Human Immunodeficiency Virus-1 Infection of the Human Promyelocytic Cell Line HL-60: High Frequency of Low-Level Infection and Effect of Subsequent Cell Differentiation

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As a model system to study the infection of early myeloid cells by human immunodeficiency virus-1 (HIV-1), we have infected the human promyelocytic cell line, HL-60, with a recombinant selectable HIV-1 clone. A fully infected population showed a relatively high frequency of low-level infection, with 40% of subcloned cells being negative by reverse transcriptase and p24 indirect immunofluorescence analysis and displaying only low levels of supernatant p24. The same treatment of a T-lymphoid cell line produced 100% productive infections. HIV-1 infection of HL-60 did not appear to alter the state of differentiation of the cells, as assessed by surface antigen expression, regardless of the level of viral expression. Furthermore, infected cells were able to respond normally to chemical inducers of differentiation. Induction of differentiation towards monocyte/macrophages by phorbol myristate acetate activated the HIV-1 long terminal repeat in a transient transfection system, and there was a corresponding increase in viral production from the infected subclones. Granulocytic differentiation, as stimulated by dimethyl sulfoxide or retinoic acid, had no effect on long terminal repeat activity and did not stimulate viral replication. These data suggest that low-level HIV-1 infections may be established at a relatively high frequency in myeloid precursor cells, and that different pathways of promyelocytic differentiation vary in their ability to stimulate HIV-1 replication.

The principal target cells of human immunodeficiency virus (HIV) infection in the body are the CD4-expressing T lymphocytes and cells of the monocyte/macrophage lineage. Although most of the circulating virus has been shown to be present in T cells, infection of monocytes and macrophages is of particular interest because of the major role that these cells play in the immune system. In addition, the reported lower cytopathicity of HIV for these cells, coupled with their lower surface expression of viral antigens, has led to the idea that these cells may serve as cellular reservoirs for HIV in vivo. Infected macrophages, with their wide tissue distribution, may allow HIV access to other cells of the body not primarily infected by HIV; there is also evidence to suggest that infection of T lymphocytes can occur by this route.

Therefore, determining the origin and nature of the infection in monocytes and macrophages is of importance to our understanding of the overall mechanism of the pathogenesis of acquired immunodeficiency syndrome (AIDS). HIV-1 has been found in the mature tissue macrophages of the lungs, central nervous system, lymph nodes, and skin of AIDS patients. These macrophages could be infected in situ or could result from the migration of infected blood monocytes into the tissues that then differentiated into macrophages. Mature macrophages have low surface expression of CD4 and the apparently nonproliferating nature of these cells is at odds with the expected retroviral requirement for host DNA synthesis to establish infection. However, it has clearly been shown that certain HIV-1 strains, such as HIV-1Bal, and HIV-1JR.FL, can grow efficiently in macrophages prepared from peripheral blood or bronchial lavage.

Myeloid cells from different stages of differentiation have been shown to be susceptible to HIV-1 infection in vitro, including hematopoietic stem cells, blood monocytes, and mature macrophages. Virus has also been isolated from these cell types in patient tissue. In addition, several human myeloid cell lines have proved infectible by HIV-1, including the immature HL-60 and U937 lines and the more mature THP-1 and Mono-Mac 6 lines. Furthermore, studies have shown that the normal process of myeloid cell differentiation may influence the susceptibility of cells to infection or affect the subsequent replication of HIV-1 in already infected cells.

As a possible route to account for HIV-1 infection of mature monocytes/macrophages, we hypothesize that early myeloid cells can be infected by HIV-1 and later differentiate into mature infected cells. Such a pathway seems feasible because some myeloid precursor cells express CD4, and virus has been detected in the hematopoietic stem cells of infected individuals. As a model system to study HIV-1 infection of such cells, we have used the human promyelocytic cell line HL-60, which we infected with a recombinant, selectable viral clone. This approach enabled us to overcome the low efficiency of infection of these cells by viral clones and to select for a fully infected population. We report here the finding of a high frequency of low-level infection of HL-60 cells by this virus (defined as supernatant p24 antigen levels <500 pg/mL in subcloned cells). This was not seen in a T-lymphoid cell line similarly infected. In addition, because this cell line has the potential to differentiate along either a monocytic or granulocytic pathway in response to specific factors, we have also studied the effects of cytokines on viral replication.

