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Vitamin D_3 inhibits TNFα-induced latent HIV reactivation in J-LAT cells

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Abstract 1,25-Dihydroxyvitamin D_3 ($1{,}25(OH)_2D_3$) is known to suppress NF-kB activity by interfering with its pathways. The aim of this study was to investigate the ability of $1{,}25(OH)_2D_3$ in reducing the reactivation of the HIV virus J-LAT cells, an established model of latently infected cells, which were treated with TNFalpha (100 ng/ml) for 2 h with or without 24 h $1{,}25(OH)_2D_3$ (100 nM) pretreatment. Reactivation of HIV RNA in J-LAT was evaluated in terms of green fluorescent protein (GFP) expression. The same experimental setting was repeated on T cells from HIV-infected patients. Treatment with TNFalpha was associated with a 16 % increase in GFP+ cells and a five-fold increase in *unspliced* HIV RNA expression ($p < 0.04$). Pretreatment of J-LAT cells with $1{,}25(OH)_2D_3$ for 24 h followed by TNFalpha (100 ng/ml) for 2 h reduced the percentage of GFP+ cells by 8 %; moreover, a 2.4-fold decrease in *unspliced* HIV RNA expression was observed ($p < 0.002$). In T cells from patients, treatment with TNFalpha significantly increased *unspliced* HIV RNA expression (sixfold increase, $p < 0.02$), whereas prestimulation with $1{,}25(OH)_2D_3$ reduced its expression (2.5-fold decrease, $p < 0.02$) compared to controls.$1{,}25(OH)_2D_3$ is able to reduce the ability of TNFalpha to upregulate the transcription of HIV RNA from latently infected cells. These data provide further understanding of the pathogenic mechanisms regulating viral reactivation from latent reservoirs, along with new insight in viral internalization.

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Keywords CD4+ · HIV · Vitamin D_3 · J-LAT

Abbreviations

CD4	Cluster of differentiation 4
TNF-α.	Tumor necrosis factor
$1{,}25\alpha(OH)_2D_3$	Vitamin D_3
Vitamin D	VitD3
SD	Standard Deviation
MO	Monocyte
LY	Lymphocyte
NFKB	Nuclear factor kappa B
GFP	Green fluorescent protein

Introduction

1α,25-dihydroxyvitamin-D_3 ($1{,}25(OH)_2D_3$) has pleiotropic effects on cellular growth control, cell differentiation, and modulation of the immune response. Several experimental evidences have been obtained in the last decade that supports the key role played by the $1{,}25(OH)_2D_3$ in the control of both innate and acquired immune responses [1–4]. Lanolin in the skin is converted to 7-dehydrocholesterol, which is converted to pre–vitamin D with exposure to ultraviolet (UV) rays from the sun. Pre-vitamin D enters the circulation and is metabolized to 25-hydroxyvitamin D, the major circulating form of the vitamin (which has a circulating half-life of approximately 15 days). It is subsequently converted to the active form, 1,25-dihydroxyvitamin D_3 (called Vitamin D_3), in the kidneys by the 25-hydroxyvitamin-D_3 1-α-hydroxylase (CYP27B1) enzyme [5]. HIV patients frequently have low Vitamin D_3 levels

[6, 7]. Additionally, patients treated with nonnucleoside reverse transcriptase inhibitors and protease inhibitors are at increased risk of Vitamin D_3 deficiency [8–11]. Therefore, Vitamin D3 deficiency is common in HIV-infected patients regardless of treatment status, viral load, or CD4+lymphocyte count. There is growing recognition of an association between Vitamin D deficiency and the pathogenesis and course of HIV disease. Vitamin D deficiency is common in HIV infection. It is present in 25–75 % of infected persons and has been associated with more rapid disease progression. Infants born from HIV-infected women with Vitamin D deficiency are at increased risk of infection and have decreased survival. A number of studies have indicated associations between low vitamin D levels and HIV disease. In the cells, Vitamin D_3 binds the nuclear Vitamin D_3 Receptor (VDR) that operates as a transcription factor activating or repressing specific target genes [12].

Several papers have shown the capacity of Vitamin D_3 to modulate the NFκB pathways. It has been shown that VDR signaling intrinsically suppresses NF-kB activation since the base-line NFκB activity is elevated in the case of genetic VDR deletion [13, 14]. It has been reported that 1,25(OH)2D3 arrests p65 nuclear translocation, blocks NFκB DNA binding, increases IkBα levels, or stabilizes IkBα protein [13–19]. It has also been shown that $1,25(OH)_2D_3$ suppresses RelB transcription [20] and reduces p105/p50 and c-rel protein levels [21]. Interestingly, p65 has been reported to physically interact with ligated VDR to modulate the transactivating activity of the VDR [22]. Despite all of these reports, a convincing mechanism to explain the relatively rapid inhibitory action of vitamin D hormone on NF-kB activity is still lacking.

