Antiviral Research 94 (2012) 57–61

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Short Communication

The 1α,25-dihydroxy-vitamin D3 reduces dengue virus infection in human myelomonocyte (U937) and hepatic (Huh-7) cell lines and cytokine production in the infected monocytes

Henry Puerta-Guardo [a], Sergio Isaac De la Cruz Hernández [a,c], Victor H. Rosales [b], Juan E. Ludert [a], Rosa María del Angel [a,*]

[a] Departamento de Infectómica y Patogénesis Molecular, México, D.F., Mexico
[b] Laboratorios centrales Centro de Investigación y Estudios Avanzados, México, D.F., Mexico
[c] Departamento de Virología, Instituto de Diagnostico y Referencia Epimiológicos (InDRE), México, D.F., Mexico

ARTICLE INFO

Article history:
Received 1 July 2011
Revised 23 January 2012
Accepted 10 February 2012
Available online 22 February 2012

Keywords:
Dengue
Dengue virus
Vitamin D
Cytokines
Antivirals

ABSTRACT

Dengue is the most important mosquito-borne viral infection in humans. Recent evidence suggests that vitamin D influences virus replication. In this work, the effect of vitamin D treatment on dengue virus infection in human hepatic Huh-7 cells and on virus infection and cytokine production in the human monocytic U937 cells was evaluated. Exposure to 1α,25-dihydroxy-vitamin D3, resulted in a significant reduction in the number of infected cells, in conditions where cell viability was not affected. Viral replication in monocytic cells was more susceptible to vitamin D3 than replication in the hepatic cells. Moreover, vitamin D3 significantly reduced the levels of proinflammatory cytokines (TNF-α, IL-6, IL-12p70 and IL-1β) produced by infected U937 cells. These results suggest that vitamin D3 may represent a potentially useful antiviral compound.

Dengue virus (DENV) is the etiological agent of the most important mosquito-borne viral disease in humans. Clinical manifestations range from asymptomatic and mild manifestations, known as dengue fever, to the hemorrhagic and more severe forms of the infection, called dengue hemorrhagic fever and dengue shock syndrome (Gubler, 2006). Currently, there are no licensed vaccines or specific antiviral drugs for dengue. For these reasons, the study of non-vaccine alternatives to prevent dengue disease or to avoid the severe forms of the infection is relevant (Noble et al., 2010; Shum et al., 2010).

The 1α,25-dihydroxy-calcitriol, vitamin D3 (VD3), is a biologically active form of the 7-dihydroxy-cholesterol in the skin and the 25-dihydroxy-vitamin D in liver and kidney, which has widespread effects on cellular differentiation and proliferation, and immune responsiveness in monocytes/macrophages, dendritic cells and lymphocytes (Bruce et al., 2010). The use of VD3 has been proposed for the treatment of autoimmune, bacterial and viral infectious diseases (Baeke et al., 2010; Zasloff, 2006). However, the evidence for the antiviral effects of the VD3 is scarce and the mechanism of its antiviral action not fully understood (Beard et al., 2011). Specifically, for DENV, there is only one study, where authors demonstrated that administration of oral VD3 reduces the severity of dengue fever (Sanchez-Valdez et al., 2009). In addition, resistance to severe dengue was observed in a Vietnamese population associated with one particular allele of the vitamin D receptor (Loke et al., 2002).

VD3 plays a role in the modulation of the innate immune response. It has been observed that VD3 treatment results in the down-regulation of the Toll-like receptors (TLRs) expression in human monocytes that affects the NF-κB/RelA activation and reduces the phosphorylation the MAPKs p38 and p42/44 (Sadeghi et al., 2006). DENV infection of human macrophages is dependent on the activation of the MAPKs JNK and p38 (Ceballos-Olvera et al., 2010) and induces the production of pro-inflammatory cytokines. In this study we evaluated the antiviral activity of VD3.

The human myelomonocytic cell line U937 (ATCC: CRL-1593.2) was grown in supplemented RPMI 1640 media and differentiated to macrophages by the addition of phorbol 12-myristate 13-acetate (PMA; Sigma Aldrich), as previously described (Puerta-Guardo et al., 2010). Cells were used unsorted, since PMA treatment resulted in the differentiation of more than 80% of U937 cells, as determined by amount of CD14+ cells by flow cytometry (FACS) assay (data not shown). Huh-7 cells, a differentiated hepatocyte

* Corresponding author. Address: Department of Infectomics and Molecular Pathogenesis, Centro de Investigación y Estudios Avanzados-IPN, Av. IPN 2508, Colonia San Pedro Zacatenco, Mexico, D.F. 07360, Mexico. Tel.: +52 55 57473800; fax: + 52 55 57473377.
E-mail address: rmangel@cinvestav.mx (R.M. del Angel).