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chemical inducers,\textsuperscript{31} we investigated the effect of differentiation along either pathway on HIV-1 long terminal repeat (LTR) activity and on viral replication.

\textbf{MATERIALS AND METHODS}

\textit{Cell culture and induction of differentiation.} The human promyelocytic cell line, HL-60, and the T-lymphocytic cell line, H9, were grown in RPMI medium, supplemented with glucose, penicillin and streptomycin, and 10\% and 20\% heat-inactivated fetal bovine serum ( Gibco Laboratories, Grand Island, NY), respectively. Cells were maintained at densities between 1 \times 10^5 and 1 \times 10^6 cells/mL. HL-60 cells were induced to differentiate along the monocytic pathway by the addition of 8 \times 10^{-8} mol/L phorbol myristate acetate (PMA) to the growth medium. Granulocytic differentiation was induced by either 1.1\% dimethyl sulfoxide (DMSO) or 10^{-6} mol/L retinoic acid.

\textit{Virus infection and production of chronically infected cell lines.} The viral clone, HIV-1 R7/Neo, is a fully infectious virus with a selectable marker. This strain is derived from the plasmid R7/Neo,\textsuperscript{32} which is identical to HXB2 and RIF-73\textsuperscript{32} except that the bacterial neo gene replaces part of the nef sequences. This construct confers resistance to G418 on the host cell, so the addition of the antibiotic to the culture medium after infection by this virus allows a population to be selected for that harbors the viral genome. Because the HIV strain HXB2 does not express the nef and ypu proteins, the recombinant HIV strain R7/Neo is identical to HXB2 in terms of the pattern of protein expression. A stock of R7/Neo virus was prepared by transfecting the human T-lymphoid cell line CEM and filtering the supernatant through a 0.45-μm pore size filter when synctia were apparent (approximately 7 days after transfection). In our preparations, the virus typically contained 1 to 5 \times 10^6 cpm/mL of reverse transcriptase activity or 10^7 to 10^8 TCID50 units (measured by syncitia formation using the human T-cell line C8166). HL-60 and H9 cells (10^6) were infected with 1 mL of this stock at 37°C for 1.2 hours, followed by the addition of media. The antibiotic G418 ( Gibco-BRL, Gaithersburg, MD) was added to the media after 14 days at a concentration of 1 mg/mL and selection continued until fully resistant populations were obtained. Cells were subcloned from these populations by limiting dilution and the properties of 10 such clones from each cell line were analyzed. Viral growth was assessed by measuring reverse transcriptase (RT) and p24 concentrations (Abbott Laboratories, Chicago, IL) in the cell culture supernatant and the percentage of infected cells was assayed by indirect immunofluorescence (IF) with anti-CD4 (Leu-3A) was purchased from Becton Dickinson (Mountain View, CA). Fluorescein-conjugated goat antimouse IgG (Cappel) was used as the second-stage antibody.

\textit{DNA transfection.} HIV-1 LTR activity in HL-60 cells under a variety of conditions was assessed by transiently transfecting cells with LTR-CAT constructs and assaying for chloramphenicol acetyltransferase (CAT) activity. Essentially, 2 \times 10^6 cells per sample-point were transfected by the diethylaminoethyl (DEAE)-dextran procedure of Grosschedl and Baltimore\textsuperscript{39} (but without the addition of chloroquine) with 2 μg of the LTR-CAT plasmid and 4 μg of a Tat expression vector. Eighteen hours later, cells were split and plated at 1 \times 10^6 cells/mL media containing appropriate inducers and harvested 24 and 48 hours postinduction. Equivalent amounts of protein were assayed for CAT activity\textsuperscript{40} and the percent conversion of 14C-chloramphenicol to its acetylated forms was determined by cutting out regions containing unreacted and acetylated forms and quantitating the amount of radioactivity in each by liquid scintillation counting. The LTR-CAT plasmid used, p938, contains the HIV-1 LTR from pU3R-III.\textsuperscript{40,41} The Tat expression vector, pCMV-Tat, was provided by D. Trono (Salk Institute, San Diego, CA) and consists of the first exon of tat on a Sal-I-Kpn I fragment from HXB2\textsuperscript{23} cloned into the multilinker site of the expression vector pCPLK.\textsuperscript{42}