The role of NFκB in activating HIV transcription has been extensively analyzed. In normal human CD4+ T lymphocytes, NFκB binding activity is low and consists predominantly of p50, but not p65, DNA binding. T cell activation results in the formation of p50–p65 NFκB complexes and enhancer-dependent HIV LTR transactivation, indicating that unstimulated CD4+ T lymphocytes offer a cellular environment of low permissiveness to HIV LTR function. In HIV-infected T cells, NFκB-dependent transactivation is essential for HIV LTR induction. Interestingly, even the function of HIV Tat in resting CD4 T lymphocytes depends on kB responsive elements in the LTR [23].

In the present study, we have evaluated the capacity of Vitamin D3 to interfere with the transcription of HIV-1 virus. In order to demonstrate our hypothesis, we used the J-LAT cell line 8.4, which has a latent HIV provirus in which GFP replaces Nef coding sequence, and CD4+ T cells from HIV drug-naïve patients with high viral load. We show that Vitamin D3 has the ability to reduce the production of HIV RNA, likely via NFκB. These results indicate that the Vitamin d3 is an excellent candidate to reduce HIV viral transcription.

Methods

Cells and HIV-1 RNA reactivation

J-LAT 8.4 cells were kindly provided by Professor Guido Poli (University of Milano). Cells (5×10^5/mL^{-1}) were cultured in Gibco RPMI-1640 media, supplemented with 10 % FBS and 5 % penicillin, streptomycin at 37 °C, and 5 % CO_2 under sterile conditions. For HIV-1 RNA reactivation experiments, cells were mixed with TNFalpha (100 ng/ml) for 2 h with or without a 24-h pretreatment with $1,25(OH)_2D_3$ (100 nM). After that, the cells were collected for flow cytometry and the RNA/protein analysis.

Primary cells

Human peripheral blood mononuclear cells (PBMCs) were obtained by Ficoll (FicollHistopaque; Sigma) density centrifugation from 45 blood samples (10 ml) of highly viremic HIV-1 drug-naïve patients (cART naïve patients) (see Table 1) (Unit of Infectious Diseases, Catania, Italy). CD4+ T cells were negatively selected using magnetic beads (CD4+ T cell isolation kit II; MiltenyiBiotec) as per manufacturer's instructions. CD14+ cells were isolated from PBMCs using the MACS CD14 isolation kit (MiltenyiBiotec) according to the manufacturer's instructions. CD4+ T cells and CD14+ were cultured in RPMI 1640 supplemented with 10 % FBS, 100 IU penicillin, 100 ng/ml streptomycin, 0.1 HEPES, and 2 mMl-glutamine. Lymphocyte and monocyte analyses were performed by multicolor flow cytometry (Cytomics FC 500, Beckman Coulter) using the following antibodies (Beckman Coulter): anti-CD14, anti-CD64, and anti-CD11c (BD Biosciences). Monocytes identified as CD14 +, CD11c+, and CD64+ cells have shown purity greater than 90–95 % (*data not shown*). The cells were treated as described in the section "*Cell treatment.*" All of the patients gave informed written consent, and this study was reviewed and approved by the Institutional Ethical Committee board of Hospital Clinic (Unit of Infectious Diseases, Catania, Italy).

Table 1 HIV-1 high viral load

Subjects recruited	45
Middle age	50 years ± 7.7
Median $CD4^+$ counts	123.5 cell/μl
Viral load average	12,480

Cells treatments

Preliminary studies were performed to assess 1α,25$(OH)_2D_3$ dose and time of treatment (data not shown). Cells were treated with different concentrations of 1α,25$(OH)_2D_3$ (100, 500, and 1000 nM) at different times (8, 16, and 24 h). Same concentrations of ethanol were used as control. Thereafter, we carried out the following cellular treatments: 1α,25$(OH)_2D_3$ (100 nM) for 24 h, TNFalpha (100 ng/ml) (Peprotech, Milan, Italy) for 2 h, and a 24-h pretreatment with 1α,25$(OH)_2D_3$ (100 nM) with a subsequent stimulation with TNFalpha (100 ng/ml) for 2 h. Treated cells with ethanol and PBS were used as a control.

Vitamin D3 [1a,25$(OH)_2D_3$]

A stock solution of 5 mM 1α,25$(OH)_2D_3$ (Sigma-Aldrich, Milan, Italy) was prepared in 100 % ethanol and stored as sterile aliquots at −20 °C. All treatments with 1α,25$(OH)_2D_3$ were carried out in dark condition.

