Enhancement of human immunodeficiency virus 1 replication in monocytes by 1,25-dihydroxycholecalciferol

(AIDS/vitamin D/calcitriol)

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ABSTRACT Human immunodeficiency virus (HIV) expression and replication are under tight regulatory control. We demonstrate that 1,25-dihydroxycholecalciferol [1,25-(OH)2D3] enhances the replication of monocyte- and lymphocyte-tropic strains of HIV-1 up to 10,000-fold in monocyte cell lines, peripheral blood monocytes, and unfraccionated peripheral blood mononuclear cells. 1,25(OH)2D3 is therefore one of the most potent regulators of HIV-1 replication described to date. Precursors of 1,25(OH)2D3 enhance HIV-1 replication in proportion to their affinity for the 1,25(OH)2D3 intracellular receptor, suggesting that 1,25(OH)2D3 influences HIV-1 replication by mechanisms involving this receptor. These studies may have important implications for the design of effective therapy of HIV-1 infection.

The monocyte is thought to play a crucial role in the pathogenesis of human immunodeficiency virus (HIV)-related illness (1). It is possible that active viral replication in monocyte/macrophages may lead to tissue injury, transmission of virus to other cell types, passage of virus between various organ systems within the body (e.g., across the blood–brain or blood–retina barrier), and eventual progression to HIV-related illness. Understanding the factors that regulate HIV-1 replication in monocytes could lead to new approaches capable of modulating disease expression in those tissues harboring HIV-1-infected monocyte/macrophages.

Expression of HIV in monocytes is under the control of several cell regulatory factors (2, 3). 1,25-Dihydroxycholecalciferol [1,25(OH)2D3], the biologically active form of vitamin D, has multiple effects on cellular differentiation and function, including regulation of surface differentiation antigens, oncogene expression, cytokine production, protein kinase C activity, phagocytosis, and heat-shock protein production (reviewed in refs. 4 and 5). Since HIV-1 replication is related to the cellul surface expression of CD4 antigen (6, 7) and the state of activation of cells (8, 9), we hypothesized that 1,25(OH)2D3 might affect HIV-1 replication. To test this hypothesis, we evaluated HIV-1 replication in a monocytoid cell line, peripheral blood monocytes, and unfraccionated peripheral blood mononuclear cells (PBMC) in the presence of 1,25(OH)2D3. HIV-1 replication was enhanced by 1,25(OH)2D3 in all of these cells at physiologic and supraphysiologic concentrations. This enhancement involves the 1,25(OH)2D3 intracellular receptor; precursors and analogs of 1,25(OH)2D3 enhance HIV-1 replication in proportion to their affinity for this receptor.

MATERIALS AND METHODS

Cell Lines and Virus Stocks. BT4A3.5 (A3.5) cells are a clone of monocytic cells that were isolated from human bone cell cultures (these cells were originally isolated and characterized by S. R. Goldring, E. P. Amento, B. Jahn, A. Duran, and S.M.K., unpublished). The cells are CD4+, HLA-DR+, CD15+ (Leu-M1+), and CD3-, they produce interleukin 1 after stimulation with endotoxin, and they are susceptible to infection with lymphocyte- and monocyte-tropic strains of HIV-1. Peripheral blood monocytes were separated from PBMC of HIV-1 seronegative donors by counterflow centrifugation (elutriation) in a Beckman JE-5.0 elutriator chamber and rotor. This method allows for the separation of large numbers of minimally activated, highly purified monocytes (10). U937 cells were obtained from the American Type Culture Collection, HIV-1-1M and H9 cells were a gift of R. Gallo (National Institutes of Health). U1 cells and HIV-1-Ba-L were provided by the National Institute of Allergy and Infectious Diseases AIDS Reagent Program. All cells used in these experiments (except U1 cells) were tested and found to be free of mycoplasma contamination.

 Cultures of A3.5 Cells. A3.5 cells were infected with HIV-1026 [multiplicity of infection (moi) = 1 × 10^-3] tissue culture infectious dose50 (TCID50) per cell) and cultured in Iscove’s modified Dulbecco’s medium supplemented with 10% heat-inactivated fetal calf serum (GIBCO or Sigma). TCID50 was determined by serial dilution of viral stock supernatant fluid and the induction of syncytium formation by C8166 cells as described (11). Every 3–4 days, culture supernatants were sampled for HIV-1 p24 antigen production by ELISA (DuPont/NEN). Supernatants fluids and cells were tested for their ability to induce syncytium formation in C8166 cells. Cell viability was determined by trypan blue exclusion. At selected times, cells from the cultures were stained by indirect immunofluorescence, after blocking Fc receptors with heat-aggregated immunoglobulins, using a polyclonal antiserum against HIV-1 and a fluorescein isothiocyanate-conjugated sheep antibody F(ab')2 fragment to human immunoglobulin (Organon Teknika–Cappel). The percentage of positive cells was determined by counting at least 300 cells using a Zeiss Axiophot MC100 microscope.