0166-3542/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2012.02.006

derived cellular carcinoma cell line, were kindly donated by Dr. Ana Maria Rivas from Autonomous University of Nuevo León, Mexico. Cells were grown in advanced DMEM supplemented with 2 mM glutamine, penicillin (5×10^4 U/ml)-streptomycin (50 μg/ml), 5% fetal calf serum, 1 ml/l of amphotericin B (Fungizone) at 37 °C and 5% CO_2. Huh-7 cells and U937 cells were used between passages 13 and 16, and between passages 38 and 41, respectively. The prototype DENV serotype 4 (DENV-4) H241 strain was propagated in BALB/c suckling mice brains and viral titers were determined by plaque assay in BHK-21 cells, as described previously (Mosso et al., 2008).

In order to determine the effect of the active metabolite VD3 (Sigma–Aldrich, St. Louis, MI) in DENV infection, 2×10^5 Huh-7 or U937 cells were treated with VD3 at concentrations ranging from 10 to 0.001 μM. Up to 48 h later for Huh-7 cells and 72 h for U937 cells, viability was evaluated by a cytotoxicity assay (CellTiter 96®AQueous Solution Cell Proliferation Assay, Promega, Madison, WI). To ensure proper levels of active VD3, cultures were replenished with the appropriate concentration of VD3 every 24 h. No significant reduction in cell viability was observed at any of the concentrations used, suggesting that the treatment conditions are non-toxic for these cell lines (Supplemental material, Fig. 1). To confirm the immunomodulatory activity of the VD3 on the cell lines, the cell surface expression of TLR-2 and -4 (Santa Cruz Biotechnology, Inc.) was determined by flow cytometry (10,000 events) as previously described by Sadeghi et al. (2006). At a concentration of 1 μM of VD3, a significant reduction of the amounts of TLR-2 and TLR-4, on the cell surface of both Huh-7 and U937 cells was observed (Table 1).

For the viral infection inhibition assays, two different protocols were used. In the first one, cells grown in 24 well plates were treated with different concentrations of VD3 for 24 h, infected with DENV-4 for 1 h at 37 °C, and subsequently treated again with VD3 until harvest time. In the second protocol, the 24-h pretreatment of the cells was omitted. After infection, cell cultures were washed with Hanks and incubated with storage media containing the different concentrations of VD3. Finally, at 72 h post-infection for U937 cells and 48 h post-infection for Huh-7 cells, the percentage of infected cells was determined by flow cytometry (10,000 events) using an antibody against the envelope protein (anti E, 4G2) and an anti-IgG FITC conjugated (Invitrogen) as described before (Puerta-Guardo et al., 2010).

A significant reduction in the percentage of infected Huh-7 and U937 cells was observed at 48 or 72 h post-infection, respectively (Fig. 1). Although the decrease in the number of infected cells was not dose-dependent, a correlation between VD3 dose and infection inhibition in both cell lines was observed, with the highest inhibition observed in cells exposed to the highest concentration of VD3 used (10 μM) (Fig. 1A and B). The inhibitory effect of VD3 on viral infection was considerably stronger in the macrophage-like than in the hepatic cells. Significant inhibition ($p < 0.05$) in DENV replication in U937 cells was already observed at 0.01 μM, while in Huh-7 cells a significant reduction ($p = 0.01$) was achieved only at a concentration of 1 μM. Unexpectedly, a significant increase ($p = 0.05$) in DENV Huh-7 infected cells was observed at the lowest VD3 concentration (0.001 μM) used. The reason for this increase is unclear but the ability of Huh-7 cells to both, metabolize and respond to vitamin D, may be responsible for this dose-dependent response (Gal-Tanamy et al., 2011). Finally, no significant differences were observed in the percentage of infected U937 or Huh-7 cells treated before and after infection, or only after infection.