RNA extraction, reverse transcription-PCR (RT-PCR), and quantitative PCR (qPCR) for expression analysis

Total RNA and DNA were extracted from cells using TRIzol reagent (Invitrogen Life Technologies, Italy) according to manufacturer's instructions. cDNA was obtained from 100 ng of total RNA using RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, Milan, Italy) in a 20 μl reaction solution. The indicated gene products were analyzed by PCR with specific oligonucleotides, followed by visualization in agarose gels. Where indicated, the quantification of gene products was performed by real-time PCR using LightCycler 480 SYBR green I master mix (Roche, Indianapolis, IN). Each value was corrected by human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expressed as relative units. Sequences of oligonucleotides used for real-time PCR are shown in Table 2. Data are presented as mean% ± SD of at least three independent experiments. Differences were analyzed by Student's t test, with $p < 0.05$ being considered statistically significant.

NFKB inhibitor treatment

J-LAT cells were pretreated at 37 °C for 45 min with 5 μM [24] of the NFκB inhibitor Bay11-7082 (Calbiochem, San Diego, CA) (2, 14), or dimethyl sulfoxide as solvent control, prior to the stimulation Bay11-7082 showed no toxicity on J-LAT cells at the tested concentrations (data not shown). Cells were then stimulated with TNFalpha (100 ng/ml) and incubated at 37 °C. At 2-h post-TNFalpha exposition, cells were collected, and total RNA was isolated using TRIzol reagent (Invitrogen Life Technologies, Italy) according to the manufacturer's protocol and the proteins were extracted with NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific, Milan, Italy). The RNA was reverse transcribed into cDNA, as previous described.

Western blot

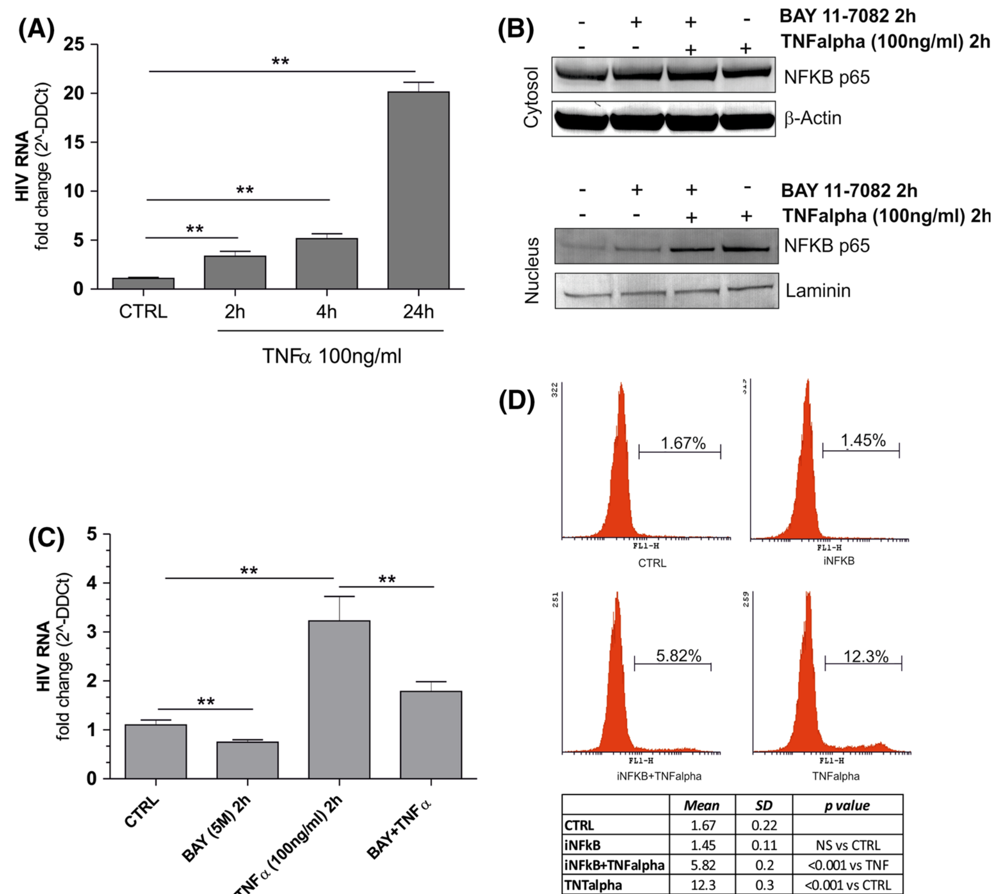
Cells were harvested by trypsinization and cytoplasmatic proteins were extracted using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Scientific, Milan, Italy). The lysates were collected for Western blot analysis. Protein concentrations were determined according to the Bradford method [25]. Protein levels were visualized by immunoblotting with antibodies against human NFKB p65 (sc-372, Santa Cruz Biotechnology, DBA, Italy), human β-Actin (sc-69879, Santa Cruz Biotechnology, DBA, Italy), and human Laminin β1 (sc-377000, Santa Cruz Biotechnology, DBA, Italy). Briefly, 40 μg of lysate supernatant was resolved by SDS/polyacrylamide gel electrophoresis on 4–20 % Mini-PROTEAN® TGX™ (BIO-RAD, Milan, Italy) and transferred to a nitrocellulose membrane trans-Blot Turbo mini nitrocellulose (BIO-RAD, Milan, Italy) using a semidry transfer apparatus (BIO-RAD, Hercules, CA). The membranes were incubated with 5 % milk in 10 mM Tris–HCl (pH 7.4), 150 mM NaCl, 0.05 % Tween 20 (TBST) buffer at 4 °C overnight. After washing with TBST, the membranes were incubated with a 1:2000 dilution of anti-NFκB p65, anti-β Actin or anti-Laminβ1 antibodies for 1 h at room temperature with constant shaking. The filters were then washed and probed with horseradish peroxidase-conjugated