Abbreviations: PBMC, peripheral blood mononuclear cells; moi, multiplicity of infection; TCID50, tissue culture infectious dose50; HIV, human immunodeficiency virus; 1,25(OH)2D3, 1,25-dihydroxycholecalciferol; 25(OH)D3, 25-hydroxycholecalciferol; LTR, long terminal repeat.

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Cultures with 1,25(OH)₂D₃ and Precursors. A3.5 cells were incubated for 24 hr in the presence of various concentrations of 1,25(OH)₂D₃. The cells were washed in phosphate-buffered saline (PBS), infected with HIV-1₁₁₁₁ (moi = 1 × 10⁻³ TCID₅₀ per cell), and then cultured with the appropriate concentration of 1,25(OH)₂D₃. Every 3–4 days, supernatant fluids were assayed for HIV-1 p24 antigen production by ELISA (DuPont/NEN) and, on selected days, were assayed for HIV-1 virus yield [performed as described (11)]. An aliquot from each sample was assayed for cell viability by trypan blue exclusion. Fresh medium was added every 3–4 days with the appropriate concentration of 1,25(OH)₂D₃. For the experiment depicted in Fig. 2B and others (including those with peripheral blood monocytes), HIV-1 (moi = 5 × 10⁻³ TCID₅₀ per cell) and 1,25(OH)₂D₃ were added simultaneously to the cells.

Cocultures of PBMC. PBMC were isolated from HIV-1 seropositive individuals by Ficoll/Hypaque density centrifugation of heparinized peripheral blood (<10 units/ml). Cocultures were performed with PBMC from HIV-1 seronegative donors prestimulated for 3–4 days with phytohemagglutinin P (Difco) and interleukin 2 (Pharmacia) as described (12). Four parallel cocultures were performed: these cocultures contained 1,25(OH)₂D₃ (24 nM). Polybrene (2 µg/ml; Sigma), 1,25(OH)₂D₃ and Polybrene (24 nM and 2 µg/ml, respectively), or no 1,25(OH)₂D₃ or Polybrene.

1,25(OH)₂D₃ and Precursors. 1,25(OH)₂D₃ was a gift of M. R. Uskokovic (Hoffmann-La Roche). Cholecalciferol and 7-dehydrocholesterol were obtained from Sigma. 25-Hydroxycholecalciferol [25(OH)D₃] was a gift of M. F. Holick (Boston University School of Medicine, Boston, MA).

Surface Marker Expression Determination. A3.5 cells were incubated for 45 min with heat-aggregated immunoglobulins to block Fc receptors and then stained by indirect immunofluorescence with the relevant monoclonal antibody and fluorescein-conjugated goat anti-mouse conjugate (13). After fixation with 1% paraformaldehyde, at least 2000 cells per sample were analyzed by flow cytometry.

RESULTS

The time course of HIV-1 p24 antigen production and cell viability after infection of A3.5 cells with HIV-1₁₁₁₁ (a lymphocyte-tropic strain of HIV-1) in the absence of 1,25(OH)₂D₃ is shown in Fig. 1. No HIV-1 p24 antigen was detectable for the first 21 days of culture after acute infection of these cells. Syncytium formation with C8166 cells occurred 7 days after infection. Chronic infection ensued with 17–60% of the cells expressing HIV-1 antigens at any given time. We thus know that this cell line is susceptible to infection with HIV-1₁₁₁₁ and can be maintained continuously in culture with ongoing HIV-1 replication. The experiments carried out in the presence of 1,25(OH)₂D₃ or its precursors in the present investigations all utilized acutely infected A3.5 cells.

