Human immunodeficiency virus type 1-infected HL-60 cells are capable of both monocytic and granulocytic differentiation

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We have used the human myelomonocytic cell line HL-60 as a model system to determine whether human immunodeficiency virus type 1 (HIV-1) infection affects differentiation of myeloid progenitor cells. HL-60 cells were infected with three HIV-1 isolates (IIIB, NL4-3 and PM213). HIV-1 antigen expression and cytopathicity in HL-60 cells infected with each of the three isolates was delayed by approximately 15 days as compared to those in the prototypic T cell line, H9. Chronically infected HL-60 cells and clonal lines derived from them were treated with dimethyl formamide (DMF) and induced to differentiate into granulocytes. Approximately the same percentage of these cells as of DMF-treated, uninfected HL-60 cells differentiated. Superoxide production by infected and uninfected DMF-induced cells was similar. Likewise, approximately the same percentage of cells in infected and uninfected cultures became adherent and were positive for non-specific esterase when monocytic differentiation was induced. The data demonstrate that HL-60 cells infected with HIV-1 are capable of morphological and functional granulocytic and monocytic differentiation.

Several lines of evidence suggest that monocytes infected by human immunodeficiency virus type 1 (HIV-1) are important to the pathogenesis of AIDS (Gendelman et al., 1989; Meltzer et al., 1990). HIV-1 can be isolated from peripheral blood monocytes and the bone marrow of AIDS patients (Gendelman et al., 1986; Treacy et al., 1987; McLerath et al., 1989); HIV-1 infection affects monocyte/macrophage function in vivo, including significantly reducing phagocytic and chemotactic activities (Spivak et al., 1984), monocyte-dependent T cell proliferation (Prince et al., 1985), Fc receptor function (Bender et al., 1988) and accessory cell function (Petit et al., 1987; Ennen et al., 1990). In addition, proliferation of granulocyte–macrophage progenitor cells from HIV-infected patients is significantly reduced as compared to that of controls (Leiderman et al., 1987). Data from studies on bone marrow suppression and the dysfunction of infected monocytes suggest that both activation and differentiation of monocytes may be altered by HIV infection (Donahue et al., 1987; Carlo-Stella et al., 1987; Groopman et al., 1987).

It has been reported that HIV-1 infects both established monocytic and promonocytic cell lines, and primary peripheral blood monocytes and myeloid progenitor cells in vitro (Folks et al., 1988; Levy et al., 1985; Ho et al., 1986; Clapham et al., 1987; Cloyd & Moore, 1990; Butera et al., 1991; Roulston et al., 1992). We have used HL-60 cells, a bipotential cell line derived from a patient with acute promyelocytic leukaemia (Collins, 1987), to examine the effect of HIV-1 infection on granulocytic and monocytic differentiation. Others have reported that HL-60 cells cannot be infected by HIV, whereas we and others find that other HL-60 cells can be infected (Clapham et al., 1987; Cloyd & Moore, 1990; Butera et al., 1991). We recognize that HL-60 cell lines differ in their differentiation potential and may differ in other biological parameters such as infectibility.

We have used three isolates of HIV-1 (NL4-3, IIIB and PM213) that grow to high titre in both H9 and HL-60 cells to compare the c.p.e. of the virus isolates on both cell types. Virus supernatants were harvested from infected H9 cells and titrated on the indicator cell line C8166 (Salahuddin et al., 1983). HL-60 and H9 cells (5 × 10⁶ cells) were infected at a multiplicity of 0.2 C8166 TCID/cell with each of the three HIV-1 isolates in the presence of 2 μg/ml polybrene. Twenty-four hours post-infection (p.i.), cells were pelleted, washed with PBS and maintained in RPMI 1640 containing 10% heat-inactivated foetal bovine serum at 1 × 10⁶ cells/ml. Cell counts were performed using a haemocytometer and viability

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was determined by trypan blue exclusion. Chronically infected cultures (> 30 days p.i.) were used for differentiation analysis.