Table 2 Primer sequence for Real-time PCR

Name	Sequence	Tm	Size
HIV-1 RNA-LTR F	GCCTCAATAAAGCTTGCCTTGA	64	101*
HIV-1 RNA-LTR R	TCCACACTGACTAAAAGGGTCTGA	70	
GAPDH F	CTGCACCACCAACTGCTTAG	62	272*
GAPDH R	AGGTCCACCACTGACACGTT	62	

* [46]

** [47]



	Mean	SD	p value
CTRL	1.67	0.22	
iNFkB	1.45	0.11	NS vs CTRL
iNFkB+TNFalpha	5.82	0.2	<0.001 vs TNF
TNTalpha	12.3	0.3	<0.001 vs CTRL

Fig. 1 TNFalpha induces the reactivation of HIV latent virus via NFκB. **a** Time point expression of HIV-1 RNA in J-LAT 8.4 treated with TNFalpha (100 ng/ml); **b** Western blotting analysis of NFKB p65 in J-LAT treated with BAY11-7082 (5 μM) and TNFalpha; **c** RNA expression of HIV-1 RNA in J-LAT treated with BAY11-7082 (5 μM) and TNFalpha (100 ng/ml); **d** Fluorescence analysis for J-LAT GFP + cells treated with BAY11-7082 (5 μM) and TNFalpha (100 ng/ml). Data are expressed as mean ± SD of at least three independent experiments. $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$, ns not significant, compared to cells untreated. The figure shows representative data from one of three replicate experiments

antirabbit IgG-HRP (sc-2030 Santa Cruz Biotechnology, DBA, Italy) for NFκB p65, goat antimouse IgG-HRP (sc-2005 Santa Cruz Biotechnology, DBA, Italy) for β-actin and Lamininβ1 at a dilution of 1:2000. Detection was performed with the TMB-Blotting 1-Step Solution according to the manufacturer's instructions (Invitrogen Life Technologies, Italy).

Flow cytometry analysis for J-LAT GFP fluorescence

GFP fluorescence was measured using a Cytomics FC500 MPL cytometer (Beckman Coulter, Fullerton, CA). A two-parameter analysis was used to distinguish GFP-derived fluorescence from the background. Fluorescence was represented on a logarithmic scale.

Statistical analysis

Statistical analysis was performed using Graph-Pad Prism 5 software. Data are expressed as mean ± standard deviation (SD). Significance was assessed by Two-tailed paired Student's *t* test. Values of $p < 0.05$ were considered statistically significant.

Results

Vitamin D_3 reduces the number of J-LAT GFP+ positive cells after stimulation with TNFalpha

In this study, we used the J-LAT 8.4 cell line, which bears a latent HIV provirus in which GFP replaces the Nef coding sequence. These cells are commonly used to assess the HIV provirus reactivation. Reactivation of HIV provirus in this system determines the expression of the green fluorescent protein (GFP). In a preliminary step, we evaluated whether the treatment with TNFalpha (100 ng/ml) for 24 h was able to induce the expression of HIV-1 RNA. It is known that treatment of J-LAT cells with TNFalpha induces the efficient expression of HIV-1 RNA via NFκB [26–28]. We observed that TNFalpha (100 ng/ml) was able to induce HIV-1 reactivation as detected by determining RNA expression levels (2 h, fold 3.36, $p < 0.05$; 4 h, fold 5.15 $p < 0.05$ and 24 h, fold 20.14, $p < 0.005$) and percentage of GFP+ cells (Fig. 1a, c). In addition, the inhibition of NFκB p65 with BAY 11-7082 (5 μM for 2 h) (Fig. 1b) reduced the internalization of p65, the expression of HIV RNA (Fig. 1c), and the percentage of GFP+ cells (Fig. 1d).

