay for productive virus infection, cell-free supernatants were obtained from overnight cultures and assayed for p24 antigen release using a commercial enzyme immunoassay (Abbott Laboratories, North Chicago, IL). Multiplicity of infection (MOI) was determined by titration assays using activated T cells.

**Cell lines.** The HL-60 promyelocytic/myeloblastic cell line14 was provided by R. Gallo (National Cancer Institute, Bethesda, MD) and the U937 promonocytic cell line15 was provided by P. Ralph (Cetus, Emeryville, CA). The THP-1 mature monocytic cell line16 was purchased from the American Type Culture Collection (Rockville, MD). The KG-1 myeloblastic cell line was established in our laboratory.17 These lines were grown in IMDM supplemented with 10% heat-inactivated fetal calf serum (FCS; Gemini Bio-
HIV INFECTION OF MYELOID LEUKEMIA CELL LINES

TPA. Following pretreatment the cells were infected with HIV-1 JR.FL, HTLV-I, or HTLV-II. The numerical designation is representative of the mean of four experiments. There was some variation in virus production, but the results were consistent within each experiment.

were maintained at 37°C in a humidified
and passed weekly.

Products Co, Calabasas, CA), 1% glutamine, and antibiotics, and
continued to produce virus for over 300 days. Morphologic examina-
were maintained mycoplasma free as determined by
agar from J-HL-60. A 30-fold increase in virus production was
Pharmaceutical Co, Ltd (Tokyo, Japan). Retinoic acid, DMSO
Genetics Institute, Inc (Cambridge, MA). Before use, each stock
1,25(OH)2D3 and retinoic acid were stored

Table 1. Effect of Differentiating Agents on HIV-1 Production in Human Leukemic Cell Lines

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>Control</th>
<th>DMSO (1.5%)</th>
<th>RA (10^-7mol/L)</th>
<th>cAMP (1mmol/L)</th>
<th>D3 (10^-1 mol/L)</th>
<th>TPA (1 ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1, H-FL</td>
<td>−</td>
<td>2+</td>
<td>4+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>HL-60</td>
<td>−</td>
<td>2+</td>
<td>4+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>U937</td>
<td>−</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>THP-1</td>
<td>4+</td>
<td>5+</td>
<td>6+</td>
<td>5+</td>
<td>5+</td>
<td>2+</td>
</tr>
<tr>
<td>KG-1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>HTLV-III, H-FL</td>
<td>−</td>
<td>3+</td>
<td>5+</td>
<td>−</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>HL-60</td>
<td>−</td>
<td>3+</td>
<td>5+</td>
<td>−</td>
<td>3+</td>
<td>2+</td>
</tr>
<tr>
<td>U937</td>
<td>−</td>
<td>2+</td>
<td>2+</td>
<td>4+</td>
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<td>THP-1</td>
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<td>4+</td>
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<td>−</td>
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<tr>
<td>KG-1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>HTLV-III, H-FL</td>
<td>−</td>
<td>4+</td>
<td>6+</td>
<td>5+</td>
<td>5+</td>
<td>−</td>
</tr>
<tr>
<td>HL-60</td>
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<td>2+</td>
<td>3+</td>
<td>4+</td>
<td>−</td>
<td>−</td>
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<td>U937</td>
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<td>4+</td>
<td>4+</td>
<td>2+</td>
<td>−</td>
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<td>5+</td>
<td>−</td>
</tr>
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<td>KG-1</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

Cells were incubated for 5 days in the presence of DMSO, retinoic acid (RA), or 1,25(OH)2D3 (D3) or for 3 days in the presence of dBcAMP (cAMP) or TPA. Following pretreatment the cells were infected with HIV-1, H-FL, HTLV-III, H-FL, or HTLV-III, H-FL. The numerical designation is representative of the mean of four experiments. There was some variation in virus production, but the results were consistent within each experiment.

*P24 (pg/mL) in supernatants on day 7 after infection are presented: −, 0-79; 1+, 80-199; 2+, 200-499; 3+, 500-999; 4+, 1,000-9,999; 5+, 10,000-99,999; 6+, >100,000.