A3.5 cells were incubated in the presence of various concentrations of 1,25(OH)₂D₃ for 24 hr and then infected with HIV-1₁₁₁₁ (moi = 1 × 10⁻³ TCID₅₀ per cell). As shown in Fig. 2A and B, HIV-1 replication was enhanced by 1,25(OH)₂D₃ in a dose-dependent manner. Enhancement was between 1000- and 10,000-fold in the presence of 24 nM or 240 nM 1,25(OH)₂D₃ after 10–14 days of culture. Virus yield assays confirmed this augmentation; for example, virus titers (TCID₅₀ per ml) after 10 days of culture in the experiment depicted in Fig. 2A were 2.1 × 10³, 1.5 × 10⁴, 1.0 × 10⁵, and 1.0 × 10⁶ in the presence of 0 M, 2.4 nM, 24 nM, and 240 nM 1,25(OH)₂D₃, respectively. These experiments were replicated nine times with changes in the protocol that included various moi of HIV-1₁₁₁₁ (6 × 10⁻⁴, 1 × 10⁻³, and 5 × 10⁻³), concentrations of 1,25(OH)₂D₃ from 12 pM to 240 nM, and either pretreatment of cells with 1,25(OH)₂D₃ or simultaneous addition of 1,25(OH)₂D₃ and HIV-1. Enhancement was seen with every protocol at every concentration of 1,25(OH)₂D₃ tested from 7–10 days after infection of the cells with HIV-1₁₁₁₁. Enhancement was 2- to 3-fold at 12–60 pM

![Fig. 1](image1)

Fig. 1. HIV-1 p24 antigen production, cell viability, and HIV-1 antigen expression after infection of A3.5 cells with HIV-1₁₁₁₁ (a lymphocyte-tropic strain of HIV-1) in the absence of 1,25(OH)₂D₃. (A) HIV-1 p24 antigen production and percentage of viable cells. (B) IF, percentage of cells staining with anti-HIV-1 antibody (% pos.) by indirect immunofluorescence.

![Fig. 2](image2)

Fig. 2. (A) HIV-1 p24 antigen production and cell viability in A3.5 cells pretreated with 1,25(OH)₂D₃ and infected with HIV-1₁₁₁₁ that were assayed on days 10 and 14 after infection (note logarithmic scale). (B) Induction of HIV-1 replication by 1,25(OH)₂D₃ in A3.5 cells treated with 1,25(OH)₂D₃ at the time of infection with HIV-1₁₁₁₁ and assayed after 7 days of culture. The Pearson correlation coefficient for B is 0.88.
and 2.5- to 10-fold at 0.12 nM. 1,25(OH)2D3 is normally present in plasma at concentrations of ~0.12 nM; therefore, enhancement of HIV-1 replication occurs at physiologic concentrations. One-hundred- to 10,000-fold enhancement was always demonstrated after 10–14 days of culture in the presence of 24 nM 1,25(OH)2D3. This may be important since local concentrations of 1,25(OH)2D3 in vivo may exceed circulating plasma levels. No effect on HIV-1 replication was observed in the presence of the 95% ethanol used to dissolve the 1,25(OH)2D3 at a dilution of 1:2000.

The effects of 1,25(OH)2D3 on HIV-1 replication were not limited to the A3.5 monocyte line. In the experiments shown in Table 1, 1,25(OH)2D3 enhancement of HIV-1 replication in U937 cells (another monocytic line) exceeded that in A3.5 cells. However, HIV-1 replication was not induced by 1,25(OH)2D3 in the U1 clone of U937 cells. The U1 clone is chronically infected with HIV-1, and HIV-1 replication can be modulated by several cytokines and other activating agents (2, 14, 15). Enhancement of HIV-1 replication was also not observed in the lymphoblastoid cell line HD (data not shown), suggesting that the effects of 1,25(OH)2D3 on HIV-1 replication may be largely limited to monocytes.

Since monocytic lines may differ from peripheral blood monocytes in their response to 1,25(OH)2D3, we studied the effects of 1,25(OH)2D3 on the replication of a monocytic-tropic strain of HIV-1 in peripheral blood monocytes. Monocytes were separated from blood of HIV-1 seronegative donors by elutriation (counterflow centrifugation), allowed to adhere for 24 hr to plastic culture wells precoated with AB+ human serum, and then infected in vitro with HIV-1 Ba.L. (16) and treated with 1,25(OH)2D3. These cells were >98% monocytes and were >98% viable as determined by morphology, α-naphthyl esterase staining, phagocytosis of latex beads, and trypan blue exclusion. A 4- to 10-fold enhancement of HIV-1 replication, compared with infected cells cultured without 1,25(OH)2D3, was seen at concentrations of 1,25(OH)2D3 ranging from 0.12 nM to 1.0 nM after 7, 10, and 14 days of culture.