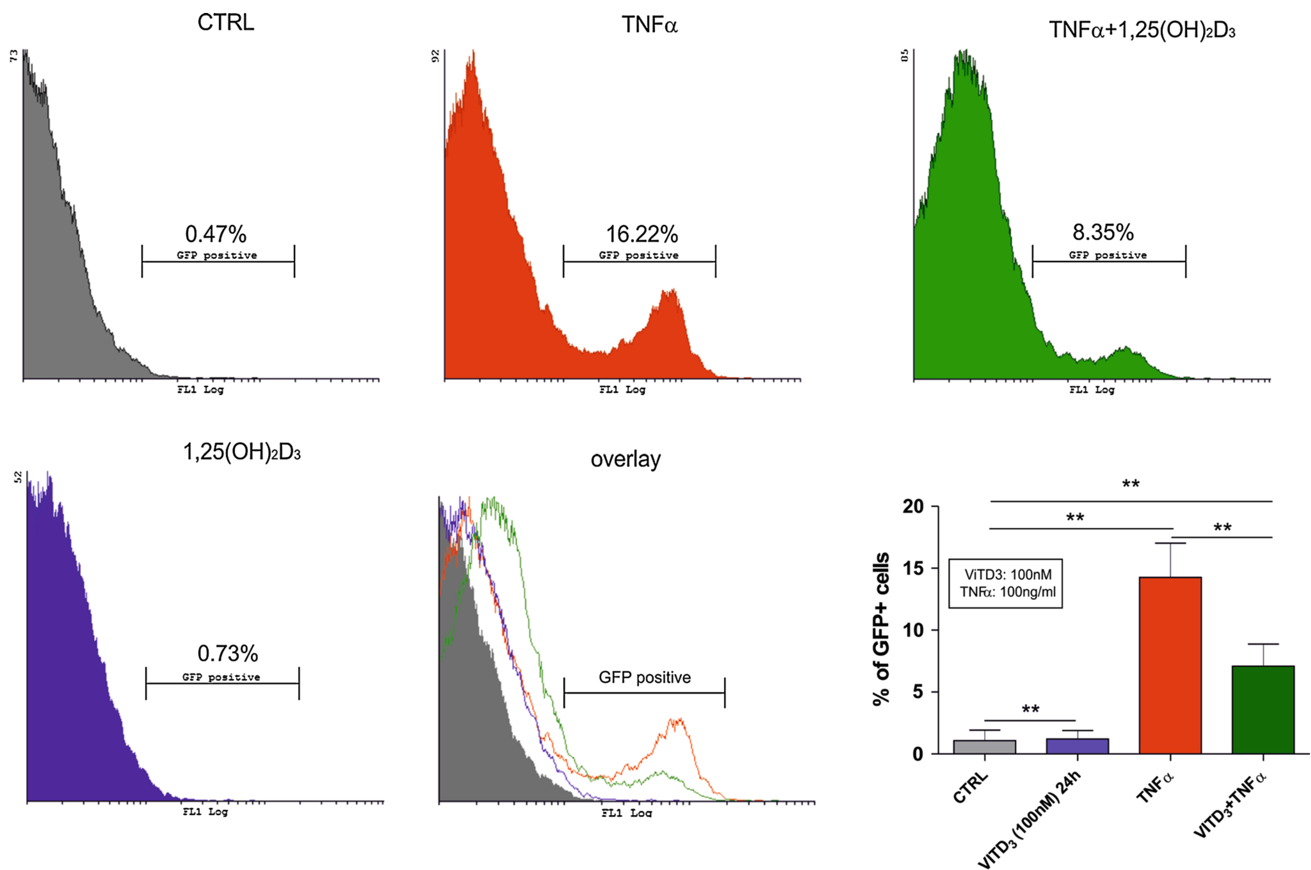


Fig. 2 Vitamin D3 interferes with the TNFalpha activation pathways of GFP fluorescence analysis in J-LAT 8.4 treated with TNFalpha (100 ng/ml) for 2 h (h) with or without 24 h pretreatment with $1,25(OH)_2D_3$ ($VitD_3$) (100 nM). TNFalpha stimulation increases the GFP+ cells after 2 h (16.22 %) and treatment with $VitD_3$ significantly reduces the number of GFP+ cells (8.35 %). The analysis was performed using a Cytomics FC500 MPL cytometer (Beckman Coulter, Fullerton, CA). A two-parameter analysis was used to distinguish GFP-derived fluorescence from the background. Fluorescence was represented in a logarithmic scale

We show that 24-h pretreatment with $1\alpha,25(OH)_2D_3$ (100 nM) and subsequent stimulation with TNFalpha (100 ng/ml) is associated to a significant reduction in the percentage of GFP+ cells (8.35 vs. 16.22 % in the control TNFalpha-treated group) (Fig. 2). The 24-h pretreatment with $1\alpha,25(OH)_2D_3$ (100 nM) significantly reduces the p65 nuclear translocation (fold 0.56, $p < 0.0002$) in J-LAT 8.4 cells under stimulation with TNFalpha (100 ng/ml for 2 h) (Fig. 3). This finding could justify the reduction of HIV-1 RNA expression (Fig. 4a). In particular, $1\alpha,25(OH)_2D_3$ treatment for 24 h significantly reduces the expression of HIV-1 RNA (fold 0.6, $p < 0.005$) compared to the control (untreated J-LAT cells) and the stimulation of J-LAT cells with TNFalpha after 24 h of $1\alpha,25(OH)_2D_3$ exposition is associated to significantly lower HIV-1 RNA levels (fold 2.1, $p < 0.005$) compared to J-LAT cells stimulated with TNFalpha alone (fold 4.9, $p < 0.00$) (Fig. 4a).