A chronically HIV-1, H-FL infected HL-60 cell line (J-HL-60) was obtained after the HIV-1, H-FL infection of retinoic acid-treated HL-60 cells. J-HL-60 cells were cultured in IMDM with 10% FCS and passed weekly. In the absence of retinoic acid the infected cells continued to produce virus for over 300 days. Morphologic examination of J-HL-60 cells indicated no difference between these cells and uninfected HL-60 cells. A subline, J-, HL-60, was cloned in soft

Products Co, Calabasas, CA), 1% glutamine, and antibiotics, and
were maintained at 37°C in a humidified 5% CO2 incubator.

Table 2. Effect of Differentiating Agents on HIV-1 Production From HL-60 Cells When Treated After Infection

<table>
<thead>
<tr>
<th>Differentiating Agents</th>
<th>Concentration</th>
<th>Incubation Time (d)</th>
<th>p24 in the Supernatants (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>10^-6 mol/L</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>DMSO</td>
<td>10^-7 mol/L</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>cAMP</td>
<td>1.5%</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>1 mmol/L</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>TPA</td>
<td>1 ng/mL</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>1,25(OH)2D3*</td>
<td>10^-7 mol/L</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

HL-60 cells were infected with HIV-1, H-FL for 2 hours in the presence of Polybrene. After washing, 5 × 10^9/mL cells were incubated with each differentiating agent. P24 in the supernatants was assayed every week until day 35 postinfection. The experiments were repeated four times and the results were consistent within each experiment. Abbreviations are the same as those in Table 1.

*Positive control; HL-60 cells treated with 1,25(OH)2D3 before infection.

Proliferative assays. Test cells (5 × 10^5) were incubated in 0.2 mL IMDM in 96-well flat-bottomed microtiter trays. Cells were incubated at 37°C in a humidified atmosphere with 5% CO2. DNA synthesis in the cultures was determined after addition of [3H]-thymidine (0.5 μCi per well) for 6 hours. The cells were recovered on glass fiber filters and the filters were counted in liquid scintillation fluid (Fluorodine, National Diagnostics, Somerville, NJ).

Immunofluorescence. Cells were washed with phosphate-buffered saline containing 0.1% sodium azide and 2% FCS, and stained using an indirect two-step immunofluorescence method, as previously described. The primary antibody was a mouse monoclonal antibody (MoAb) (anti-Leu-3a (anti-CD4; Immunoglobulin G1[IgG1] isotype) purchased from Becton Dickinson Immunocytochemistry Systems (Mountain View, CA). The second antibody was a fluorescein isothiocyanate (FITC)-conjugated goat F(ab')2, antimouse IgG, obtained from Cooper Biomedical Inc (Malvern, PA). Control studies were performed with a nonbinding control mouse IgG1 isotype antibody (Becton Dickinson). Analysis of fluorescence was performed on a FACS flow cytometer (Becton Dickinson).

Purified recombinant human GM-CSF was provided by Genetics Institute, Inc (Cambridge, MA). Before use, each stock solution was diluted with culture medium to the required concentration. The final concentration of ethanol was no more than 0.1%, which had no effect on HL-60 cell proliferation or differentiation. The concentration of differentiating agent used was determined following dose-response studies and the designated concentration yielded maximal HIV-1 production and minimal cytotoxic effect.

Unless otherwise indicated, cell differentiation was induced by treating cells for 5 days at a concentration of 2 × 10^5 cells/mL in supplemented IMDM with the designated final concentration of differentiating agent. Differentiation was monitored by nonspecific esterase and Giemsa staining of cyto centrifuge preparations of untreated and treated cells. Cells staining positive for α-naphthyl butyrate esterase were classified as monocyte/macrophage-lineage cells.

Differentiating agents and induction of differentiation. 1,25(OH)2D3 (1 mmol/L) in ethanol was provided by Chugai Pharmaceutical Co, Ltd (Tokyo, Japan). Retinoic acid, DMSO (endotoxin free), dBcAMP, and TPA were all purchased from Sigma. Stock solutions of 1,25(OH)2D3 and retinoic acid were stored at −20°C. Purified recombinant human GM-CSF was provided by Genetics Institute, Inc (Cambridge, MA). Before use, each stock solution was diluted with culture medium to the required concentration. The final concentration of ethanol was no more than 0.1%, which had no effect on HL-60 cell proliferation or differentiation. The concentration of differentiating agent used was determined following dose-response studies and the designated concentration yielded maximal HIV-1 production and minimal cytotoxic effect.