\textit{Electrophoretic mobility shift assays (EMSA).} Cellular extracts were prepared from HL-60 cells grown in medium alone or after incubation with PMA or DMSO for 2 days, and EMSA was performed as described by Baeuerle and Baltimore.\textsuperscript{43} Approximately 1.5 \times 10^7 cells were harvested, washed with ice-cold phosphate-buffered saline (PBS), and transferred to eppendorf tubes. Cells were resuspended in buffer containing 20 mmol/L HEPES (pH 7.9), 0.35 mol/L NaCl, 20\% glycerol, 1% NP-40, 1 mmol/L MgCl₂, 1 mmol/L DTT, 0.5 mmol/L EDTA, 0.1 mmol/L EGTA, 1% aprotinin (Sigma, St Louis, MO), and 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), and lysed on ice for 10 minutes. Particular material was removed by centrifugation at 4°C for 15 minutes and the amount of protein in the resulting supernatant was quantitated with bicinchoninic acid (Micro BSA Protein Assay Reagent No. 23235; Pierce, Rockford, IL). For the EMSA, 10 μg of protein was incubated with a 32P-labeled β oligonucleotide, prepared as described by Sen and Baltimore.\textsuperscript{44} Specific DNA-protein complexes were identified after electrophoresis through a 5% acrylamide gel by competition with unlabeled wild-type and mutant oligonucleotides. The nucleotide sequences of the oligonucleotides used were:

Wild-type β oligonucleotide:

\textbf{GATCCACTTCGATCGGACATTTCCAGGAGGA}

\textbf{GAGCCGACCCCTGAAAGTCCCTCCTCTAG}

Mutant β oligonucleotide:

\textbf{***}

\textbf{GATCCACTTCGATCGGACATTTCCAGGAGGA}

\textbf{GAGCCGACCCCTGAAAGTCCCTCCTCTAG}

(***, mutations introduced).

\textit{Analysis of cell surface antigens.} Fluorescein-activated cell sorter (FACS) analysis was performed as previously described.\textsuperscript{45} MoAbs to CD11b (903) and CD14 (MY4) were provided by J.D. Griffin (Dana-Farber Cancer Institute, Boston, MA); antibody to CD18 (10F12) was provided by J. Ritz (Dana-Farber Cancer Institute); and antibody to CD4 (Leu-3A) was purchased from Becton Dickinson (Mountain View, CA). Fluorescein-conjugated goat antimag mouse IgG (Cappel) was used as the second-stage antibody.

\textbf{RESULTS}

\textit{HL-60, but not H9 cells, display a high frequency of low-level infection by HIV-1.} To obtain a population of HL-60 cells fully infected with HIV-1, we have used a recombinant virus, R7/Neo, that can be selected for in the presence of G418.\textsuperscript{32} R7/Neo is a fully infectious viral clone that infects the T-lymphocytic H9 cell line as efficiently as its parental virus, HXB2,\textsuperscript{33} although it replicates slightly more slowly (data not shown). In the presence of G418, a fully resistant population was established and subclones were derived from this population by limiting dilution. These resistant cell lines grew with the same doubling times as the parental HL-60. Also, the pattern of the expression of many cell surface
markers used in this study was essentially identical between the parental and selected lines (see below). These results suggest that the G418 selection procedure itself had no significant effect on the experiments we describe below.