All three viruses caused rapid death of H9 cells (Fig. 1). Approximately 90% of the infected H9 cultures died within 12 days p.i. The mean survival time (MS50), defined as the time at which the culture was 50% viable, was approximately 7 days for H9 cell cultures and was independent of the virus isolate tested. The percentage of infected H9 cells expressing HIV surface antigens was determined by indirect immunofluorescence (IFA). Fig. 2(a) shows the results of infecting H9 cells with NL4-3. Similar results were obtained with IIIB and PM213 (data not shown). Briefly, $1 \times 10^5$ cells were applied to a glass slide, air-dried and fixed in methanol for 5 min. The fixed cells were incubated with HIV-positive pooled patient serum at 37 °C in 5% CO₂ for 40 min, washed with PBS, incubated with fluorescein-conjugated goat anti-human IgG (Sigma) at 37 °C in 5% CO₂ for 40 min, washed with PBS and stored at 4 °C in PBS:glycerol (1:1). The number of fluorescent cells per 1000 cells was determined. As the percentage of viable cells decreased, the proportion of HIV-1 antigen-expressing cells and the level of detectable reverse transcriptase (RT) (assay performed as described by Kusch & Wigdahl, 1989) increased (Fig. 2a).

The effect of HIV infection on HL-60 cells was very different (Fig. 1). After infection, the cells continued to proliferate. Greater than 90% of the culture was viable for the first 10 days p.i. and 2 to 5% of the cells were positive for HIV antigen expression (Fig. 2b). At approximately day 15 p.i., the viability of the culture began to decrease (Fig. 1). By day 30 a majority of the cells were dead. The MS50 for HIV-infected HL-60 cells was approximately 22 days. Both HIV-1 antigen-expressing cells and RT activity present in the culture medium increased as the viability of the culture decreased (Fig. 2b). As with H9 cells, each of the three HIV-1 isolates killed HL-60 cells with similar kinetics (Fig. 1) and results shown in Fig. 2(b) are typical of infection with either NL4-3, IIIB or PM213.

The difference in the observed kinetics of cell death of HIV-infected HL-60 and H9 cells could be due to differences in the percentage of cells that express CD4 on the cell surface. This was analysed by fluorescence-activated cell sorting (FACS). Cells ($2 \times 10^6$) were incubated with an anti-CD4 antibody (OKT4; Ortho-Diagnostics Systems) and stained with fluorescein-conjugated goat anti-mouse IgG (Sigma). The percentage of CD4-positive cells was similar in H9 and HL-60 cell cultures (> 85% of each culture was CD4⁺; see Table 1). In addition, the fluorescence intensity of HL-60 and H9 cells differed by 13% (based on median channel intensity), indicating that the number of receptors per
CD4 antibody (Leu3a; Becton-Dickinson) (250 ng/ml) blocked HIV NL4-3 infection of H9 and HL-60 cells for 24 days p.i. (Table 1). Therefore it is likely that infection of both H9 and HL-60 cells occurs via CD4–HIV-1 envelope interactions.

To determine whether HIV-1 infection affects the bipotential differentiation of HL-60 cells, cultures were infected with HIV IIIIB, NL4-3 or PM213. On day 36 p.i., granulocytic differentiation was induced (1 × 10⁶ cells/ml) by addition of 80 mM-dimethyl formamide (DMF) (Sigma). On day 6 after induction, cell differential counts were performed on Wright-Giemsa-stained cells. The percentage of cells at each stage of granulocytic differentiation was scored on the basis of morphology (Holland et al., 1989). It was shown that the percentage of cells in each stage of differentiation in uninfected and infected HL-60 cell cultures was approximately the same (Table 2) in three independent experiments. Thus, granulocytic differentiation of chronically infected HL-60 cells is indistinguishable from that of uninfected HL-60 cells.