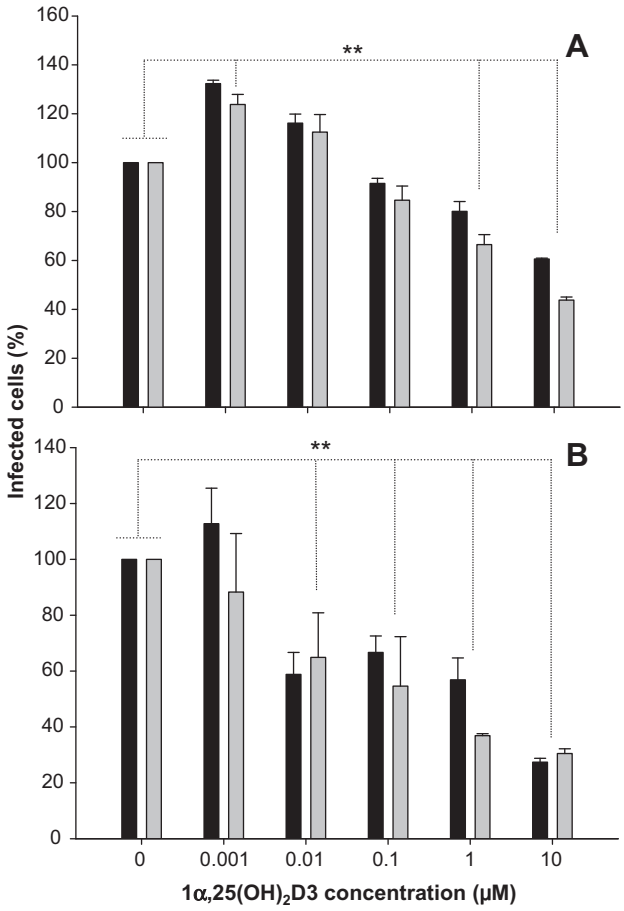

Fig. 1. Effect of VD3-treatment in DENV-4 infection of cultured cells. (A) Huh-7 and (B) U937 cells were treated with different concentrations of VD3 (μM) after infection (black bars) or before and after infection (grey bars) and the percentage of infected cells was evaluated after 48 and 72 h post-infection respectively by flow cytometry (10,000 events) using an anti-E (4G2) as primary antibody and an anti-mouse antibody coupled to FITC. Pretreatment was carried out for 24 h while treatment after infection was continuous until harvest time. Differences between the diverse VD3 treatments and control groups were evaluated using the statistical program Sigma-Plot 11. In all cases, a one tailed analysis of variance (ANOVA) was performed. For all tests a $p \leqslant 0.05$ was considered significant. Each bar represents mean ± SD of three independent experiments. Non-treated cells (0 μM) were used as 100% of infection. $^{**}p < 0.05$.

To further evaluate the effect of VD3 on DENV replication, virus yield was determined in VD3-treated cells. The extracellular virus production from infected Huh-7 cells supernatants were determined by the standard plaque assay in BHK-21 cells (Mosso et al., 2008). Since cytokines present in supernatants of U937 cells interfere with standard plaque assays, virus yield determination in U937 cells was performed by the *q*RT-PCR assay. For viral amplification, a TaqMan CFX96 system assay (Bio-Rad Laboratories) was used as described by Johnson et al. (2005). The amount of viral RNA transcripts was calculated by generating a standard curve

Table 1
TLR (-2 and -4) expression dependent of VD3 treatment in U937 and Huh-7 cells.

Cell type	% reduction in TLR expression (mean ± SD)[a]		Significance analysis (one way ANOVA)
U937 (macrophage)	TLR -2	15.6 ± 2.1	$p < 0.001$
	TLR -4	10.9 ± 5.8	$p = 0.001$
Huh7 (hepatocyte)	TLR -2	14.0 ± 3.0	$p = 0.001$
	TLR -4	9.4 ± 3.7	$p = 0.001$

[a] Data represent % reduction obtained with 1 μM of VD3. $n = 3$. Non-treated cells were taken as control (100%).

from 10-fold dilutions of RNA isolated from a DENV-4 preparation titrated in BHK-21 cells and expressed as plaque forming units equivalents per milliliter (PFU equivalents/ml) (Callahan et al., 2001; Johnson et al., 2005).

VD3 treatment induced up to 65% reduction in viral yield in Huh-7 cells (Fig. 2A) and up to 2 logs reduction in U937 cells (Fig. 2B), with a clear dose-dependent effect observed for U937 cells. These results confirm the inhibitory effect of VD3 on viral infection observed by flow cytometry. A stronger inhibitory effect of VD3 was observed in macrophage-like than in the hepatic cells.

To determine the effect of VD3 treatment on the production of cytokines secreted from infected U937 cells, cells were treated with 1.0 μM of VD3 both before and after infection, or only after infection. Mock-infected cells treated or not with VD3 were included as controls. Cytokines in cell supernatants were determined using a CBA-based system array (FlowCytomix Human Th1/Th2 11plex kit, Bender Medsystems®) following the Manufacturer's instructions. Secreted cytokines were quantified from a standard curve by using FlowCytomix Pro software (Bender Medsystems®). Sensitivity for tumor necrosis factor-α (TNF-α) and the interleukins: -1 beta (IL-1β), -6 (IL-6), -8 (IL-8), -10 (IL-10) and -12p70 (IL-12p70) are 3.2, 4.2, 1.2, 0.5, 1.9 and 1.5 pg/ml respectively. In agreement with previous reports (Carr et al., 2003; Lin et al., 2002), a significant increase ($p < 0.05$) in the production of TNF-α, IL-6, IL-12p70 and IL-1beta was observed in DENV-infected U937 cells in comparison with control, mock-infected cells (Fig. 3). However, in infected cells treated with VD3, the expression levels of these cytokines were significantly reduced ($p \leqslant 0.05$) and reached control levels (Fig. 3). In addition, the effect of VD3 treatment only after infection on cytokine production was also tested. A significant ($p < 0.05$) reduction in the production of cytokines TNF-α, IL-6, IL-12p70 and IL-1β was observed in treated cells in relation to non-treated cells, albeit the reduction in cytokine production in the cells treated with VD3 only after infection was less marked than the reduction observed in cells treated before and after infection (Fig. 3). Finally, no differences in the synthesis of IL-10 and IL-8 between infected cells treated or not with regard to control cells were found.