When fully resistant to G418, the HL-60 and H9 cell populations were markedly different. The T-lymphocytic H9 cells were 100% positive for HIV-1 infection, as assessed by p24 immunofluorescence analysis, whereas the promyelocytic HL-60 population was only 60% positive. These ratios were stable over a period of 8 weeks, and the populations remained of similar composition when G418 selection was discontinued. Subclones were derived from both populations by limiting dilution techniques, and 10 randomly chosen clones from each population were analyzed in further detail. The H9-derived subclones were all highly positive for p24 expression by immunofluorescence analysis, and had RT levels in the range of 10^5 to 10^6 cpm/mL. However, the 10 HL-60 subclones displayed a range of levels of infection (Table 1). Four of the subclones analyzed had low levels of HIV-1 infection, being negative for supernatant RT activity and for expression of p24 by IF analysis. The levels of supernatant p24 antigen in these cultures was also very low (<500 pg/mL), comparable to the levels seen in the cell lines ACH2 and U1, which are well-characterized models of low-level infection. The levels of viral expression in all the subclones were more or less constant at a given cell concentration throughout the culture. These results are consistent with the population p24 IF ratios.

The doubling times of both the high- and low-level-infected HL-60 subclones were comparable, and all 10 subclones contained HIV-1-specific RNA (see below), which argues against spontaneous G418 resistance having developed in uninfected HL-60 cells. By comparison with the H9 population, which was 100% productively infected, it is apparent that this high frequency (40%) of low-level HIV-1 infection is a property of the HL-60 cell line and not the R7/Neo virus.

Expression of viral RNA in HIV-1–infected HL-60 clones. To gain a better understanding of the differences in viral gene expression in HL-60 and H9 cells, we have analyzed the pattern of HIV-1–specific RNA transcripts present in the subclones from each population (Fig 1). As controls, we also examined RNA from H9 cells infected by HIV-1mb and by HXB2 (Fig 1, lanes 11 and 12). In each case, HIV-1 produced three specific size classes of RNA, corresponding to the genomic size RNA (band 1); the singly spliced messages, including the env message (band 2); and the small multiply-spliced messages coding for the regulatory proteins (band 3 for HIV-1R7/Neo and band 4 for HIV-1mb and HXB2). The presence of the additional 1 kb of neo DNA at the 3’ end of the genome in R7/Neo produced multiply-spliced RNA transcripts that were markedly larger than the corresponding HIV-1mb or HXB2 species (compare bands 3 and 4). An effect on the size of the other two RNA bands is not apparent, which could be a result of the gel conditions used or could be possibly caused by an additional splice acceptor site being present in the HIV-1R7/Neo genome, which could alter the final size of the transcripts.

Previous analyses of RNA from cells infected productively and at low level by HIV-1 have suggested a difference in the ratios of the three size classes of RNA in the two types of
infection. In the latently infected T-lymphoid ACH-2 cell lines, a pattern of RNA expression has been observed in which the smaller, multiply-spliced transcripts predominate over the genomic size species. Of the four HL-60 clones that were negative for surface p24 expression, clones 1, 7, and 11 demonstrated a similar pattern of RNA transcription to these latently infected cell lines, i.e., the smaller transcripts were more prevalent than the genomic transcripts (Fig 1, lanes 7, 9, and 10). In contrast, most of the productively infected HL-60 clones, and all of the H9 clones, displayed patterns more typical of a productive infection, with larger amounts of genomic RNA. Furthermore, HL-60 clones 9 and 10, which had the highest overall levels of supernatant RT activity, contained the greatest amount of HIV-1 RNA (Fig 1, lanes 1 and 2). (However, it has to be noted that this correlation between viral production and the ratio of genomic to subgenomic transcripts is not always the case, because clone 3, despite being a low producer, expressed significant levels of genomic RNA, whereas the high producer clone 4 had very little of the genomic transcript.) These data suggest a variation in the nature of the infections that establish in HL-60 cells. The relatively high frequency of establishment of such low-level infections (40% of the clones) is a property of the HL-60 cell line and did not occur in T cells infected in the same manner.