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<td>DMSO</td>
<td>10^-7 mol/L</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>cAMP</td>
<td>1.5%</td>
<td>5</td>
<td>−</td>
</tr>
<tr>
<td>1,25(OH)2D3</td>
<td>1 mmol/L</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>TPA</td>
<td>1 ng/mL</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>1,25(OH)2D3*</td>
<td>10^-7 mol/L</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

HL-60 cells were infected with HIV-1, H-FL for 2 hours in the presence of Polybrene. After washing, 5 × 10^9/mL cells were incubated with each differentiating agent. P24 in the supernatants was assayed every week until day 35 postinfection. The experiments were repeated four times and the results were consistent within each experiment. Abbreviations are the same as those in Table 1.
Fig. 1A. Effect of differentiating agents on CD4 expression in HL-60. Cells were incubated for 5 days in the presence of retinoic acid or 1,25(OH)$_2$D$_3$ for 3 days in the presence of dBcAMP or TPA. Cells were stained with either Leu3a (solid line) or IgG1 (isotype control for nonspecific binding; dotted line), followed by FITC-conjugated goat anti-mouse IgG for FACS analysis, as described in Materials and Methods. Shown in the insets are the results for the mean channel linear fluorescence (MCLF) of total Leu3a binding. The experiment was repeated three times and the results were consistent within each experiment. We show one representative experiment.
HIV INFECTION OF MYELOID LEUKEMIA CELL LINES

Untreated
MCLF=2.9

TPA
MCLF=2.66

Fluorescence Intensity

Southern blot analysis. Chromosomal DNA was prepared from J774-HL-60 cells by Hirt DNA extraction. Residual RNA was removed by RNase A digestion (20 μg/mL) for 30 minutes at 37°C, followed by phenol/chloroform extraction and ethanol precipitation. DNA, 20 μg, was digested with EcoRI, BamHI (Bio Labs, Beverly, MA), or SstI (Bethesda Research Laboratories, Gaithersburg, MD), and fractionated by electrophoresis through 0.8% agarose gels. Gels were transferred to nitrocellulose filters (Schleicher & Schuell, Keene, NH) and hybridized to 32P-labeled DNA probes as previously described. A 0.6-kilobase (Kb) HindIII fragment from pKBH10S (ERC BioServices Co. Rockville, MD) was radioactively labeled using the random priming procedure.

RESULTS

Effect of differentiating agents on viral production in human leukemic cell lines. The monocytotropic HIV-1 isolates (HIV-1JRFL and HTLV-IIIbL) productively infected THP-1 cells as well as peripheral monocytes and activated T cells, but did not infect untreated HL-60, U937, and KG-1 cells (Table 1). HL-60 cells were rendered susceptible to productive infection by these virus isolates after treatment with DMSO, retinoic acid, dBcAMP, 1,25(OH)2D3, or TPA. The HL-60 cells acquired morphologic and cytochemical characteristics of mature granulocytes in the presence of DMSO, retinoic acid, or dBcAMP, and characteristics of monocyte/macrophages in the presence of 1,25(OH)2D3 or TPA. Pretreatment with DMSO, retinoic acid, or 1,25(OH)2D3 for at least 3 days was necessary for virus production (data not shown). On the other hand, virus production was undetectable when HL-60 cells were differentiated after exposure to virus (Table 2). Exposure to virus did not perturb the HL-60 cells because they differentiated normally in response to the various agents.

U937 cells were also susceptible to virus infection if treated with DMSO, retinoic acid, and dBcAMP. 1,25(OH)2D3-treated U937 cells were infected with neither HIV-1JRFL nor HTLV-IIIbL. THP-1 cells did not require pretreatment for productive HIV-1 infection, although the differentiating agents enhanced viral production in these cells. In response to TPA, KG-1 cells differentiated along the macrophage pathway, but treated cells were not susceptible to productive HIV-1 infection.

