Institut Pasteur
Microbes and Infection 18 (2016) 510–516
www.elsevier.com/locate/micinf

Short communication

Antiviral molecules correlate with vitamin D pathway genes and are associated with natural resistance to HIV-1 infection

Wbeimar Aguilar-Jimenez [a], Wildeman Zapata [a,b], María T. Rugeles [a,*]

[a] Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia UdeA, Medellín, Colombia
[b] Grupo Infettare, Facultad de Medicina, Universidad Cooperativa de Colombia, Medellín, Colombia

Received 14 February 2016; accepted 30 March 2016
Available online 12 April 2016

Abstract

The relationship between the immunomodulatory effects of Vitamin D (VitD) and the expression of anti-HIV-1 molecules has not been explored in HIV-1-exposed seronegative individuals (HESNs). Higher mRNA levels of cathelicidin and HAD-4 in oral-mucosa and peripheral-blood, along with higher CYP24A1 mRNA in vaginal-mucosa and lower TLR2 mRNA in endocervical-mucosa were found in HESNs compared to non-exposed controls. Furthermore, the mRNA of anti-HIV molecules Elafin, TRIM5, Cathelicidin, HAD-4 and RNase7, previously associated with natural resistance to HIV-1 infection, positively correlated with the mRNA expression of VDR in HESNs, suggesting the potential participation of VitD in natural resistance to HIV-1.

Keywords: HIV-1; Vitamin D; HIV-1-exposed seronegative individuals (HESNs); Antiviral agents; Natural resistance to HIV-1

1. Introduction

Exposure to HIV-1 does not always lead to infection. Several immune components, host genetic variants, as well as soluble factors have been associated with resistance to HIV-1 infection on HIV-1-exposed but seronegative (HESN) individuals. Some of the most important soluble factors exhibiting anti-HIV-1 activity, identified by us and others in mucosa or peripheral blood mononuclear cells (PBMCs) of HESNs, include the human alpha defensin (HAD)-1, human beta defensins (HBD)-2 and -3 [1,2], the antiproteases Elafin, SerpinA1 and the secretory leukocyte protease inhibitor (SLPI) [3–5], the intracellular anti-HIV-1 restriction factors APOBEC3G, TRIM5α and SAMHD1 [4,6] and peptides with ribonuclease activity such as RNase 1, RNase 7, Eosinophil Derived Neurotoxin (EDN) and Angiogenin (ANG) [2,4].

However, other antiviral molecules, not yet evaluated in the Colombian cohort, that have been reported to reduce HIV-1 infection *in vitro* such as cathelicidin (*CAMP*) [7], HAD-4 [8], or to modulate an anti-HIV-1 response such as *TLR2* and *TLR4* [9,10] could also be associated with natural resistance to HIV-1 infection.

Recently, it has been shown that beyond its role in calcium metabolism, vitamin D (VitD) has immunomodulatory effects [11,12]. Indeed, keratinocytes and immune cells are endorsed with metabolic machineries such as the 1α-hydroxylase (CYP27B1) and the vitamin D receptor (VDR), allowing activation and use of VitD as transcription factor of a substantial number of genes [11,13]. Certainly, it induces transcription of some of the anti-HIV-1 peptides mentioned above, in several cell populations [13,14], suggesting its possible participation in the resistance against HIV-1 infection.

Remarkably, we recently found higher plasma VitD as well as higher VDR mRNA levels in peripheral blood mononuclear

* Corresponding author. Grupo Inmunovirología, Facultad de Medicina, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín, Colombia. Tel.: +57 4 2196551; fax: +57 4 2196482.
E-mail address: maria.rugeles@udea.edu.co (M.T. Rugeles).

http://dx.doi.org/10.1016/j.micinf.2016.03.015
1286-4579/

cells (PBMCs) and mucosa from HESNs compared to non-exposed healthy controls (HCs) [15]. Moreover, mRNA expression of VDR was positively correlated with that of the anti-inflammatory cytokine IL-10 and the antimicrobial peptides HBD-2 and -3 [15], further supporting the protective role that VitD may have during HIV-1 exposure.