HIV-1 infection of HL-60 does not alter the expression of a number of differentiation-associated cell surface markers. Differentiation of myeloid cells is associated with characteristic changes in cell surface markers. In HL-60 cells, the expression of surface antigens CD4, CD11b, CD14, and CD18 are useful indicators of the state of cellular differentiation of the cells. The CD11b and CD14 antigens are markers associated with mature myeloid cells, and their expression is correspondingly low on undifferentiated HL-60 cells, whereas CD18 is expressed on HL-60 cells at all stages of differentiation. Induction of differentiation in HL-60 cells along either the monocytic or granulocytic pathway results in an increase in the expression of all three antigens with the increase in CD14 expression being especially characteristic of monocytic differentiation. CD4 expression is unchanged by granulocytic differentiation, but is markedly reduced upon monocytic differentiation by the addition of PMA.

FACS analysis was performed on uninfected, uninduced cells to establish the pattern of expression of these surface antigens in HL-60 cells. CD11b and CD14 antigens were present at low levels (less than 15% of the cells), CD18 was expressed by 100% of the cells, and CD4 was present on approximately 90% of the population (Fig 2). The levels of these markers in the HIV-1-infected subclones were also studied and compared with the levels in the uninfected population.

Figure 2 shows a comparison between the uninfected population and one representative clone (subclone 2; Table 1). CD4 expression was downregulated in this infected clone, as is commonly observed in HIV-infected cells, but the levels of expression of CD11b, CD14, and CD18 were comparable between the two populations, indicating that HIV-1 infection did not alter the state of differentiation of the HL-60 cells. Similar results were also obtained for a number of other infected subclones for CD11b, CD14, and CD18. CD4 expression was downregulated in all of the productively infected subclones, but 3 of 4 of the low level infections (clones 1, 7, and 11) still expressed surface CD4. These data suggest that in our experimental system, HIV-1 infection of HL-60 cells did not induce cell differentiation.

HIV-1–infected HL-60 cells respond normally to inducers of differentiation. Although our data suggested that HIV-1 infection did not alter the state of differentiation of HL-60 cells, it is possible that chronic viral infection could prevent these cells from responding normally to differentiation signals. FACS analysis was used to determine the antigen changes associated with the differentiation of uninfected HL-60 cells in response to PMA or DMSO. Differentiation along both pathways resulted in expected increases in the expression of the CD11b, CD14, and CD18 antigens (Fig 2). The same
pattern was observed when several HIV-1–infected subclones were treated in the same way, and the results for one representative subclone are shown (Fig 2). In addition, cell differentiation was assessed by cytochemical assays for monocyte- and granulocyte-specific esterase enzymes, with the infected subclones demonstrating the same pattern of enzyme activity as the uninfected cells, both before and after induction of differentiation (data not shown). This demonstrates that chronic HIV-1 infection did not impair the ability of HL-60 cells to respond normally to chemical inducers of differentiation.

Effect of cell differentiation on HIV-1 LTR activity. The effect of HL-60 differentiation on HIV-1 LTR-driven gene expression was studied by transiently cotransfecting LTRCAT and Tat expression plasmids into undifferentiated HL-60 cells. Eighteen hours later, the cells were induced to differentiate along either the monocytic pathway, by addition of 8 × 10⁻⁸ mol/L PMA, or the granulocytic pathway, by addition of 1.1% DMSO or 10⁻⁶ mol/L retinoic acid. Cells were harvested at 1, 2, and 3 days postinduction and assayed for CAT activity (Fig 3). Treatment with retinoic acid or DMSO had no significant effect on LTR activity, giving levels of CAT activity of only onefold to twofold above that of the uninduced control. Induction of monocytic differentiation by PMA, however, led to a marked increase in CAT activity, with increases of 10- to 30-fold over the uninduced level. These results were reproducible in several independent experiments. Transcription from the HIV-1 LTR was therefore highly stimulated by PMA treatment, but was unaffected by DMSO or retinoic acid induction.