Unlike HIV-1JRFL or HTLV-IIIbL, the HTLV-IIIb strain was capable of infecting HL-60, U937, and THP-1 cells irrespective of treatment with differentiating agents. However, neither KG-1 cells nor 1,25(OH)2D3-treated U937 cells were susceptible to infection with HTLV-IIIb. A cytocidal effect was observed when HL-60 cells were infected with HTLV-IIIb. This effect was enhanced after treatment with dBcAMP and TPA.

Effect of differentiating agents on CD4 expression in HL-60 cells. In an effort to determine whether virus entry via CD4 could explain the susceptibility to virus replication, the level of CD4 expression in untreated and treated HL-60 cells was determined. As shown in Fig 1A, a subpopulation of HL-60 cells do express CD4 antigens. Expression of the CD4 antigen on HL-60 cells remained unchanged following incubation with retinoic acid, increased in the presence of dBcAMP, and decreased after treatment with TPA or 1,25(OH)2D3. CD4 expression on KG-1 cells was not detected on untreated cells or following TPA-induced differentiation (Fig 1B). Both U937 and THP-1 cells expressed CD4 (data not shown).

Kinetic analysis of HIV-1 production. [3H]-thymidine incorporation and cell morphology in HIV-infected HL-60 cells. 1,25(OH)2D3 and retinoic acid-treated cells were analyzed after infection for cell proliferation, differentiation, and virus production. Supernatants were harvested and assayed for virus p24 antigen to determine the kinetics of
HIV-1 production and [3H]-thymidine incorporation to determine the extent of proliferation (Fig 2). After treating HL-60 cells with 1,25(OH)2D3 for 5 days, most cells were differentiated and nonspecific esterase positive. At 5 days, [3H]-thymidine incorporation was reduced as compared with untreated HL-60 cells. Virus production was initially detected in the supernatant fluid on day 2, peaked on day 7, and reverted to undetectable levels by day 13 (Fig 2A). Despite an overall increase in [3H]-thymidine incorporation after day 7, at that time the amount of virus decreased as the percentage of mature cells decreased. A similar result was obtained with retinoic acid-treated HL-60 cells after HIV-1JR.FL infection (Fig 2B). Thus, peak virus production does not correlate with enhanced cell proliferation. Exposure to virus appeared to have little or no effect on the proliferation and differentiation of untreated HL-60 cells.

Chronically HIV-1JR.FL infected HL-60 cells and effect of differentiating agents on virus production. A chronically infected HL-60 cell line, J-HL-60, was established following retinoic acid treatment and HIV-1JR.FL infection (see Materials and Methods). Replication-competent virus was continually produced by the J-HL-60 cell line, and this virus was able to replicate in both monocytes and lymphocytes. The J-HL-60 cells differentiated normally in response to differentiating agents such as retinoic acid, DMSO, dBcAMP, 1,25(OH)2D3, or TPA, and treatment enhanced virus production (Fig 3). TPA and 1,25(OH)2D3 induced monocyte/macrophage differentiation and enhanced virus production more than 10-fold. Although hematopoietic growth factors such as GM-CSF enhance virus production in peripheral monocytes, they did not have an effect on virus production in J-HL-60 cells. A similar result was obtained following colony-stimulating factor-1 (CSF-1) and interleukin-3 (IL-3) stimulation of J-HL-60 cells (data not shown).

HIV-1 production from differentiated, adherent J-HL-60 cells. After treatment with 1,25(OH)2D3 for 2 weeks, J-HL-60 cells were divided into two populations: adherent and suspension (nonadherent). The adherent, differentiated cell population produced a 21-fold greater amount of virus than the nonadherent, immature cell population (Fig 4).