To study a more homogeneous population of cells, a series of subclones of either HL-60 cells or chronically infected HL-60 (IIIIB) cells were established. Infected

### Table 1. CD4 expression and blocking on HL-60 and H9 cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CD4* (%)</th>
<th>HIV†</th>
<th>Leu3a‡</th>
<th>Day 3</th>
<th>Day 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9</td>
<td>96</td>
<td>−</td>
<td>−</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>19</td>
<td>82</td>
</tr>
<tr>
<td>HL-60</td>
<td>85</td>
<td>−</td>
<td>−</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Percentage of the culture which was CD4-positive as determined by FACS analysis.
† HIV-NL4-3 was used to infect cells at a multiplicity of 0.5 TCID/cell. –, Mock-infected; +, HIV-infected.
‡ Cells were preincubated with Leu3a (250 ng/ml) 10 min prior to addition of virus. –, No Leu3a added; +, Leu3a added. After 24 h cultures were pelleted, washed and suspended in medium without Leu3a.
§ Percentage of IFA-positive cells determined by IFA staining.

### Table 2. Granulocytic differentiation of HIV-infected HL-60 cells

<table>
<thead>
<tr>
<th>Cell line</th>
<th>DMF*</th>
<th>IFA-positive (%)‡</th>
<th>Promyelocyte (%)§</th>
<th>Myelocyte (%)§</th>
<th>PMN (%)§</th>
<th>Superoxide§</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-60</td>
<td>−</td>
<td>0</td>
<td>98</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>90</td>
<td>84</td>
</tr>
<tr>
<td>PLB-985</td>
<td>−</td>
<td>0</td>
<td>97</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>82</td>
<td></td>
</tr>
</tbody>
</table>
| Chronically infected cell lines¶
| HL-60 IIIIB | −    | 19               | 97                | 3              | 0       | 0         |
|           | +    | 21               | 0                 | 12             | 88      | 158       |
| HL-60 NL4-3 | +    | 14               | 96                | 4              | 0       | 251       |
|           | +    | 19               | 0                 | 13             | 87      |           |
| HL-60 PM213 | −    | 59               | 98                | 2              | 0       |           |
|           | +    | 55               | 0                 | 10             | 90      | 141       |
| PLB-985 IIIIB | −    | 15               | 98                | 2              | 0       |           |
|           | +    | 16               | 0                 | 18             | 82      |           |
| Clonal lines**
| HL-60 C1   | −    | 0                | 98                | 2              | 0       | ND        |
|           | +    | 0                | 0                 | 13             | 87      | ND        |
| HL-60 IIIB-1 | −    | 33               | 97                | 3              | 0       | 84        |
|           | +    | 36               | 0                 | 9              | 91      |           |
| HL-60 IIIB-2 | −    | 19               | 99                | 1              | 0       |           |
|           | +    | 24               | 0                 | 20             | 80      | 106       |

* Cells scored 6 days after treatment with DMF. –, Untreated cells; +, treated with 80 mM-DMF.
† Percentage of IFA-positive cells 6 days after treatment.
‡ Percentage of cells at each stage of differentiation identified by morphological observation after Wright-Giemsa staining. The values represent an average of three determinations of >100 cells scored in two separate induction experiments.
§ NBT reduction assay. The values are expressed as a percentage of normal control PMNs. The superoxide values were >0.04 A₅₁₅/15 min/10⁶ cells.
¶ Cultures 36 days p.i.
∥ ND, Not determined.
** Expansion of single cell clones of uninfected, HL-60 (C1) or chronically infected HL-60 IIIIB cell lines.
clones were isolated by performing two sequential limiting dilutions (0-5 cells/well). Clones were isolated at a frequency of approximately 5% in the first dilution (five of 96) and 9% (18 of 192) in the second serial dilution. All clones examined induced syncytia when co-cultivated with C8166 cells (15 lines). Of 10 clones tested for RT activity, nine were RT-positive. The cloning efficiency and tests of viral expression suggest that the lines are clones and represent a population of infected cells. The percentage of IFA-positive cells in the clonal lines (Table 2) suggests, in addition, that the population contains some cells expressing viral antigens and other cells not expressing viral antigens. This finding is consistent with a previous report that HIV DNA in HL-60 cells is extrachromosomal and therefore is not faithfully transmitted during clonal expansion (Butera et al., 1991).