The transient transfection assays demonstrated that PMA stimulation of HL-60 cells increased LTR-driven gene expression. PMA treatment of HL-60 cells has previously been shown to stimulate NF-κB binding activity,⁵⁰ and as the LTR contains two κB sites, induction of NF-κB binding leads to enhanced LTR transcription.⁵⁰ We here confirmed the induction of NF-κB in HL-60 cells in response to PMA by electrophoretic mobility shift analysis of a ³²P-labeled oligonucleotide probe corresponding to the LTR κB sites. This was incubated with cell extracts from uninduced and PMA-treated cells, and the induction of NF-κB binding at 2 days' poststimulation was shown by the appearance of a specific protein-DNA complex on an acrylamide gel (Fig 4). Treatment of HL-60 cells with DMSO did not induce NF-κB.

Viral replication is stimulated by monocytic but not granulocytic differentiation. The establishment of chronically infected HL-60 subclones allowed us to study the effects of cellular differentiation on viral replication at various time-points postinduction. We therefore treated both high- and low-level-infected HL-60 subclones with PMA or DMSO, and analyzed the changes in viral replication by measuring p24 concentration in the cell culture supernatant. PMA stimulation increased supernatant p24 antigen levels by threefold to fivefold from both high- and low-level producers over a control population (Fig 5), whereas treatment with DMSO had no significant effect. In the case of high producer lines, this induction of threefold to fivefold was also observed when RT activity in the cell culture supernatant was measured (data not shown). (It should be noted that PMA treatment arrests cell division and kills up to 50% of the HL-60 cells within 48 hours. This makes it difficult to draw a firm conclusion from the data for the low producer clones, which produce only small levels of p24 antigen and have undetectable levels of RT in the culture supernatant.) The increases in viral production from several HL-60 subclones were of a similar range to the fourfold induction previously reported for a chronically infected Molt-4 cell line treated with PMA,⁵³ but were considerably less than the induction obtained for U1 or ACH-2 cells treated with PMA.⁶⁴ Our results indicate that monocytic, but not granulocytic, differentiation enhances viral replication, at least in the case of high-level–infected HL-60 subclones.

**DISCUSSION**

Using the human promyelocytic cell line, HL-60, we have studied various aspects of HIV-1 infection of early myeloid cells. To reduce variability in our experiments, our approach has been to infect HL-60 cells with a selectable viral clone, to establish a fully infected population, and to derive infected subclones from this population. This system has enabled us to compare the frequency of establishment of productive infections in early myeloid and T-lymphoid cell lines, and also to investigate the effect of myeloid cell differentiation on the replication of HIV-1.

This system, which allows the selection of both low level and productive infections, has demonstrated that HIV-1 infection of the early myeloid cell line, HL-60, appears to be remarkably different from that of the T-lymphoid H9 cell
population composition remained similar in the absence of G418 selection. In addition, the doubling times of all the HL-60 subclones were comparable.

The nature of these low-level infections remains unclear. The predominance of the multiply-spliced transcripts in three of the four low-level subclones is reminiscent of the situation in the U1 and ACH-2 cell line models of latent infection. Alternatively, these subclones may harbor defective proviruses incapable of making wild-type virions, as has been reported to occur with other retroviral infections. Attempts to determine whether this low-level phenotype was caused by defective virions or was the result of a specific interaction between the virus and the host cell were hampered by the loss of infectivity of all of the HL-60 clones (both high- and low-level producers) over time (data not shown). We are currently exploring the reasons for this observation. Whether these low-level infections are latent or defective, they are established at a relatively high frequency, and are only seen with the HL-60 cell line. We believe that that greater tendency for these low-level infections to establish in early myeloid cells than in mature T lymphocytes could have relevance to HIV-1 infection in vivo.

HIV-1 infection of early myeloid cell lines has previously been proposed to induce differentiation, although this remains controversial. Analysis of the cell surface markers in a number of infected HL-60 subclones demonstrated the retention of an almost identical pattern to the parental population, arguing against HIV-1 infection stimulating differentiation in HL-60 cells and further demonstrating that HIV-1 did not selectively infect cells of greater or lesser maturity from within the population. Treatment of both infected and uninfected HL-60 cells with PMA or DMSO induced differentiation and led to characteristic changes in cell surface antigens. In addition, PMA treatment caused the cells to rapidly become adherent and develop a macrophage-like morphology. Therefore, it appears that infection by HIV-1 did not prevent HL-60 from differentiating normally in response to these chemical inducers.