Vitamin D_3 reduces the expression of HIV RNA in HIV patients CD4+T cells

In order to confirm the data obtained using the J-LAT cells, we replicated the same experimental conditions on CD4+ T cells isolated from HIV drug-naive patients with high viral load (>10,000 RNA copies/ml).

We show that the CD4+ lymphocytes (LY) from HIV-1 patients present significantly higher levels of HIV RNA (fold 53.94, $p < 0.005$) compared to monocytes (MO). Vitamin d3 treatment significantly reduced HIV RNA expression levels (fold 21.74 $p < 0.005$) compared to the untreated cells. TNFalpha stimulation significantly increased the levels of LTR after 2 h of exposure (fold 79.53, $p < 0.005$) compared to control cells and LTR levels were reduced in the cells pretreated for 24hs with Vitamin D_3 (fold 34.03, $p < 0.005$) (Fig. 4b). These data

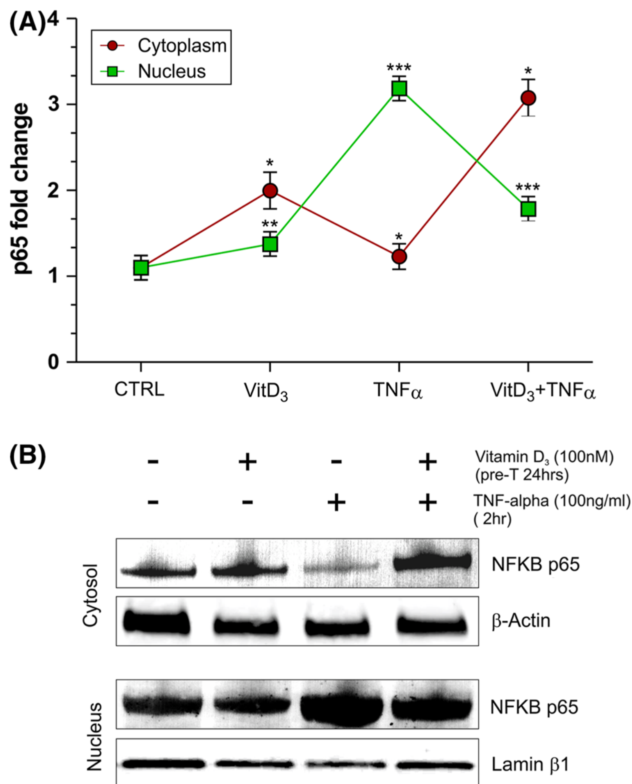


Fig. 3 p65 nuclear translocation in J-LAT with and without Vitamin D3 exposition. J-LAT treated with TNFalpha (100 ng/ml) significantly increases the p65 nuclear translocation (fold 2.5, $p < 0.0001$). J-LAT pretreatment with Vitamin D3 (100 nM) reduces significantly the p65 nuclear translocation (fold 0.8, $p < 0.0001$). In J-LAT co-stimulated with Vitamin D3 and TNFalpha, we have shown that the p65 nuclear translocation was reduced significantly (fold 0.56, $p < 0.0001$). Data are expressed as mean ± SD of at least three independent experiments. $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$ compared to untreated cells. The figure shows representative data from one of three replicate experiments