Integration of virus DNA. Southern blot analysis of J2,-HL-60 DNA showed a single integration site for the
HIV INFECTION OF MYELOID LEUKEMIA CELL LINES

Fig 3. Effect of differentiating agents on HIV-1 production from J-HL-60 cells. J-HL-60 cells (2 x 10^5 cells/mL) were incubated in 24-well plates for 6 days with the following concentrations of differentiating agents: retinoic acid, 10^-7 mol/L; DMSO, 1.25%; dBcAMP, 0.1 mmol/L; 1,25(OH)_2D_3, 10^-7 mol/L; TPA, 0.1 ng/mL; GM-CSF 100 pmol/L. HIV-1 p24 antigen in the supernatants was assayed on day 5 in triplicate. The experiment was repeated six times and we show one representative experiment. Data are presented as the mean and standard deviation of four measurements.

HIV-1 JR.FL provirus, confirming the clonal origin of this cell line (Fig 5). Unintegrated proviral DNA was not detected in these cells. The integrated provirus appears to be intact (unpublished results) and encodes a replication competent virus (see above). The internal Sst I cleavage site for the provirus is consistent with the HIV-1 JR.FL map as previously reported.13

Fig 4. HIV-1 infection of adherent, differentiated cells derived from J-HL-60 treated with 1,25(OH)_2D_3. J-HL-60 cells were incubated for 14 days in the presence of 10^-7 mol/L 1,25(OH)_2D_3. After removal of 1,25(OH)_2D_3, the cells were continuously cultured for 14 days. On day 28 after the treatment, two populations of J-HL-60 cells were obtained in the same culture flask: one consisting of adherent, differentiated cells (Ad) and the other consisting of nonadherent, immature cells (Non-Ad). Cells, 10^6, from each population were cultured for 24 hours and the supernatants were assayed for p24 viral protein. The experiment was repeated three times. There was some variation in virus production between experiments, but the results were consistent within each experiment. We show one representative experiment.

DISCUSSION

Hematologic abnormalities are common in patients with acquired immunodeficiency syndrome (AIDS).22-24 Defects in hematopoiesis and destruction of mature cells result in cytopenias, which decrease host defense and complicate treatment with antiviral agents and antibiotics which are in themselves myelosuppressive.25,26 In addition to quantitative abnormalities in myelopoiesis, defects in mature neutrophil and macrophage function have been described.27,28 Although the mechanism of disordered hematopoiesis in AIDS is not clearly understood, infection of stem cells is thought to occur.5 While the primary target for HIV-1 is the mature helper T lymphocyte, infection of cells of the mononuclear phagocyte lineage is believed to be important in the pathogenesis of AIDS.10,29,30 Direct infection of monocytes and macrophages has been clearly demonstrated, but it is not certain to what degree infection of stem cells and precursor cells contributes to virus production by more mature progeny.

We used myeloid leukemic cell lines as models to examine the relationship between differentiation/maturation and the ability to support productive infection of monocytotropic strains of HIV-1. We found that promyelocytic HL-60 cells and promonocytic U-937 cells could not be productively infected with the monocytotropic strains of HIV-1 unless the cells were first exposed to differentiating agents such as
Fig 5. Southern blot analysis of J2r-HL-60 cells. (A) Restriction endonuclease cleavage map for HIV-1 provirus. Abbreviation: S, Ssr I. A 0.6-kb HindIII fragment of pBKBH105S was used to identify HIV-1 sequences. The corresponding region within the HIV-1 provirus is indicated by a black box. There are no EcoRI nor BamHI restriction enzyme sites in the HIV-1 provirus.15 (B) Detection of HIV-1 DNA sequences in J2r-HL-60 cells. Chromosomal DNA from J2r-HL-60 cells was cut with restriction endonuclease EcoRI, BamHI, or SsrI and Southern blot analysis was performed. Chromosomal DNA from uninfected HL-60 cells was used as a control. The position of molecular weight markers is indicated.

DMSO, retinoic acid, cAMP, 1,25(OH)2D3, or TPA. Laboratory-maintained strains of HIV-1 such as HTLV-IIIls readily infected HL-60 cells. This result may be due to their long passage in the laboratory, and such laboratory-maintained strains may not be applicable for studying the relationship between differentiation and infectivity. Additionally, HTLV-IIIls had a cytocidal effect on some cells following treatment with differentiating agents.

