1,25-Dihydroxyvitamin D3 Upregulates Functional CXCR4 Human Immunodeficiency Virus Type 1 Coreceptors in U937 Minus Clones: NF-κB-Independent Enhancement of Viral Replication

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U937 cell clones which sustain efficient or poor replication of human immunodeficiency virus type 1 (HIV-1) (referred to herein as plus clones and minus clones, respectively) have been previously described. 1,25-Dihydroxyvitamin D3 (vitamin D3) potently induced HIV-1 replication and proviral DNA accumulation in minus clones but not in plus clones. Vitamin D3 did not induce NF-κB activation but selectively upregulated CXCR4 expression in minus clones. The CXCR4 ligand stromal-cell derived factor-1 induced Ca2+ fluxes and inhibited both constitutive and vitamin D3-enhanced HIV replication in minus clones.

Chemokine receptors have been recently shown to act as human immunodeficiency (HIV) coreceptors together with CD4 for viral entry (10, 26). Understanding which physiological factors regulate HIV-coreceptor expression in CD4+ cells is of great importance for developing strategies aimed at curtailing or preventing viral spreading in vivo.

The human promonocytic cell line U937 (34), which expresses both CD4 and CXCR4, is a well-known target for CXCR4-using (X4) T-cell-line-adapted (TCLA) strains of HIV type 1 (HIV-1) (1, 6, 12, 19, 23, 31). Efficient and inefficient patterns (referred to herein as plus patterns and minus patterns, respectively) of X4 virus replication have been described among U937 cellular clones by us (2, 13) and others (4, 18). Recently, the deficient ability of U937 minus clones (i.e., those demonstrating minus patterns) has been explained by the lack of fusogenic capacity for TCLA viruses in spite of good levels of CXCR4 expression (23).

1,25-Dihydroxyvitamin D3 (vitamin D3), a well-characterized differentiating agent for myelomonocytic cells (28), was previously reported to increase HIV expression in chronically infected U937 cells (21), including stimulated U1 cells (14). Plus or minus U937 cells (2 × 10⁵/ml) were adsorbed with pelleted HIV-1_LAI/IIIB propagated on the H9 T cell line (Advanced Biotechnology, Inc., Columbia, Md.) for 1 h at 37°C, washed, and seeded in duplicate cultures in the presence or absence of vitamin D3 in 48-well plastic plates. Culture supernatants were tested for Mg2+-dependent reverse transcriptase (RT) activity (13). Strikingly, stimulation with vitamin D3 upregulated HIV replication in minus clones to levels comparable to those observed in parallel cultures of infected plus cells. In contrast, vitamin D3 did not modulate viral replication in U937 plus clones (Fig. 1).

Modulation of NF-κB activation by vitamin D3 has been documented in different cell types (9, 37). Therefore, time course experiments were performed (5, 13) with plus and minus clones stimulated with tumor necrosis factor alpha (TNF-α), phorbol 12,myristate-13,acetate (PMA), or vitamin D3. However, unlike TNF-α or PMA, vitamin D3 stimulation did not induce activation of NF-κB in either type of U937 clones, whether they were uninfected (Fig. 2) or infected with HIV-1 (data not shown).

Lack of fusogenic capacity of X4 HIV-1 in spite of functional CXCR4 receptors has been reported as a correlate of the so-called resistance of U937 minus clones to supporting viral replication (23). U937 minus cells showed mean fluorescence intensities for CXCR4 lower, although more stable, than those of plus clones, whereas a relative downmodulation of the chemokine receptor occurred in plus cells after 4 to 5 days of culture (Fig. 3A). In agreement with a previous study (23), CXCR4 was a functional chemokine receptor in U937 minus clones in that Ca2+ fluxes were promptly demonstrated after stimulation by its ligand stromal cell-derived factor-1 (SDF-1) (Fig. 3B).

Vitamin D3 has been shown to upregulate CXCR4 mRNA in the promyelocytic cell line HL-60 (22). Consistent with its effect on HIV replication, a selective upregulation of CXCR4 mRNA expression (not shown) and cell surface density was observed in vitamin D3-treated minus clones (Fig. 4A) but not in U937 plus cells (data not shown). In contrast, vitamin D3 did not affect CD4 surface expression in minus clones (data not shown).

Minus U937 cells were differentiated for 72 h with vitamin D3 and then infected with DNase-treated HIV-1_LAI/IIIB previously propagated and titered on phytohemagglutinin-blasts (multiplicity of infection = 1). After virus adsorption for 1 h at 37°C, excess virus was removed by extensive washing. Accumulation of HIV-1 proviral DNA was quantified by real-time PCR (16) with an ABI Prism 7700 Sequence Detector (Perkin-Elmer Applied Biosystems, Foster City, Calif.). The following primer pair set and probe in gag were used: forward primer, for 5'-ACA TCA AGC AGC CAT GCA AAT-3'; reverse primer, rev 5'-ATC TGG CCT GGT GCA ATG GG-3'; and probe, probe 5'[FAM] CAT TGA GGA AGC TGC AGA ATG GGA TAG A (TAMRA)-3'. Accelerated kinetics and higher levels of HIV proviral DNA accumulation were observed within a time frame compatible with a single round of...
HIV replication (12 to 24 h) in vitamin D3-stimulated cells compared to unstimulated cells (Fig. 4B).