Therefore, the purpose of this study was to evaluate the transcription expression level of the antiviral molecules Cathelicidin, HAD-4, TLR2 and TLR4 and of the VitD pathway genes CYP27B1, CYP27A1 and CYP24A1 in PBMCs, in oral, endocervical and vaginal mucosa of HESNs, in HIV-1-infected individuals and HCs from Colombia. In addition, and with the purpose of determining the potential relationship of the VitD pathway with the phenomenon of natural resistance to HIV-1 infection, we correlated the relative mRNA levels of these molecules and the levels of the mRNAs of anti-HIV-1 soluble factors, previously reported in the same cohort [2–4], with the VDR mRNA expression, also formerly described [15].

2. Material and methods

2.1. Population

This is a cross-sectional study involving cDNA samples from a cohort of Colombian sexual serodiscordant couples composed of 58 HESNs and 43 chronically HIV-1-infected subjects, hereafter called seropositives (SPs); they were recruited from the HIV-1 comprehensive care programs in Santa Marta and Medellín, Colombia. The inclusion criteria for HESN subjects were previously reported [1]; briefly, the HESNs in this study had an average of 8 unprotected sexual intercourses per month with an SP partner with detectable viral load (median [interquartile range]: 2569 [400–25,250]), within at least 2 years of follow up, negative HIV-1 ELISA and proviral HIV DNA tests at sampling [15]. Most individuals (80%) were heterosexuals and the remaining were bisexuals. None of the individuals was Δ32-homozygous nor had any sexually transmitted disease (STD) at sampling. However, 27% of HESNs reported previous events of STDs, which could have increased even more their risk to acquire HIV-1; however, they preserved the seronegative status.

The similar age (mean years ± standard deviation: 34.9 ± 10.3 for HESNs and 33.7 ± 7.1 for SPs), gender (41.4% and 53.5% males for HESNs and SPs respectively) as well as ancestry component and pair-wise fixation index (FST) values in the SPs and HESNs [15,16] indicated that there was not intra-cohort stratification by ethnicity.

We also included cDNA samples from 59 HCs with similar demographic backgrounds and citizenship as the HESN and SP individuals (32.8 ± 9.8 years and 42.4% males). HC individuals had negative HIV-1 ELISA and proviral HIV DNA tests, fewer than 2 sexual partners in the last 2 years and self-reported no risk behaviors for HIV-1 infection.

All individuals signed an informed consent prepared according the Colombian Legislation; this study was approved by the Ethical Committee CBE-SIU of Universidad de Antioquia (certificate 13-08-520).

2.2. mRNA quantification by real time RT-PCR

cDNA samples previously obtained from oral, vaginal and endocervical mucosa as well as from PBMCs from all individuals were used for the analysis of gene expression. Briefly, mucosal samples were taken by rubbing a cytobrush against the inner mucosa surfaces [1]. PBMCs were isolated by gradient centrifugation, from 17 HESNs, 15 SPs and 38 HCs out of the total cohort [15]. RNA isolation was performed using TRizol Reagent (Invitrogen) plus DNase I treatment (Thermo Scientific) followed by retro-transcription using the Revertaid H Minus Retrotranscriptase Kit (Thermo Scientific) [15].