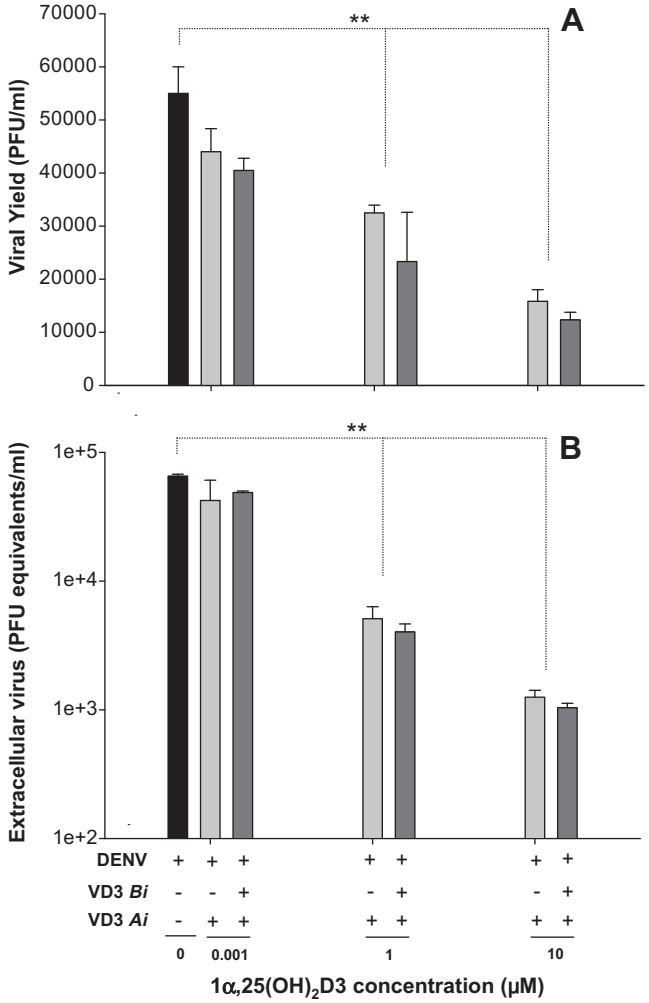

Fig. 2. Effect of VD3-treatment in virus yield of DENV-4 infected cells. (A) Huh-7 and (B) U937 cultures were treated with different concentrations of VD3 (μM) after infection (light grey bars) or before and after infection (dark grey bars) during 48 or 72 h post-infection, respectively. Supernatants were removed and extracellular virus production was determined by plaque assay for Huh-7 and by a Singleplex Real-Time RT-PCR (TaqMan) Assay for U937 cells. Differences between the diverse VD3 treatments and control groups were evaluated using the statistical program Sigma-Plot 11. In all cases, a one tailed analysis of variance (ANOVA) was performed. For all tests a $p \leqslant 0.05$ was considered significant. Each bar represents the main ± SD of two independent experiments. $^{*}p < 0.05$. VD3 *Bi*, treatment with VD3 24 h before DENV-infection; VD3 *Ai*, treatment with VD3 for after DENV infection.

Novel evidence suggests that VD3 plays a major role regulating the immune system (Baeke et al., 2010). These findings prompted further studies about the role of VD3 in the modulation of bacterial and viral infections (Cannell et al., 2008; Bang et al., 2010; Beard et al., 2011; Gal-Tanamy et al., 2011). Herein, it is shown that treatment with VD3 of two human cell lines, macrophages (U937) and hepatocytes (Huh-7) inhibits DENV infection *in vitro*. The inhibitory effect of the VD3 treatment was dependent on the cell line, with inhibition being stronger in monocytes than in hepatocytes. The mechanisms involved in the inhibition of the DENV infection by VD3 treatment are unclear, mainly due to the many different activities and functions described for VD3. Recent studies have proposed the induction of some anti-microbial peptides associated with TLRs and the synthesis of human β defensin 2 as a probable anti-viral mechanism modestly up-regulated in monocytes after VD3 treatment (Liu et al., 2006). Alternatively, a significant reduction in the expression of TLRs, as well as a decrease in NFκB-activation and the p38 and p42/44 phosphorylation have also been reported as a consequence of the immunoregulatory activity of VD3 (Sadeghi et al., 2006). In this study, variable modulation in the expression of TLR-2 and TLR-4 after treatment with a 1 μM VD3 concentration was observed. In addition, the activation of the host cell signaling pathways, including the MAPKs p38 and JNK at early stages of DENV infection, is necessary for efficient replication in human isolated monocytes (Ceballos-Olvera et al., 2010). Thus, it is likely that VD3 would inhibit DENV infection by interfering with the activation of several host cell signaling pathways reported previously to be important for DENV replication.