In order to demonstrate that HIV-1 infection and spreading in U937 minus clones occurs in a CXCR4-dependent manner (3, 24), U937 minus clones were adsorbed with HIV-1LAI/IIIB (propagated on H9 cells) and then cultured either alone or in the presence of vitamin D3, SDF-1, or both agents. Unlike what was previously shown in a fusogenicity assay (23), SDF-1 strongly inhibited both constitutive and vitamin D3-enhanced HIV replication in U937 minus clones (Fig. 4C).

Vitamin D3 has been previously shown to exert both positive and negative effects on primary monocytic cells or cell lines, including U937 cells (7, 14, 21, 25, 30, 32). However, at least in U937 minus clones, vitamin D3 restored the inefficient HIV replication without inducing either TNF-α secretion (not shown) or NF-κB activation. Thus far, CXCR4 is the only chemokine receptor known to act as a coreceptor for TCLA HIV-1 (11). Little is known, however, of the physiologic regulation of CXCR4, although interleukin-4 has been recently shown to increase its expression (17). In our study, vitamin D3 selectively enhanced the density of CXCR4 receptors on the cell surface of minus clones but not plus clones. Consistent with the lower constitutive levels of CXCR4 expressed by minus clones compared to plus clones, this effect was responsible, at least in part, for the potent enhancement of HIV replication induced in U937 minus clones by vitamin D3 stimulation. These conclusions were supported by the kinetic quantitative analysis of proviral DNA synthesis. Of interest, Moriuchi et al. (23) have shown that both SDF-1 and the anti-CXCR4 12G5 monoclonal antibody (MAb) poorly inhibited HIV-1 Env-mediated fusion in one U937 plus clone, suggesting the existence of additional, though unidentified, entry cofactors (23). We have confirmed that SDF-1 minimally induced Ca2+ fluxes and poorly inhibited HIV replication in plus clones (2a). However, we here dem-

![FIG. 1. Vitamin D3 selectively upregulates viral replication in U937 minus clones. One minus clone and one plus clone were infected with H9-derived HIV-1LAI/IIIB in the absence of vitamin D3 (unstimulated [Unst.]) or presence of 10 nM vitamin D3 (Vit. D3). Mean values of duplicate cultures are shown. The data are representative of four independently performed experiments, including two additional minus clones and one plus clone. Low but detectable levels of viral replication were observed in minus clones (peak RT activity, 930 cpm/μl at day 17 postinfection).](http://jvi.asm.org/)
clones. U937 minus clones infected with HIV-1LAI/IIIB (propagated on H9 cells) both constitutive and vitamin D3-enhanced viral replication in U937 minus cells, likely as a result of an accelerated spreading of infection.

(C) SDF-1 inhibits regression (r) of proviral DNA copy number in U937 minus cells. (A) U937 minus clones either unstimulated (Unst.) or stimulated with 10 nM vitamin D3 (Vit. D3) were cultured with medium alone (unstimulated [unst.]), 10 nM vitamin D3 (Vit. D3) or medium alone (unstimulated [unst.]) before infection with HIV-1LAI/IIIB grown and titered on phytohemagglutinin-blasts. The thermal cycling conditions were 50°C for 2 min, 95°C for 12 min, and 40 cycles of 95°C for 15 s and 65°C for 1 min. The DNA extracted from serially diluted chronically infected ACH-2 T cells, containing one proviral DNA copy per cell (27), was used as a standard. The DNA extraction and amplification conditions were optimized to yield a linear relationship between the number of copies of proviral DNA and the fluorescence intensity in the range of 2 to 31,250 cells.

FIG. 4. Vitamin D3 enhances CXCR4 expression and HIV infection in U937 minus cells. (A) U937 minus clones either unstimulated (Unst.) or stimulated with 10 nM vitamin D3 (Vit. D3) or medium alone (unstimulated [unst.]), 10 nM vitamin D3 (Vit. D3) or medium alone (unstimulated [unst.]) before infection with HIV-1LAI/IIIB grown and titered on phytohemagglutinin-blasts. The thermal cycling conditions were 50°C for 2 min, 95°C for 12 min, and 40 cycles of 95°C for 15 s and 65°C for 1 min. The DNA extracted from serially diluted chronically infected ACH-2 T cells, containing one proviral DNA copy per cell (27), was used as a standard. The DNA extraction and amplification conditions were optimized to yield a linear relationship between the number of copies of proviral DNA and the fluorescence intensity in the range of 2 to 31,250 cells.

cannot exclude the possibility that vitamin D3 is able to alter cell death in chronic infection of T cells. Do tumor necrosis factor alpha and gamma interferon selectively kill HIV-infected cells? J. Virol. 68:2598–2604.

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