Real time RT-PCR was performed in a final volume of 15 μL, using 2 μL cDNA, 1X Maxima SYBR green qPCR master mix kit (Thermo Scientific) and 260 μM of the following specific primers: Cathelicidin (Fw: 5′-GGATGCTAACCTCTACCGC-3′ and Rv: 5′-AGGGTCACTGTCCCCATACA-3′); HAD-4 (Fw: 5′-GCTCTTCAGGTTTCAGGCTCA-3′ and Rv: 5′-TCACACCACCAATGAGGCAG-3′); TLR2 (Fw: 5′-GAGTTCTCCCAGTGTTTGGTG-3′ and Rv: 5′-CCAGTGCTTCAACCCACAACT-3′); TLR4 (Fw: 5′-TTATCACGGAGGTGGTTCCT-3′ and Rv: 5′-TGGTTGAGAAGGGGAGGTTGT-3′); CYP27A1 (Fw: 5′-TGGACACGACATCCAACACG-3′ and Rv: 5′-GACCACAGGGTAGAGACGCA-3′); CYP27B1 (Fw: 5′-GTCCAGACAGCACTCCACTC-3′ and Rv: 5′-ACCACAGGGTACAGTCTTAGC-3′); and CYP24A1 (Fw: 5′-CGCAAATACGACATCCAGGC-3′ and Rv: 5′-AATACCACCATCTGAGGCGT-3′). In addition, the expression of the reference genes β-actin (Fw: 5′-CTTTGCCGATCCGCCGC-3′ and Rv: 5′-ATCACGCCCTGGTGCCTGG-3′), and Phosphoglycerate Kinase 1 (PGK-1) (Fw: 5′-GTTGACCGAATCACCGACC-3′ and Rv: 5′-TCGACTCTCATAACGACCCGC-3′), were used to normalize the amount of RNA. The cycling profile in all the experiments was: 95 °C for 10 min, followed by 40 cycles at 94 °C for 10 s, and annealing/extension for 40 s at 60 °C. Duplicate assays were performed (SDs were less than 0.5 cycle in all assays). The Bio-Rad CFX manager 3.1 (Bio-Rad) was used to acquire the cycle thresholds (Ct), determined in each sample using a regression fit in the linear phase of the PCR amplification curve. The relative expression was calculated by the ΔCt method. The results are presented as median of the mRNA relative expression units (RUs) to the reference genes. Samples that did not amplify target genes were excluded from the analysis. The percentage of samples analyzed is described in the figure legends.

In addition, mRNAs RU of anti-HIV-1 factors HAD-1, HBD-2, HBD-3, Elafin, SerpinA1, SLPI, APOBEC3G, TRIM5α, SAMHD1, RNase 1, RNase 7, EDN and ANG analyzed in the mucosa of this HESN cohort in our previous studies [2–4] were also used to correlate with the previously quantified VDR mRNA levels [15].

2.3. Statistical analysis

According to the results of the Shapiro–Wilk test, a non-parametric test (Mann–Whitney *U*-two-tailed test) was used to compare the mRNA expression of each gene between HESNs vs HCs or HESNs vs SPs. The correlations between VDR mRNA and transcript levels of the molecules analyzed was evaluated using the Spearman coefficient rank (r). A p value < 0.05 was considered statistically significant. The statistical tests were performed using the GraphPad Prism version 6.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. The mRNA levels of Cathelicidin and HAD-4 were higher in PBMCs and oral mucosa of HESNs than of HCs

The relative mRNA expression of Cathelicidin was 5.2 and 1.8-fold higher in PBMC and oral mucosa of HESN individuals compared to HCs ($p = 0.0007$ and $p = 0.0210$ for PBMCs and oral mucosa, respectively). In addition, the Cathelicidin mRNA expression level in PBMCs of HESNs was 4.0-fold higher than in SPs ($p = 0.0323$) (Fig. 1A).

Similarly, 13.3 and 1.7-fold higher mRNA relative expression level of HAD-4 was found in PBMCs and oral mucosa of HESNs compared to HCs ($p = 0.0002$ and $p = 0.0376$ for PBMCs and oral mucosa, respectively). In addition, the HAD-4 mRNA expression level in PBMCs of HESNs was 6.9-fold higher than of SPs ($p = 0.0346$) (Fig. 1B).

3.2. Low mRNA levels of TLR2 in endocervical mucosa along with high mRNA levels of TLR4 in oral mucosa were observed in HESNs compared to HCs

The mRNA relative expression level of TLR2 was 2- and 2.7-fold lower in endocervical mucosa of HESN individuals compared to HCs ($p = 0.0360$) and SPs ($p = 0.0410$) respectively (Fig. 1C). Conversely, the TLR4 mRNA expression level was 2.2-fold higher in oral mucosa of HESNs than of HCs ($p = 0.0074$) (Fig. 1D).

3.3. High mRNA levels of the VitD target hydroxylase CYP24A1 in vaginal mucosa of HESNs

We found that the baseline mRNA expression level of cytochrome P450 VitD-hydroxylases (CYP27A1, CYP27B1 and CYP24A1) varied depending on the tissue analyzed. Whereas, CYP27A1 mRNA was similar in all evaluated tissues (Fig. S1), the CYP27B1 and CYP24A1 mRNA expression level was 10- and 33-fold lower, respectively, in PBMCs than in mucosa and only detected in 30% of the samples (Fig. 1E, F). Significant differences between HESNs and HCs were only found for CYP24A1 mRNA that was 4.8-fold higher in vaginal mucosa of HESNs compared to HCs ($p = 0.0091$. Fig. 1F).