Plasma leakage and massive hemorrhagic manifestations are hallmarks for the severe manifestations of dengue disease (DHF/DSS) (Green and Rothman, 2006). These have been linked in clinical and *in vitro* studies to the elevated secretion of several pro-inflammatory mediators by infected dendritic cells and macrophages (Chaturvedi et al., 2000). In accord with this notion, increased level of proinflammatory cytokines were observed in U937 cells infected with DENV with respect to mock-infected cells (Ubol et al., 2008; Bozza et al., 2008; Chaturvedi et al., 1999). Interestingly, an important reduction in the concentrations of the proinflammatory cytokines TNF-α, IL-1β, IL-6 and IL-12p70 was observed when DENV infected cultures were previously (VD*Bi*) and subsequently (VD*Ai*) treated with VD3. These results agree with previous reports showing VD3 inhibition of cytokine production in monocytes, human trophoblasts and macrophages (Sadeghi et al., 2006; Lemire et al., 1995; Almerighi et al., 2009; D'Ambrosio et al., 1998).

Different levels of inhibition were observed for TNF-α and IL-6, but not for IL-12p70 or IL-1β, depending on the VD3 treatment

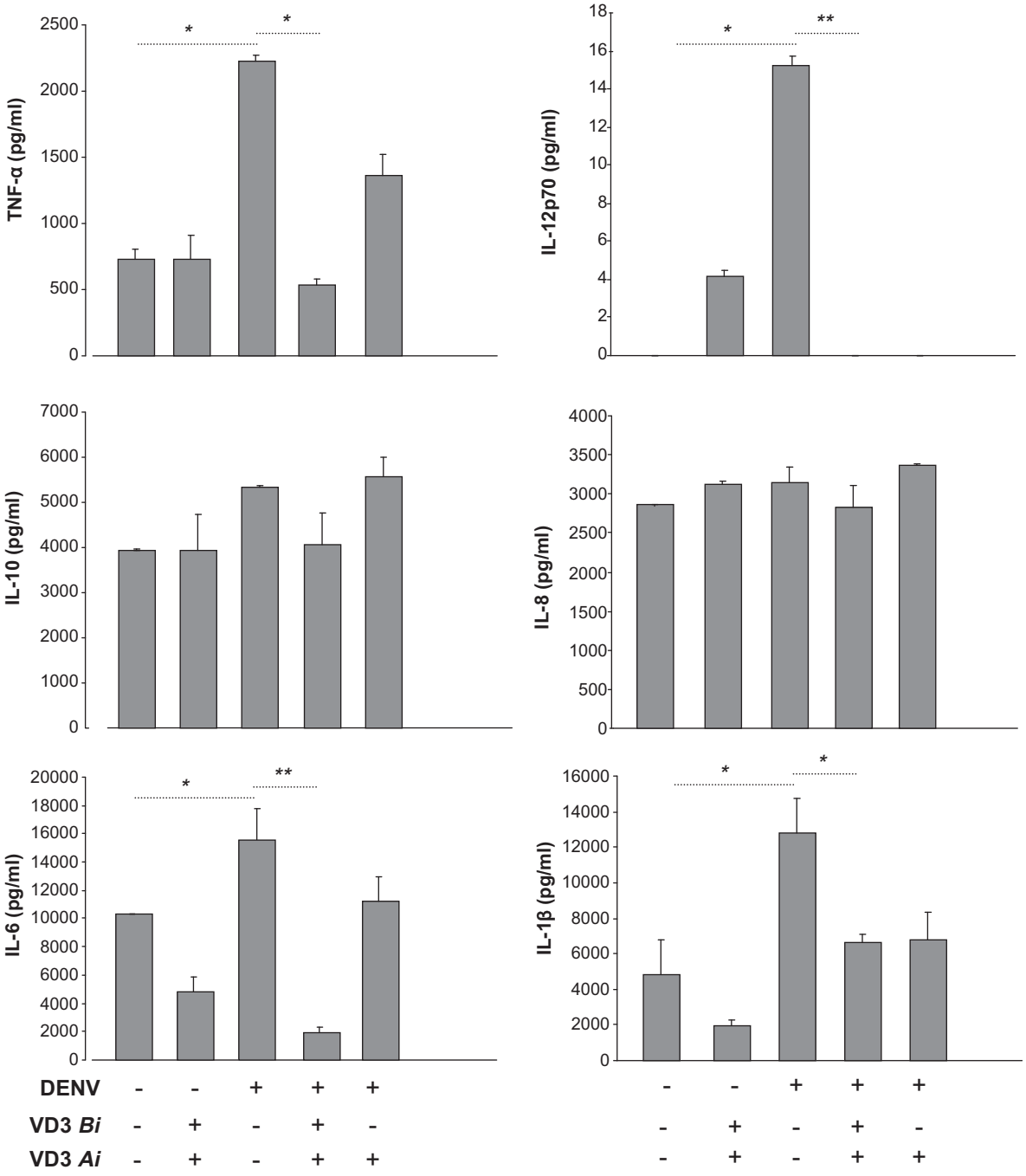

Fig. 3. Cytokine production in VD3-treated and untreated DENV infected U937 cells. Supernatants from U937 cultures previously treated or not with VD3 (1 μM) and infected with DENV-4, were removed at 48 h post infection and cytokine quantification carried out using a CBA assay. Differences between cytokine production levels were evaluated using the statistical program Sigma-Plot 11. In all cases, a one tailed analysis of variance (ANOVA) was performed. For all tests $p \leqslant 0.05$ was considered significant. Each bar represents the mean ± SD of two independent experiments. $^{**}p < 0.05$. VD3 *Bi*, treatment with VD3 24 h before DENV-infection; VD3 *Ai*, treatment with VD3 for 48 h after DENV infection.

conditions used. In addition, levels of IL-12p70 were non-detectable in supernatants obtained from infected cultures treated with VD3, while measurable levels of this cytokine were observed in cultures of non-infected, treated cells. In activated macrophages, the expression of several cytokines, including TNF-α and IL-6, is predominantly dependent on the activation of the Nuclear Factor kappa-B (NFκ-B), a pathway known to be modulated by VD3 (Sadeghi et al., 2006; Cohen-Lahav et al., 2006; Li and Verma, 2002). In contrast, distinct regulatory pathways (i.e. MAPKs and STATs), control the induction of IL-1β and IL-12p70 (O'Neill, 2002; Watford et al., 2003). Variations in the pathways inducing the expression of the studied cytokines may be related, at least partially, to the differences observed in response to VD3 treatment.