Enhancement of HIV-1 replication by 1,25(OH)2D3 was also demonstrated in cocultures of PBMC from HIV-1 seropositive individuals with PBMC from seronegative subjects. HIV-1 replication was enhanced in five of seven cultures carried out in the presence of 1,25(OH)2D3 compared with cocultures performed in the presence of Polybrene or no drug. In two of these five instances, HIV-1 was recovered only in the presence of 1,25(OH)2D3 but not when cocultures were performed with Polybrene alone or no drug. In the other three cultures, HIV-1 was detected earlier or at higher titers in the 1,25(OH)2D3-treated cells than could be detected in cultures containing Polybrene alone or no drug. Polybrene addition did not increase virus yield over that induced by 1,25(OH)2D3 alone.

The mechanism of HIV-1 enhancement by 1,25(OH)2D3 was initially investigated by determining if alterations in the expression of surface antigens by 1,25(OH)2D3 might correlate with increased HIV-1 replication; we hypothesized that alterations in monocyte CD4 expression might affect HIV-1 replication. The expression of CD14, CD11b, and CD15 antigens in A3.5 cells increased in the presence of 1,25(OH)2D3; the expression of CD4 and HLA-DR was unchanged (Table 2). The expression of CD14 increased in a dose-dependent fashion after treatment with 1,25(OH)2D3 in concentrations ranging from 24 pM to 240 nM (data not shown). Therefore, modulation of CD4 expression was not correlated with enhancement of HIV-1 replication by 1,25(OH)2D3 in A3.5 cells. It should be noted, however, that the effects of 1,25(OH)2D3 on cell surface antigen expression in different monocyte cell lines and peripheral blood monocytes can vary. For example, CD4 and HLA-DR expression are reduced in peripheral blood monocytes, whereas CD14 and class I HLA antigen expression are unaffected after exposure to 1,25(OH)2D3 (17).

To determine whether the 1,25(OH)2D3 intracellular receptor might be involved in the enhancement of HIV-1 replication by 1,25(OH)2D3, we studied the effects of 1,25(OH)2D3 precursors on HIV-1 replication. These precursors of 1,25(OH)2D3 either bind with greatly reduced affinity (104-fold) to the 1,25(OH)2D3 intracellular receptor (cholecalciferol and 7-dehydrocholesterol) or bind with somewhat reduced (103 to 104-fold) affinity (25(OH)D3; refs. 5, 18, 19). When A3.5 cells were infected with HIV-1 and cultured in the presence of cholecalciferol or 7-dehydrocholesterol at concentrations ranging up to 26 nM, no enhancement of HIV-1 replication was observed. In contrast, HIV-1 replication was enhanced in the presence of 25(OH)D3. The potency of 25(OH)D3 was ~200- to 500-fold less than that of 1,25(OH)2D3.

Table 1. 1,25(OH)2D3 enhances HIV-1 replication in U937 and A3.5 cells but not in U1 cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>1,25(OH)2D3, nM</th>
<th>HIV-1 p24 % viable</th>
<th>HIV-1 p24 % viable</th>
<th>HIV-1 p24 % viable</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3.5</td>
<td>0</td>
<td>0.10</td>
<td>98</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>14.5</td>
<td>76</td>
<td>492.0</td>
</tr>
<tr>
<td>U937</td>
<td>0</td>
<td>0.37</td>
<td>94</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>33.0</td>
<td>97</td>
<td>1107.0</td>
</tr>
<tr>
<td>U1</td>
<td>0</td>
<td>0.13</td>
<td>86</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.07</td>
<td>93</td>
<td>0.18</td>
</tr>
</tbody>
</table>

A3.5 and U937 cells were infected with HIV-1 (moi = 1 × 10−5) and treated at the start of the experiment and thereafter with 24 nM 1,25(OH)2D3. U1 cells (chronically infected with HIV-1) were cultured in the presence of various concentrations of 1,25(OH)2D3 (only 24 nM is depicted) without additional virus input. HIV p24 antigen production is expressed as ng per 106 cells. Cell viability is expressed as percentage of viable cells (% viable). ND, not done.