3.4. mRNA levels of the anti-HIV molecules elafin, TRIM5, cathelicidin, HAD-4 and RNASE7 were positively correlated with the mRNA expression levels of VDRs in HESNs, whereas TLR2 and VDR mRNAs were negatively correlated

Since VitD induces transcription of antimicrobial molecules [13,14], we subsequently evaluated if the VDR mRNA [15] correlates with the mRNA of anti-HIV-1 soluble factors. We found that VDR mRNA was positively correlated with Elafin ($r = 0.65$, $p = 0.0059$) and Cathelicidin ($r = 0.76$, $p = 0.0015$) in PBMCs (Fig. 2A, B), with HAD-4 ($r = 0.57$, $p = 0.0142$) and RNase7 ($r = 0.50$, $p = 0.0419$) in endocervical mucosa (Fig. 2C, D) and with TRIM5 in vaginal mucosa of HESNs ($r = 0.48$, $p = 0.0289$; Fig. 2E). Furthermore, a negative correlation between the mRNA of VDR and TLR2 in endocervical mucosa of HESNs was also observed ($r = -0.53$, $p = 0.0192$; Fig. 2F).

4. Discussion

The study of protective factors against HIV-1 is of capital interest to improve preventive and immunomodulatory approaches in view of protecting high-risk individuals. In particular, we studied the natural resistance to HIV-1 infection in a cohort of serodiscordant couples from Colombia in the last 10 years. From the 14 antiviral molecules analyzed in this cohort, we have found higher expression of Elafin in PBMCs [4], of HAD-1, HBD-2, HBD-3, Elafin, SAMHD1, Serpin1, SLPI and APOBEC3G in oral mucosa [1–4], and of Elafin, SAMHD1, TRIM5α, ANG, EDN, RNase-1 and -7 in genital mucosa of HESNs compared to HCs [2,4]. In addition, in the present study we found higher transcription expression of the antimicrobial peptides HAD-4 and cathelicidin in HESNs compared to HCs and SPs, and higher TLR4 in HESNs compared to HCs in PBMCs and oral mucosa. Furthermore, we found that in vaginal and endocervical mucosa the mRNA expression level of the antimicrobial peptides cathelicidin and HAD-4 was similar in HESNs and HCs, but TLR2 mRNA was lower in HESNs than HCs.

Altogether, our findings suggest that in general HESNs have a pronounced anti-HIV-1 effector-like profile in PBMCs, oral and genital mucosa, most likely contributing to the natural resistance to HIV-1 infection.

Considering that the main aim of the study is to define associations with natural resistance to HIV-1 infection, the appropriate comparison is between HESN and HC, since it might reflect a genetic trait associated with HIV-1 resistance, revealed by viral exposure. Although we found significant differences between the levels of mRNA for cathelicidin, HAD-4 and TLR2, between HESNs and SPs, the expression of these soluble factors in SP could have been influenced by the chronic immune stimulation induced by HIV-1 infection or by the immunological exhaustion as previously reported [17].

Interestingly, VitD seems to be a key element to immunoregulate the pathogenic immune response against HIV-1; in fact, its deficiency has been associated with HIV/AIDS