U937 is not a susceptible target cell for monocytotropic HIV-1 viruses.13 However, Asjo et al reported that a subclone of U937 (U937-2) was susceptible to infection by primary viral isolates.31 Although the parental U937 cell line used in our studies expressed CD4, as does U937-2,32 our U937 was not susceptible to infection by monocytotropic strains unless exposed first to differentiating agents. HTLV-IIIls, on the other hand, was capable of infecting untreated U937 cells as well as HL-60 cells. These findings indicate that both cellular and viral factors are important in determining the potential for productive HIV-1 infection.

CD4 receptor expression is an important cellular factor believed to be necessary for HIV-1 entry in human neoplastic cell lines.8 Myelomonocytic leukemia cell lines such as HL-60, U937, and THP-1, which express CD4 receptors, were susceptible to infection after differentiation. However, KG-1 cells, which do not express CD4 receptors, were not susceptible to infection with any HIV-1 strains even after the differentiation. Virus production in HL-60 cells increased following induced differentiation without an increase in CD4 expression. These data suggest that the CD4 receptor is necessary but not sufficient for productive HIV-1 infection in myeloid leukemia cell lines. Thus, virus production may not be regulated at the level of virus entry but later in the retroviral life cycle.

Virus production in HL-60 cells exposed to differentiating agents did not coincide with thymidine incorporation but rather with cell maturation. Proliferating, untreated HL-60 cells were resistant to virus infection. Thus, maximal p24 production could be dissociated from proliferation. This result is consistent with our results from the 1,25(OH)2D3-treated chronically-infected J-HL-60 line, where maximal virus production was associated with the adherent, mature cell population, not with the more proliferative, nonadherent cells. Moreover, only the more mature monocytic cell line, THP-1, was susceptible to virus infection without any pretreatment. Thus, differentiation along the myelomonocytic pathway appears to enhance virus production despite a decrease in DNA synthesis.

We demonstrate that differentiating agents permit HIV-1 infection of myeloid leukemia cell lines. Our data suggest that these differentiating agents may act at two levels of HIV-1 replication. The first is presumably after virus entry and before proviral integration. HIV-1 may enter untreated HL-60 cells via CD4 receptors but would be unable to continue on in the life cycle. Pretreatment with various differentiating agents may supply a factor necessary for integration and continuation of the virus life cycle; whether this factor is an intracellular one resulting from differentiation or a secreted one arising from the more mature cell population has yet to be determined. The more differentiated THP-1 cells may constitutively express this factor.

The second level at which differentiating agents may act is after proviral integration. Our studies with J-HL-60 suggest that once proviral integration has occurred, virus production can be enhanced by treatment with various agents resulting in differentiation. This increase presumably occurs via enhanced provirus transcription, because virus production in J-HL-60 cells is not affected by reverse transcriptase inhibi-
tors such as azidothymidine (AZT). This result is consistent with a report by Griffin et al. who showed that HIV-1 expression increases during monocytoytic differentiation. They postulate this may occur via an increase in expression of the transcription factor NF-κB. Differentiating agents such as retinoic acid have also been shown to enhance retroviral transcription of a Moloney murine leukemia virus LTR. Our results suggest that hematopoietic stem cells may not be productively infected by HIV-1 without differentiation.

We have used immortalized myeloid cell lines and monocytoytic HIV-1 strains to examine the stage of myeloid differentiation most conducive to productive virus infection. The results suggest that the mature cells of the monocytic lineage and intermediate cells of the granulocytic lineage are optimal stages for HIV-1 production. Less mature cells, including stem cells, might be infected; however, substantial virus expression may not occur until intermediate levels of myeloid differentiation are reached. Our results show that differentiation inducers were required for infection of immature myeloid leukemic cell lines by monocytopathic HIV-1 strains, and productive infection was achieved if immature myeloid cells are differentiated along either the granulocytic or monocytic pathways. This result provides some evidence that granulocyte precursors may also harbor HIV-1.

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REFERENCES

8. Popovic M, Read-Connoise E, Gallo RC: T4 positive human neoplastic cell lines susceptible to and permissive for HTLV-III. Lancet 2:1472, 1984

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