Table 2. Cell surface marker modulation by 1,25(OH)2D3 and 25(OH)D3 in A3.5 cells

<table>
<thead>
<tr>
<th>Antigen</th>
<th>No drug</th>
<th>1,25(OH)2D3, 24 nM</th>
<th>24 nM</th>
<th>2.4 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.5</td>
<td>3.5</td>
<td>4.3</td>
<td>10.8</td>
</tr>
<tr>
<td>CD4</td>
<td>93 (73)</td>
<td>94 (59)</td>
<td>98 (71)</td>
<td>97 (63)</td>
</tr>
<tr>
<td>CD15</td>
<td>99 (190)</td>
<td>99 (234)</td>
<td>98 (179)</td>
<td>97 (183)</td>
</tr>
<tr>
<td>CD11b</td>
<td>6.4 (198)</td>
<td>52 (51)</td>
<td>2.4</td>
<td>76 (59)</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>83 (120)</td>
<td>85 (100)</td>
<td>86 (103)</td>
<td>74 (91)</td>
</tr>
<tr>
<td>CD14</td>
<td>5.5</td>
<td>96 (450)</td>
<td>4.4</td>
<td>93 (390)</td>
</tr>
<tr>
<td>MOP9</td>
<td>6.3D3</td>
<td>89 (137)</td>
<td>4.3</td>
<td>93 (145)</td>
</tr>
</tbody>
</table>

A3.5 cells were cultured in the presence or absence of 1,25(OH)2D3 ranging from 24 pM to 240 nM (only 24 nM is depicted) or 25(OH)D3 (24 nM and 2.4 μM) and collected at 24, 48, and 72 hr. After incubation with heat-aggregated immunoglobulins to block Fc receptors, the cells were stained by indirect immunofluorescence for CD4, HLA-DR, M1, CD11b, and CD14 antigens and counted using a fluorescence-activated cell sorter.

*Mean fluorescence is given in parentheses.

1 Fluorescein isothiocyanate conjugate alone.
1,25(OH)_{2}D_{3}; 500 nM 25(OH)_{2}D_{3} enhanced HIV-1 p24 antigen production to the same extent as 0.96 nM and 2.4 nM 1,25(OH)_{2}D_{3} on days 7 and 10 of culture, respectively. These results are consistent with a mechanism of induction of replication that involves the 1,25(OH)_{2}D_{3} receptor. Typical 1,25(OH)_{2}D_{3} receptors demonstrated previously in U937 cells were also found in the A3.5 cells (data not shown; method as in ref. 20).

DISCUSSION

These studies demonstrate that 1,25(OH)_{2}D_{3} enhances HIV-1 replication in monocyte cell lines, peripheral blood monocytes, and cocultures of PBMC from HIV-1 seropositive individuals to a degree similar to or greater than that observed with the most potent regulator of HIV-1 expression (the HIV-1 tat protein) described to date. We consistently observed enhancement at physiologic concentrations of 1,25(OH)_{2}D_{3} (∼0.12 nM) in the experiments with the A3.5 cell line and peripheral blood monocytes separated by elutriation (e.g., Fig. 2B [normalized to control values without 1,25(OH)_{2}D_{3}]). This enhancement occurred in a dose-dependent manner. It should be mentioned that local concentrations of 1,25(OH)_{2}D_{3} may be higher than circulating levels. In addition, UV irradiation can transiently increase circulating levels of 1,25(OH)_{2}D_{3} in normal volunteers (25%) and dramatically increase 1,25(OH)_{2}D_{3} levels in vitamin D-deficient patients (∼300%; ref. 21). Vitamin supplements increase 1,25(OH)_{2}D_{3} and 25(OH)_{2}D_{3} levels above the physiologic level; our in vitro data indicate that this might be an undesirable effect. After performing dose–response experiments in each cell type, we chose to study higher doses in subsequent experiments to more easily measure differences in the effects on these cells.

The cellular and molecular events underlying the immunomodulatory effects of 1,25(OH)_{2}D_{3} have not been fully elucidated. One of the major effects of 1,25(OH)_{2}D_{3} is the induction of changes in calcium flux across cell membranes; this may occur after binding of 1,25(OH)_{2}D_{3} to its cytosolic receptor or more rapidly, without transcriptional effects, after interaction of 1,25(OH)_{2}D_{3} with the cell membrane (22, 23). Several lines of evidence indicate that HIV infection of cells, or the binding of HIV-1 gp120 to CD4, also results in increased levels of intracellular calcium and inositol triphosphate, which may be associated with modulation of HIV replication or pathogenic effects of HIV (24). HIV-1 gp120 induces an increase in concentration of intracellular free calcium that is associated with toxicity to rodent retinal ganglion cells and hippocampal neurons; these effects are abrogated by the calcium channel blocker nimodipine (25). Diphencylhydantoin inhibits the HIV-induced increase in concentration of intracellular calcium and potentiates the anti-retroviral activity of zidovudine (AZT) in some cell lines (26).