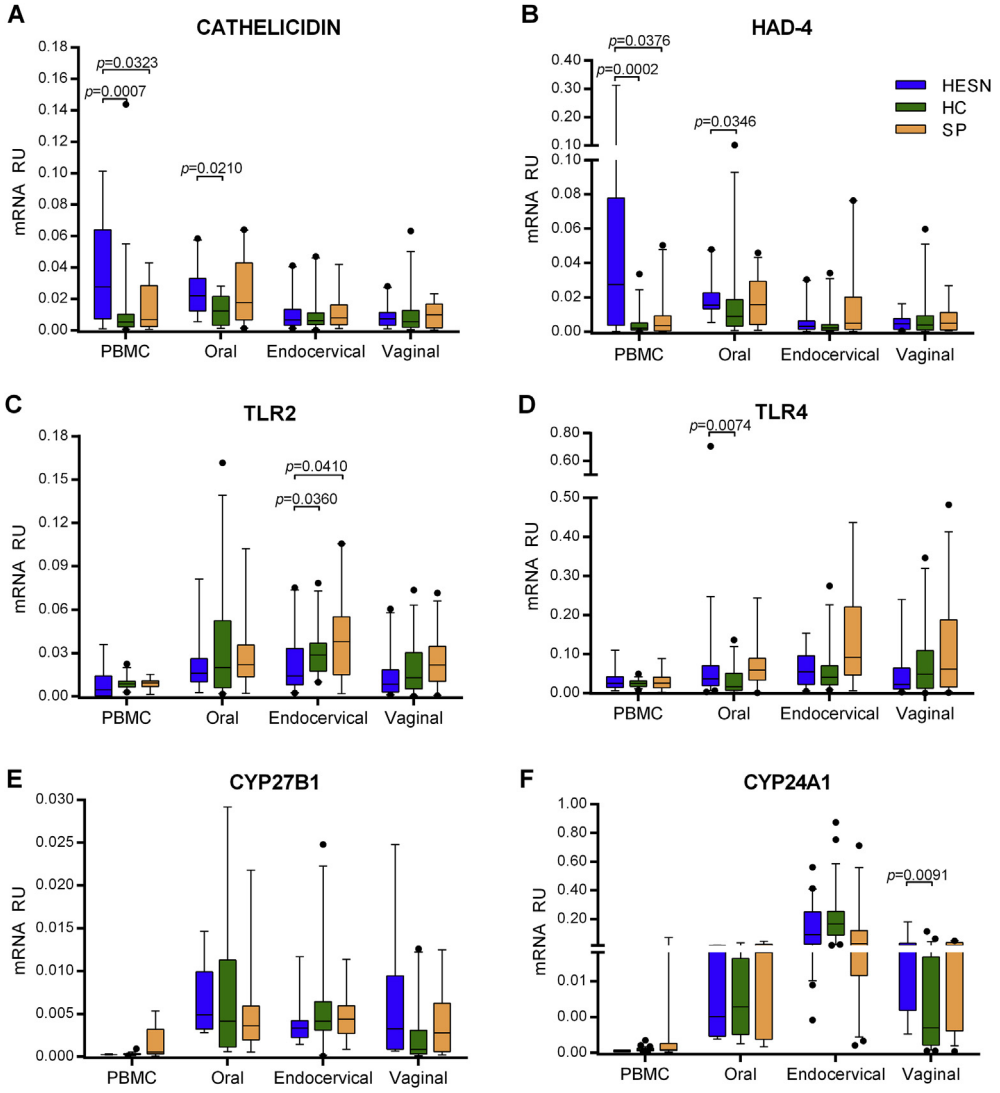

Fig. 1. The box and whisker plots show the median value and 5–95 percentiles of mRNA relative units (RU; normalized to β-actin and PGK1 mRNA) of Cathelicidin (A), HAD-4 (B), TLR2 (C), TLR4 (D), CYP27B1 (E) and CYP24A1 (F) in PBMCs (88% samples were correctly amplified for all genes), and oral (73%), endocervical (83%) and vaginal (80%) mucosa of HESNs, HCs and SPs. A non-parametric test (Mann–Whitney *U*-two-tailed test) was used to compare mRNA RU between HESNs vs HCs or HESNs vs SPs. Outliers are plotted as black circles and significant *p* values are displayed in each graph. Significantly higher mRNA RU of Cathelicidin and HAD-4 in PBMC and oral mucosa (A–B), and significantly lower TLR2 mRNA in endocervical mucosa (C) of HESNs compared to HCs and SPs are shown. Furthermore, significantly higher TLR4 mRNA in oral mucosa (D) and CYP24A1 mRNA in vaginal mucosa of HESN than HCs (F) are also displayed.

progression and mortality, whereas its supplementation could be a simple, cost-effective intervention, particularly in resource-poor settings, to reduce HIV-1 risk and disease progression [18]. Indeed, we have found that VitD reduces HIV-1 infection of PBMCs in vitro (manuscript submitted for publication).