Increased levels of several vasoactive factors such as tumor growth factor alpha (TGF)-α (Chaturvedi et al., 2000), tumor growth factor (TGF)-β (Agarwal et al., 1999), IL-8 (Raghupathy et al., 1998), IL-6 (Juffrie et al., 2001), IL-10 (Gagnon et al., 2002), IL-1β (Bozza et al., 2008) and others, have been reported in the bloodstream of patients with DHF. Recently, increased expression of proinflammatory cytokines, such as TNF-α, was observed in

human dendritic cells infected with a DENV isolated from a fatal case as compared with an isolate from a mild clinical case (Silveira et al., 2011). Our results demonstrate a differential modulation in the cytokines produced by infected U937 cells cultures treated with VD3 (1 μM) with respect to the mock-untreated DENV infected cells, especially in the concentration of some well-known pro-inflammatory cytokines such as TNF-α, IL-6, IL-12p70 and IL-1β (Green et al., 1999; Cardier et al., 2005; Carr et al., 2003). Thus, our results taken together, suggest that the utility of VD3 treatment for therapeutic or prophylactic interventions at some stage in DENV infection merits to be evaluated.

Acknowledgments

Authors thank Dr. Juan B. Kouri for the generous gift of Vitamin D. This work was partially supported by CONACYT Grant number 103783 and ICyT-DF Grant number PIFUTP09-284 to J.E.L. and CONACYT Grant number 127447 and ICyT-DF Grant number PIFUTP8-152 to R.M.A.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.antiviral.2012.02.006.

References

Agarwal, R., Kapoor, S., Nagar, R., Misra, A., Tandon, R., Mathur, A., Misra, A.K., Srivastava, K.L., Chaturvedi, U.C., 1999. A clinical study of the patients with dengue hemorrhagic fever during the epidemic of 1996 at Lucknow, India. Southeast Asian J. Trop. Med. Publ. Health 30, 735–740.

Almerighi, C., Sinistro, A., Cavazza, A., Ciaprini, C., Rocchi, G., Bergamini, A., 2009. 1Alpha,25-dihydroxyvitamin D3 inhibits CD40L-induced pro-inflammatory and immunomodulatory activity in human monocytes. Cytokine 45, 190–197.

Baeke, F., Takiishi, T., Koft, H., Gysemans, C., Mathieu, C., 2010. Vitamin D: modulator of the immune system. Curr. Opin. Pharmacol. 10, 482–496.

Bang, U.C., Shakar, S.A., Hitz, M.F., Jespersen, M.S., Andersen, O., Nielsen, S.D., Jensen, J.E., 2010. Deficiency of 25-hydroxyvitamin D in male HIV-positive patients: a descriptive cross-sectional study. Scand. J. Infect. Dis. 42, 306–310.

Beard, J.A., Bearden, A., Striker, R., 2011. Vitamin D and the anti-viral state. J. Clin. Virol. 50, 194–200.

Bozza, F.A., Cruz, O.G., Zagne, S.M., Azeredo, E.L., Nogueira, R.M., Assis, E.F., Bozza, P.T., Kubelka, C.F., 2008. Multiplex cytokine profile from dengue patients: MIP-1beta and IFN-gamma as predictive factors for severity. BMC Infect. Dis. 8, 86.