It is well documented that treatment of infected cell lines with phorbol ester stimulates HIV-1 replication and that the differentiation of infected monocytes to macrophages

**Fig 4.** NF-xB induction by PMA. Ten micrograms of protein from unstimulated HL-60 cells (lane 1) or those treated with DMSO (lane 2) or PMA (lane 3) for 2 days were incubated at room temperature for 20 minutes with a 32P-labeled oligonucleotide corresponding to the enhancer sequence of the HIV-1 LTR. Gel electrophoresis demonstrated the presence of a specific protein-DNA complex induced by PMA treatment (arrow) whose specificity was confirmed by incubation of the day 2 sample with 30 ng of unlabeled wild-type (W) or mutant (M) oligonucleotide (lanes 4 and 5). FP, the unreacted probe.

**Fig 5.** PMA stimulates viral production from infected HL-60 subclones. Several HL-60 subclones were split, plated in fresh media at a density of 2 x 10^5 cells/mL, and treated with PMA (10^-8 mol/L) or DMSO (1.1%) or left untreated as a control population. After 24 or 48 hours, viral activity in both groups was assessed by supernatant p24 antigen levels. Note that the p24 concentration of the high producer (clone 2) is shown as nanograms per milliliter, whereas the low producer (clone 3) is shown as picograms per milliliter.
in vitro is accompanied by an increase in viral replication.\textsuperscript{8,23} PMA treatment of both high- and low-level-infected clones resulted in a threefold increase in viral production, whereas DMSO-treated cells retained the same level of viral replication as the uninduced control population. Furthermore, transient transfection analyses showed a 10- to 30-fold enhancement of LTR transcription in cells treated with PMA above the control or DMSO-treated populations. Enhancement of HIV-1 replication and LTR transcription in PMA-treated cells has previously been attributed to the induction of NF-\kappaB. PMA increases \kappaB-binding activity in HL-60 cell extracts (as first reported by Griffin et al.\textsuperscript{31} and confirmed here by our work), which could act to increase LTR transcription. In contrast, NF-\kappaB binding is not increased by DMSO treatment. At present, we cannot account for the discrepancy in the level of PMA enhancement of LTR-directed transcription and viral replication, but it is probable that additional, posttranscriptional processes, such as viral assembly and release, limit the final rate of virus production.

If cells of the monococyte/macrophage lineage can be infected at the myeloid stem cell stage, then it is possible that mature neutrophils could also be infected in this manner and harbor virus. Neutrophils lack the CD4 receptor and infection of these cells has not been demonstrated. Furthermore, a study by Spear et al.\textsuperscript{80} to determine whether proviral DNA sequences were present in neutrophils was inconclusive. However, impaired neutrophil function has been reported in AIDS patients.\textsuperscript{58,59} so it is possible that neutrophils are infected with HIV in vivo, but at a very low level. In addition, HIV infection of neutrophils may be more difficult to detect because of their short half-life relative to the macrophage. Induction of granulocytic differentiation in HL-60 cells by the addition of DMSO or retinoic acid neither increased levels of LTR-driven CAT activity in our transient transfection assays nor stimulated viral production above control levels from our infected subclones. It is therefore possible that any in vivo infection of neutrophils or their precursor cells would be nonproductive.

Our observations with the HL-60 cell line suggest that infection of early myeloid cells by HIV-1 may frequently be relatively nonproductive, and that myeloid precursors could therefore serve as reservoirs of viral infection in the body. Noncytopathic infection of myeloid stem cells has preceded in other retroviral infections, including Visna virus infection of sheep\textsuperscript{60} and FeLV infection of cats.\textsuperscript{61} Furthermore, the completion of the Visna life-cycle and the production of mature virions is dependent on the maturation of these infected stem cells to fully differentiated macrophages.\textsuperscript{62} It is therefore possible that a similar situation occurs in the case of HIV-1, with early myeloid cells serving as a route of entry into the more quiescent, mature macrophage, and that viral replication would be stimulated by monocytic, but not by granulocytic differentiation of these precursor cells.

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