Remarkably, in this study we found higher mRNA levels of CYP24A1, a well-established gene indicator for the presence of active VitD (calcitriol), in vaginal mucosa from HESNs compared to HCs, suggesting an immune-regulated environment induced by VitD that could boost the control of HIV-1 entry.

Indeed, we found that mRNA levels of VDR, previously quantified in the individuals from the same cohort [15], were positively correlated with the antiviral factors elafin and cathelicidin in PBMCs, with HAD-4 and RNase-7 in endocervical mucosa, and with TRIM5 in vaginal mucosa of HESNs. Furthermore, we had previously reported a positive correlation between mRNA expression levels of VDR and HBD-2 and -3 in oral mucosa of HESNs [15], supporting the hypothesis of VitD involvement in the expression of anti-HIV-1 molecules in HESNs.

Remarkably, the correlations between VDR and cathelicidin, HBD-2 and -3 are supported by in vitro assays and/or the presence of VitD response elements in their promoters [13,14]. Likewise, we observed a significant increase of Elafin mRNA that was also correlated with VDR mRNA after a VitD treatment that suppress in vitro HIV-1 infection in PBMCs (manuscript submitted for publication).

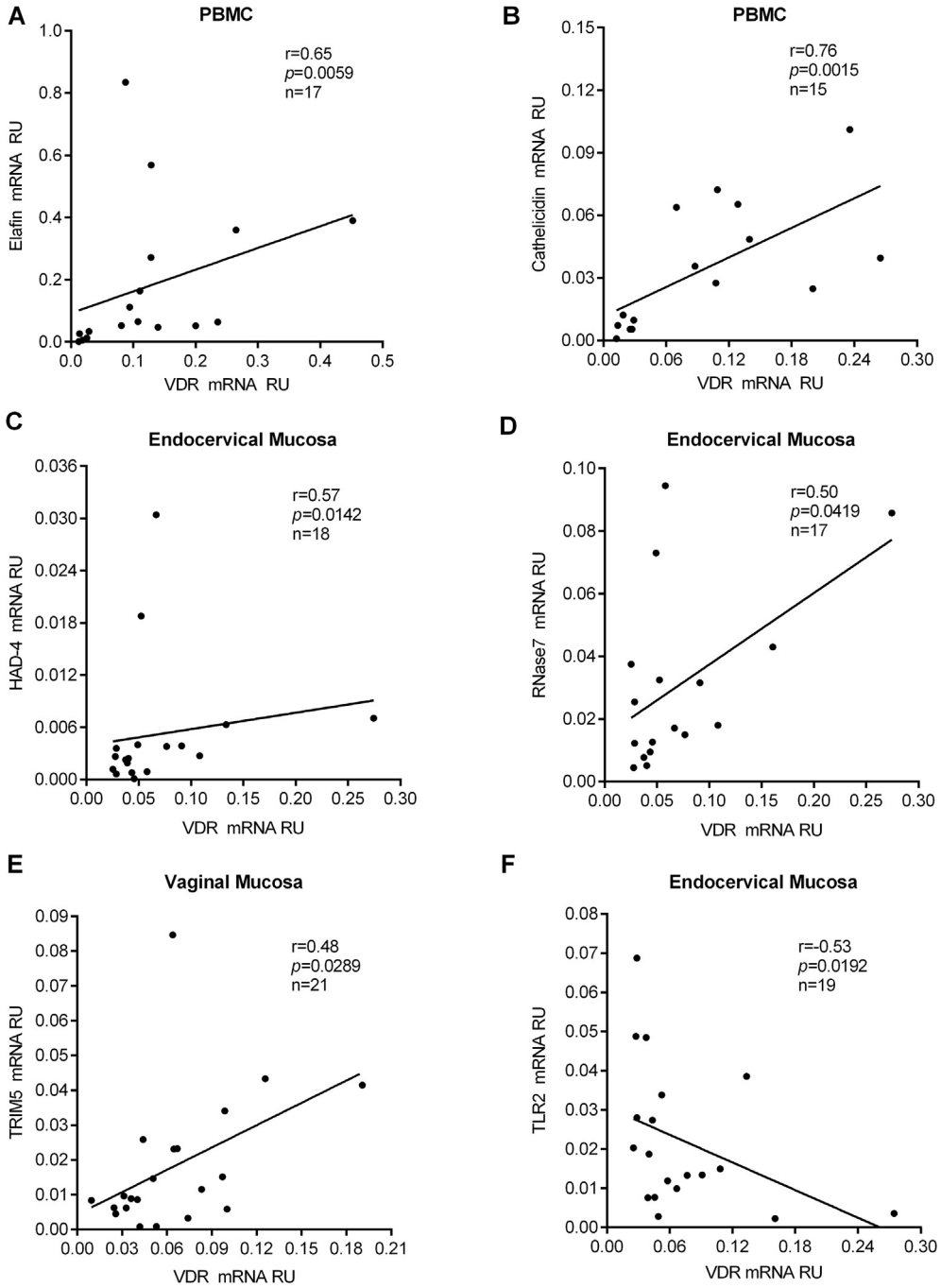

Fig. 2. The VDR mRNA [15] was positively correlated with the mRNA of Elafin (A) and Cathelicidin (B) in PBMCs, with HAD-4 (C) and RNase7 (D) in endocervical mucosa and with TRIM5 in vaginal mucosa (E) of HESNs. Furthermore, a negative correlation between mRNA of VDR and TLR2 in endocervical mucosa of HESNs was also observed (F). The correlations were evaluated using the Spearman coefficient ranks (r), which are displayed in each graph with the best linear fit lines, *p* values and number of samples (n).

Considering that genital mucosa is the main port for viral entry, factors associated with protection should be present mainly in these tissues; however, PBMCs are circulating immune cells that migrate around the entire body including mucosas, and thus they may reflect, at least partially, the ongoing response of these surfaces [19,20]. On the other hand, although HIV-1 oral sex is not an efficient route of infection, it does carry a small risk [21,22] pointing to the protective role of these molecules during oral sexual exposure to HIV-1, as previously proposed [21,23].