Steroid hormones influence transactivation of promoter regions in other retroviral systems. The mouse mammary tumor virus long terminal repeat (LTR) contains a cis-acting target [glucocorticoid response element (GRE)] for the glucocorticoid–receptor complex (27, 28). This DNA–protein/receptor interaction increases transcription from linked initiation sites relatively independently of the orientation of the GRE or its distance from heterologous promoters (27). Cellular transcription factors (the mouse homolog of NF-1, F-1) may also be important in this interaction (29, 30). NF-1 may influence the effect of various steroids on the relevant hormone response element (HRE) in this system by binding to regions outside of HRE (31).

Steroid hormones may also affect HIV-1 replication. Tamoxifen can block phorbol 12-myristate 13-acetate (PMA)-induced HIV-1 replication in the U1 clone, and tamoxifen and dexamethasone block PMA enhancement of expression of a HIV-LTR chloramphenicol acetyltransferase construct cotransfected with the tat gene in cells of monocyte and CD4+ lineage (32, 33). Some investigators have reported inhibitory effects of 1,25(OH)_{2}D_{3} on HIV-1 replication in U937 cells (34). Others have shown that 1,25(OH)_{2}D_{3} facilitates HIV-1 infection and replication in HL-60 and chronically infected U937 cells (35, 36). Our investigations demonstrate enhancement of HIV-1 replication in acutely infected monocyte cell lines (A3.5 and U937 cells) and peripheral blood monocytes; however, the U1 cell line behaves quite differently than its “parental” U937 cell line and the A3.5 cell line in experiments involving induction by 1,25(OH)_{2}D_{3}. Monocytoid cell lines often differ from each other in several ways, including cytokine production, the detailed kinetics of HIV-1 replication, cell surface marker modulation, and kinetics of cellular proliferation. Our experiments showing enhancement of HIV-1 replication in peripheral blood monocytes may be particularly important in this regard as they suggest that models showing enhancement of HIV-1 replication by 1,25(OH)_{2}D_{3} may more closely parallel the in vivo situation.

Our investigations also suggest the importance of the 1,25(OH)_{2}D_{3} intracellular receptor in these effects. Intracellular receptors for 1,25(OH)_{2}D_{3} are found in many tissues, including brain, thymus, bone marrow, intestine, colon, kidney, bone, muscle, and lung (4). Monocytes and activated, but not resting, T cells have this receptor. Several possible explanations for the effects of 1,25(OH)_{2}D_{3} observed in the present study are possible. The steroid hormone and its intracellular receptor may bind to the relevant response elements within the genome and, in the case of 1,25(OH)_{2}D_{3}, alter intracellular calcium levels or other relevant cellular correlates of 1,25(OH)_{2}D_{3} activity. Alternatively, the receptor–ligand complexes could bind to regulatory regions within the HIV-LTR that share sequence homology to the relevant steroid hormone response elements (37, 38). It is also known that steroid receptor binding sites are often found clustered with binding sites for other cellular transcription factors [e.g., NF-kB and Sp-1 (39)]; these may act synergistically to enhance HIV-1 replication or steroid hormone effects.

UV light can induce HIV-1 expression, perhaps through a mechanism involving heat-shock proteins (40, 41). UV light activation of a HIV-LTR construct in transgenic mice has been demonstrated (42). Enhancement of HIV-1 replication by 1,25(OH)_{2}D_{3} may be relevant in this in vivo system, since UV light is known to promote 25-hydroxylation of cholecalciferol in skin.

Our studies may have important biological and clinical implications. We have described a monocyte cell line that can be infected with HIV-1 and provides a model to study factors that influence HIV-1 replication during acute infection. Studies with chronically infected cells should also be possible. The addition of 1,25(OH)_{2}D_{3} to cell cultures should optimize recovery of HIV-1 and aid in the diagnosis of HIV infection. 1,25(OH)_{2}D_{3} or compounds that generate 1,25(OH)_{2}D_{3} (e.g., cholecalciferol and ergocalciferol) might have adverse effects in individuals infected with HIV-1. These compounds are used to treat several diseases, and some are readily available in health-food stores as vitamin supplements. Finally, specific antagonists of the effects of 1,25(OH)_{2}D_{3} might inhibit virus replication. Such compounds could provide novel therapeutic agents for HIV-1-infected individuals.

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