Bruce, D., Ooi, J.H., Yu, S., Cantorna, M.T., 2010. Vitamin D and host resistance to infection? Putting the cart in front of the horse. Exp. Biol. Med. 235, 921–927.

Callahan, J.D., Wu, S.J., Dion-Schultz, A., Mangold, B.E., Peruski, L.F., Watts, D.M., Porter, K.R., Murphy, G.R., Suharyono, W., King, C.C., Hayes, C.G., Temenak, J.J., 2001. Development and evaluation of serotype- and group-specific fluorogenic reverse transcriptase PCR (TaqMan) assay for dengue virus. J. Clin. Microbiol. 39, 4119–4124.

Cannell, J.J., Zasloff, M., Garland, C.F., Scragg, R., Giovannucci, E., 2008. On the epidemiology of influenza. Virol. J. 5, 29.

Cardier, J., Mariño, E., Romano, E., Taylor, P., Liprandi, F., Bosch, N., Rothman, A., 2005. Proinflammatory factors present in sera from patients with acute dengue infection induce activation and apoptosis of human microvascular endothelial cells: possible role of TNF-α in endothelial cell damage in dengue. Cytokine 30, 359–365.

Carr, J.M., Hocking, H., Bunting, K., Wright, P.J., Davidson, A., Gamble, J., Burrell, C.J., Li, P., 2003. Supernatants from dengue virus type-2 infected macrophages induce permeability changes in endothelial cell monolayers. J. Med. Virol. 69, 521–528.

Ceballos-Olvera, I., Chávez-Salinas, S., Medina, F., Ludert, J.E., del Angel, R.M., 2010. JNK phosphorylation, induced during dengue virus infection, is important for viral infection and requires the presence of cholesterol. Virology 396, 30–36.

Chaturvedi, U.C., Elbishbishi, E.A., Agarwal, R., Raghupathy, R., Nagar, R., Tandon, R., Pacsa, A.S., Younis, O.I., Azizieh, F., 1999. Sequential production of cytokines by dengue virus-infected human peripheral blood leukocyte cultures. J. Med. Virol. 59, 335–340.

Chaturvedi, U.C., Agarwal, R., Elbishbishi, E.A., Mustafa, A.S., 2000. Cytokine cascade in dengue hemorrhagic fever: implications for pathogenesis. FEMS Immunol. Med. Microbiol. 28, 183–188.

Cohen-Lahav, M., Shany, S., Tobvin, D., Chaimovitz, C., Douvdevani, A., 2006. Vitamin D decreases NFkappaB activity by increasing IkappaBalpha levels. Nephrol. Dial. Transplant. 21, 889–897.

D'Ambrosio, D., Cippitelli, M., Cocciolo, M.G., Mazzeo, D., Di Lucia, P., Lang, R., Sinigaglia, F., Panina-Bordignon, P., 1998. Inhibition of IL-12 production by 1,25-dihydroxyvitamin D3. Involvement of NF-kappaB downregulation in transcriptional repression of the p40 gene. J. Clin. Invest. 101, 252–262.

Gagnon, S.J., Mori, M., Kurane, I., Green, S., Vaughn, D.W., Kalayanarooj, S., Suntayakorn, S., Ennis, F.A., Rothman, A.L., 2002. Cytokine gene expression and protein production in peripheral blood mononuclear cells of children with acute dengue virus infections. J. Med. Virol. 67, 41–46.

Gal-Tanamy, M., Bachmetov, L., Ravid, A., Koren, R., Erman, A., Tur-Kaspa, R., Zemel, R., 2011. Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. Hepatology 54, 1570–1579.

Green, S., Vaughn, D.W., Kalayanarooj, S., Nimmannitya, S., Suntayakorn, S., Nisalak, A., Lew, R., Innis, B.L., Kurane, I., Rothman, A.L., Ennis, F.A., 1999. Early immune activation in acute dengue illness is related to development of plasma leakage and disease severity. J. Infect. Dis. 179, 755–762.

Green, S., Rothman, A., 2006. Immunopathological mechanisms in dengue and dengue hemorrhagic fever. Curr. Opin. Infect. Dis. 19, 429–436.

Gubler, D.J., 2006. Dengue/dengue haemorrhagic fever: history and current status. Novartis Found. Symp. 277, 3–16.

Johnson, B.W., Russell, B.J., Lanciotti, R.S., 2005. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J. Clin. Microbiol. 43, 4977–4983.

Juffrie, M., Meer, G.M., Hack, C.E., Haasnoot, K., Sutaryo, Veerman, A.J., Thijs, L.G., 2001. Inflammatory mediators in dengue virus infection in children: interleukin-6 and its relation to C-reactive protein and secretory phospholipase A2. Am. J. Trop. Med. Hyg. 65, 70–75.

Lemire, J.M., Archer, D.C., Beck, L., Spiegelberg, H.L., 1995. Immunosuppressive actions of 1,25-dihydroxyvitaminD3: preferential inhibition of Th1 functions. J. Nutr. 125, 1704S–1708S.