The production of cathelicidin by monocytes, natural killer (NK), B and γδT cells [24] could support its observed expression in PBMCs. In contrast, neutrophils and the intestine seem to be the biological source of HAD-4 [25]. Remarkably, to the best of our knowledge, this is the first study identifying the anti-HIV-1 molecules cathelicidin and HAD-4

overexpressed not only in PBMC but also in oral mucosa of HESNs compared to healthy controls. According to the expression of the human alpha defensins HAD-1 to -3, we hypothesized that HAD-4 could be expressed in monocytes, NK, T and B cells, explaining its expression in PBMCs [24].

Interestingly, the previously reported anti-inflammatory effects of VitD [11,12] could be represented by the negative correlation between VDR mRNA and the lower levels of TLR2 observed in the endocervical mucosa of HESNs. Moreover, we previously reported lower mRNA of the pro-inflammatory cytokine TNF-α in oral and genital mucosa of HESNs compared to SPs, and higher mRNA levels of anti-inflammatory cytokine IL-10 in genital mucosa of HESNs compared to HCs with positive correlations between VDR and IL-10 mRNA levels in PBMCs and genital mucosa of HESNs [15].

The differences in the mRNA expression profile among all the tissues might be due to compartmentalization issues and differences in the frequency of various cell subpopulations, such as immune cells in peripheral blood versus epithelial cells in mucosa, or different shapes and structures of the epithelial tissue such as the stratified squamous epithelium in oral and vaginal mucosa vs single-layered columnar epithelium of endocervix [26]. Indeed, it is well-known that the expression of the antiviral molecules are tightly regulated and highly dependent on the type of tissues or cell subpopulations [23,24,27,28]. Unfortunately, protein levels were not measured due to sample constraints, representing a limiting condition of this study. However, an overall positive correlation between mRNA and protein expression levels explaining more than 85% of the variation in steady-state protein levels have been reported [29], increasing the confidence in the use of mRNA expression for biological discovery. Furthermore, previous studies have found protein expression particularly of cathelicidin and HAD-4 in human oral, rectal an genital mucosa [27,30] strengthening the biological significance of our findings.