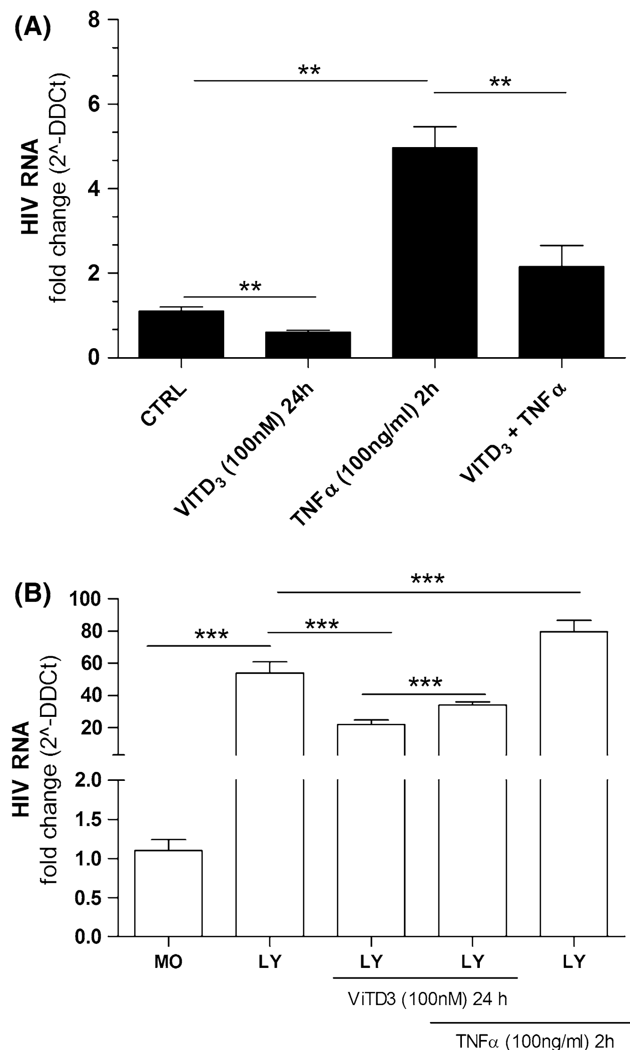


Fig. 4 Modulation of HIV RNA by Vitamin D3 in J-LAT cells and HIV CD4+Tcells. **a** PCR analysis in J-LAT 8.4 treated with TNFalpha (100 ng/ml) for 2 h with or without 24 h pretreatment with 1,25(OH)$_2$D$_3$ (VitD3) (100 nM). VIT D3 treatment for 24 h significantly reduces the expression of HIV-1 RNA (fold 0.6, $p < 0.005$) compared to the control (J-LAT untreated) and J-LAT stimulation with TNFalpha after 2 h of Vitamin D3 exposition produces significantly lower levels (fold 2.1, $p < 0.005$) compared to J-LAT treated with TNFalpha alone (fold 4.9, $p < 0.00$). **b** PCR analysis in CD4+T cells isolated from HIV naive patients with high viral load. TNFalpha stimulation significantly increases the levels of HIV-1 RNA after 2 h of exposition (fold 79.53, $p < 0.005$) compared to monocyte (MO), lymphocyte (LY) and was reduced in the cells treated for 24hs with vitamin D3 (fold 34.03, $p < 0.005$). Data are expressed as mean ± SD of at least three independent experiments. $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$ compared to untreated cells

confirm the antiviral role played by the Vitamin D$_3$ in HIV infection.

Discussion

In this article, we demonstrate that Vitamin D$_3$ is able to modulate the expression of HIV-1 RNA in J-LAT cells and in HIV-1 drug-naïve patients CD4 T cells (Fig. 5). Our results show that treatment of J-LAT cells with TNFalpha determines an increase in the expression of HIV provirus. Furthermore, the inhibition of NFκB significantly reduces the expression of LTR and the percentage of GFP+ cells in the J-LAT cell line. These results are in accordance with the evidence that the NFκB signal transduction pathway is essential for viral transcription [29]. Previous studies have found that the intracellular efficiency of HIV-1 gene expression and replication is due in part to the ability of HIV-1 to co-opt host signaling pathways to activate viral transcription [30]. The promoter-proximal (enhancer) region of the HIV-1 long terminal repeat (LTR) contains two adjacent NFκB binding sites (–109 to –79) that play a central role in mediating inducible HIV-1 gene expression. In fact, transdominant mutants of IkBa that block NFκB