Li, Q., Verma, I.M., 2002. NF-κB regulation in the immune system. Nat. Rev. Immunol. 2, 725–734.

Lin, Y.W., Wang, K.J., Lei, H.Y., Lin, Y.S., Yeh, T.M., Liu, H.S., Liu, C.C., Chen, S.H., 2002. Virus replication and cytokine production in dengue virus-infected human B lymphocytes. J. Virol. 76, 12242–12249.

Liu, P.T., Stenger, S., Li, H., Wenzel, L., Tan, B.H., Krutzik, S.R., Ochoa, M.T., Schauber, J., Wu, K., Meinken, C., Kamen, D.L., Wagner, M., Bals, R., Steinmeyer, A., Zügel, U., Gallo, R.L., Eisenberg, D., Hewison, M., Hollis, B.W., Adams, J.S., Bloom, B.R., Modlin, R.L., 2006. Toll-like receptor triggering of a vitamin D-mediated antimicrobial response. Science 311, 1770–1773.

Loke, H., Bethell, D., Phuong, C.X., Day, N., White, N., Farrar, J., Hill, A., 2002. Susceptibility to dengue hemorrhagic fever in Vietnam: evidence of an association with variation in the vitamin D receptor and Fc gamma receptor IIa genes. Am. J. Trop. Med. Hyg. 67, 102–106.

Mosso, C., Galván-Mendoza, I., Ludert, J.E., del Angel, R.M., 2008. Endocytic pathway followed by dengue virus to infect the mosquito cell line C6/36 HT. Virology 378, 193–199.

Noble, C.G., Chen, Y.L., Dong, H., Gu, F., Lim, S.P., Schul, W., Wang, Q.Y., Shi, P.Y., 2010. Strategies for development of dengue virus inhibitors. Antiviral Res. 85, 450–462.

O'Neill, L.A., 2002. Signal transduction pathways activated by the IL-1 receptor/toll-like receptor superfamily. Curr. Top Microbiol. Immunol. 270, 47–61.

Puerta-Guardo, H., Mosso, C., Medina, F., Liprandi, F., Ludert, J.E., del Angel, R.M., 2010. Antibody-dependent enhancement of dengue virus infection in U937 cells requires cholesterol-rich membrane microdomains. J. Gen. Virol. 91, 394–403.

Raghupathy, R., Chaturvedi, U.C., Al-Sayer, H., Elbishbishi, E.A., Agarwal, R., Nagar, R., Kapoor, S., Misra, A., Mathur, A., Nusrat, H., Azizieh, F., Khan, M.A., Mustafa, A.S., 1998. Elevated levels of IL-8 in dengue hemorrhagic fever. J. Med. Virol. 56, 280–285.

Sadeghi, K., Wessner, B., Laggner, U., Ploder, M., Tamandl, D., Friedl, J., Zügel, U., Steinmeyer, A., Pollak, A., Roth, E., Boltz-Nitulescu, G., Spittler, A., 2006. Vitamin D3 down-regulates monocyte TLR expression and triggers hyporesponsiveness to pathogen-associated molecular patterns. Eur. J. Immunol. 36, 361–370.

Sanchez-Valdez, E., Delgado-Aradillas, M., Torres-Martínez, J.A., Torres-Benítez, J.M., 2009. Clinical response in patients with dengue fever to oral calcium plus vitamin D administration: study of 5 cases. Proc. West. Pharmacol. Soc. 52, 14–17.

Shum, D., Smith, J.L., Hirsch, A.J., Bhinder, B., Radu, C., Stein, D.A., Nelson, J.A., Früh, K., Djaballah, H., 2010. High-content assay to identify inhibitors of dengue virus infection. Assay Drug Dev. Technol. 8, 553–570.

Silveira, G.F., Meyer, F., Delfraro, A., Mosimann, A.L., Coluchi, N., Vasquez, C., Probst, C.M., Báfica, A., Bordignon, J., Dos Santos, C.N., 2011. Dengue virus type 3 isolated from a fatal case with visceral complications induces enhanced proinflammatory responses and apoptosis of human dendritic cells. J. Virol. 85, 5374–5383.

Ubol, S., Masrinoul, P., Chaijaruwanich, J., Kalayanarooj, S., Charoensirisuthikul, S., Kasisith, J., 2008. Differences in global gene expression in peripheral blood mononuclear cells indicate a significant role of the innate responses in progression of dengue fever but not dengue hemorrhagic fever. J. Infect. Dis. 197, 1459–1467.

Watford, W.T., Moriguchi, M., Morinobu, A., ÓShea, J.J., 2003. The biology of IL-12: coordinating innate and adaptive immune response. Cytokine Growth Factor Rev. 14, 361–368.

Zasloff, M., 2006. Fighting infections with vitamin D. Nat. Med. 12, 388–390.