One control uninfected HL-60 clone, HL-60 C1, and two HL-60 IIIB-infected cell clones, HL-60 IIIB-1 and HL-60 IIIB-2, were chosen for further analysis (Table 2). The two HIV-infected clones were determined to be HIV-1-positive by FACS analysis and determination of RT activity. Like the parental uninfected HL-60 cell line, HL-60 IIIB-1 and HL-60 IIIB-2 cells were predominantly promyelocytic as determined by Wright-Giesma staining, and were used with three chronically infected HL-60 cell populations to examine differentiation of HIV-infected myeloid cells. When HL-60 IIIB-1 and HL-60 IIIB-2 cells were treated with DMF their differentiation was similar to that of uninfected or chronically infected HL-60 cells (Table 2).

To determine whether these results are unique to HL-60 cells, identical experiments were performed with another human myelomonocytic cell line, PLB-985 (Tucker et al., 1987). These cells are less differentiated than HL-60 cells but are also bipotential and differentiable in response to DMF and phorbol 12-myristate-13-acetate (PMA) (Tucker et al., 1987). The MS50 for HIV-IIIIB-infected PLB-985 cells was approximately 20 days (Fig. 1). Although there was a slight increase in the rate of cell death of infected PLB-985 cells in comparison to HL-60 cells, both the kinetics of HIV-1 infection (Fig. 1) and differentiation (Table 2) were similar to those of HL-60 cells.

To test the function of differentiated HL-60 cell cultures infected with HIV-1, superoxide (O2-) production was monitored spectrophotometrically (A515) following reduction of nitroblue tetrazolium (NBT) (Bachner & Nathan, 1968; Newburger et al., 1984). The ability of DMF-treated HL-60 cells (day 6 after treatment) to generate O2- was compared to that of normal human peripheral mononuclear cells (PMNs). Superoxide production by HIV-infected HL-60 cells was equal to or greater than that of uninfected HL-60 cells (Table 2). Therefore, HIV-1 infection affects neither the granulocytic differentiation potential nor the functional capability of differentiated HL-60 cells.

Chronic and clonal lines of infected HL-60 cells and HIV-IIIB-infected PLB-985 cells (1 x 10^6 cells/ml) were induced to differentiate into monocytes by addition of PMA (62 ng/ml). Cultures were monitored from 1 to 3 days after treatment. Greater than 90% of both the infected and uninfected cultures became adherent after differentiation. An equally high proportion of cells (>89%) in each culture stained positive for the monococyte-specific enzyme non-specific esterase (Yam et al., 1971). The results indicate that HIV-1 infection affects neither differentiation of HL-60 cells into monocytes nor the production of a monocyte-specific enzyme.

These data demonstrate that HIV-1 infection of HL-60 cells does not induce differentiation of these cells. Neither does HIV-1 infection abolish or inhibit the response of HL-60 cells to either PMA or DMF treatment. This is in contrast to recent evidence that HIV-1 infection of the promonocytic cell line U937 (a more differentiated cell line than HL-60) induces differentiation of these cells and that the stage of cellular differentiation of U937 cells may determine the pattern of virus replication (Pauza et al., 1988) and production (Pautrat et al., 1990). In addition, Roulston et al. (1992) have reported that chronically infected lines of PLB-IIIB cells are more monocytic than the parental PLB-985 cells and non-responsive to granulocytic differentiation induced by dibutyryl cAMP. The difference between these results and our results may be due to a difference in the cells examined or to the agent used to induce differentiation. Both of the previous studies were performed using cell lines established from populations of cells that survive an HIV infection. We induced differentiation in chronically infected HL-60 and PLB-985 cells or chronically infected cloned HL-60 cells lines by treatment with DMF. Alternatively, the stage of myelocytic differentiation of haematopoietic cells may determine whether HIV-1 infection affects differentiation of the cells.

These in vitro findings suggest that the granulocytopenia seen in vivo may not be due to an effect on the differentiation of HIV-1-infected granulocytic precursor cells and suggest that either the c.p.e. of HIV infection on the granulocytic precursor is the primary cause of the granulocytopenia, or that the differentiation of haematopoietic cells may be altered in vivo at a stage earlier than that represented by HL-60 cells or by factors not present in the cultures.
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References


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