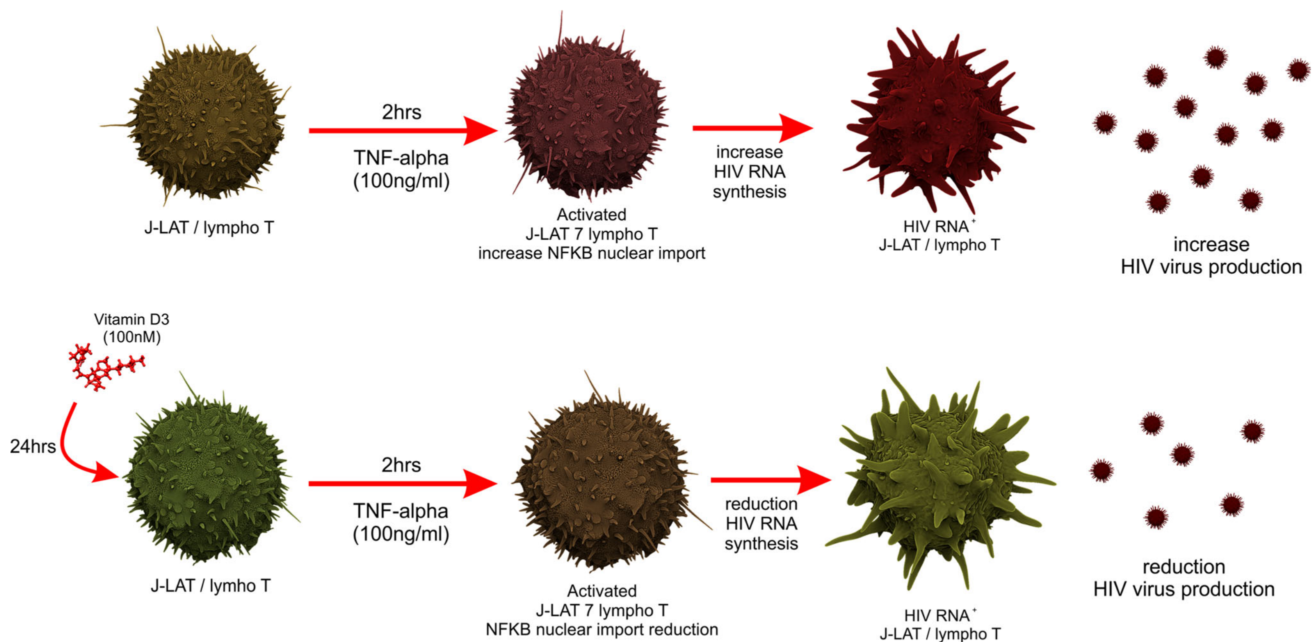


Fig. 5 Graphical representation of Vitamin D3 action in J-LAT and in HIV-1 CD4+Tcells. The graphical representation shows the modulation role played by the Vitamin D3 in J-LAT and in HIV-1 CD4+T cells isolated from HIV-1 naive patients with high viral load

induction also inhibit *de novo* HIV-1 infection in T cells by interfering with viral transcription [31–33]. Additional evidences come from the internalization of the p50/p65 complex. In normal human CD4+T lymphocytes, NFκB binding activity is low and consists predominantly of p50, but not p65, binding to the DNA. T cell activation results in the formation of p50-p65 NFκB complexes and enhancer-dependent HIV-1 LTR transactivation [29], indicating that unstimulated CD4+T lymphocytes offer a cellular environment of low permissiveness to HIV-1 LTR function. In HIV-1-infected T cells, the NFκB-dependent transactivation is essential for HIV-1-LTR induction. Interestingly, even the function of HIV-1 Tat in resting CD4+T lymphocytes depends on kB responsive elements in the LTR sequence. Furthermore, CD4+T lymphocytes carrying an infectious HIV-1 provirus with point mutations in these elements fail to transcribe viral RNA upon cell activation [23]. However, other studies indicate that NFκB sites are not absolutely required for viral growth, since HIV-1 will grow, albeit slowly, in the absence of NFκB domains [34].

In light of this evidences, it seems clear that the inhibition of NFκB pathways is a hotspot for the transcription of HIV-1 virus. It is evident that the use of natural compounds that interfere with the NFκB pathways is ideal for the treatment of virus replication. Vitamin D3 is one of the most potent natural inhibitors of NFκB. Consistently, many studies have shown that 1,25$(OH)_2D_3$ down-regulates a variety of genes, including IL-12, IL-8, MCP-1, PAI-1, angiotensinogen, and microRNA-155 by blocking NFκB activation [35, 36].

Severe hypovitaminosis D is common among HIV patients. Ansemant and collaborators have shown in a cross-sectional study that 36 % of HIV-infected outpatients suffer from severe hypovitaminosis D [37]. Low serum levels of 25-hydroxyvitamin D are associated with impaired CD4 recovery following HAART [38], possibly due to Vitamin D_3-associated production of naive CD4 cells that occur during immune reconstitution [39]. Importantly, low serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels correlate with HIV-1 disease progression and mortality [39] and low 25-hydroxyvitamin D plasma levels seem to affect the probability of being infected with HIV-1. Indeed, Mehta et al. [40] observed that the risks of HIV-1 infection and neonatal death are higher in children born to women with hypovitaminosis D.

Finally, independent reports suggest a protective role of Vitamin D in TB and opportunistic infections in HIV-1 patients [41, 42], partly by inducing autophagy and by inhibiting the expression, secretion and activity of MMP7, MMP9, and MMP-10 [43–45].

In conclusion, the effects of vitamin D appear to be manyfold. On one hand, vitamin D supplementation in HIV-infected subjects can promote improved antibacterial immunity. On the other hand, vitamin D inhibits viral replication upon immune activation, by blocking the NFκB pathway. Our data support the role for Vitamin D_3 in the control of

HIV-1 infection, and provide a biological explanation for the benefits of Vitamin D_3 in HIV-1 patients. Therefore, results from this study provide support for the usefulness of vitamin D supplementation in HIV-1 patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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