Taken together, these findings suggest that VitD may favor an immune quiescence phenotype in genital tissue, protecting against infection by limiting HIV-1 target cells and substrates available for HIV-1 replication, while promoting an antiviral response as previously shown for other viral infections [12,31].

Acknowledgments

The authors thank patients and volunteers who kindly participated in this study. Thanks go to “Grupo de Vida” foundation in Medellin – Colombia and to the staff of HERES Health, from Santa Marta – Colombia for their collaboration in patient recruitment. Thanks to Anne-Lise Haenni from the Université Paris-Diderot for her positive revision and English proofreading.

This work was supported by the Universidad de Antioquia (Estrategia Sostenibilidad), Universidad Cooperativa de Colombia (Sostenibilidad 2015–2016, Grupo Infettare) and Banco de la República de Colombia (Grant 3377). Wbeimar Aguilar-Jimenez is recipient of a doctoral scholarship from Colciencias. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.micinf.2016.03.015.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Zapata W, Rodriguez B, Weber J, Estrada H, Quiñones-Mateu M, Zimermman P, et al. Increased levels of human beta-defensins mRNA in sexually HIV-1 exposed but uninfected individuals. Curr HIV Res 2008; 6:531–8.

[2] Zapata W, Aguilar-Jimenez W, Feng Z, Weinberg A, Russo A, Potenza N, et al. Identification of innate immune antiretroviral factors during in vivo and in vitro exposure to HIV-1. Microbes Infect 2015;18: 211–9.

[3] Taborda N, Zapata W, Montoya CJ, Rugeles MT. Increased expression of secretory leukocyte protease inhibitor -SLPI- in oral mucosa of Colombian HIV-1-exposed seronegative individuals. AIDS Res Hum Retroviruses 2012;28:859–62.

[4] Gonzalez SM, Taborda NA, Feria MG, Arcia D, Aguilar-Jimenez W, Zapata W, et al. High expression of antiviral proteins in mucosa from individuals exhibiting resistance to human immunodeficiency virus. PLoS One 2015;10:e0131139.

[5] Iqbal SM, Ball TB, Levinson P, Maranan L, Jaoko W, Wachihi C, et al. Elevated elafin/trappin-2 in the female genital tract is associated with protection against HIV acquisition. AIDS 2009;23:1669–77.

[6] Biasin M, Piacentini L, Lo Caputo S, Kanari Y, Magri G, Trabattoni D, et al. Apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G: a possible role in the resistance to HIV of HIV-exposed seronegative individuals. J Infect Dis 2007;195:960–4.

[7] Bergman P, Walter-Jallow L, Broliden K, Agerberth B, Soderlund J. The antimicrobial peptide LL-37 inhibits HIV-1 replication. Curr HIV Res 2007;5:410–5.

[8] Wu Z, Cocchi F, Gentles D, Ericksen B, Lubkowski J, Devico A, et al. Human neutrophil alpha-defensin 4 inhibits HIV-1 infection in vitro. FEBS Lett 2005;579:162–6.

[9] Cote SC, Plante A, Tardif MR, Tremblay MJ. Dectin-1/TLR2 and NOD2 agonists render dendritic cells susceptible to infection by X4-using HIV-1 and promote cis-infection of CD4(+) T cells. PLoS One 2013;8:e67735.

[10] Schlaepfer E, Rochat MA, Duo L, Speck RF. Triggering TLR2, -3, -4, -5, and -8 reinforces the restrictive nature of M1- and M2-polarized macrophages to HIV. J Virol 2014;88:9769–81.

[11] Trochoutsou AI, Kloukina V, Samitas K, Xanthou G. Vitamin-D in the immune system: genomic and non-genomic actions. Mini Rev Med Chem 2015;15:953–63.

[12] Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. J Immunol 2010;184: 965–74.

[13] Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 2004;173:2909–12.

[14] Dai X, Sayama K, Tohyama M, Shirakata Y, Hanakawa Y, Tokumaru S, et al. PPARgamma mediates innate immunity by regulating the 1alpha,25-dihydroxyvitamin D3 induced hBD-3 and cathelicidin in human keratinocytes. J Dermatol Sci 2010;60:179–86.
[15] Aguilar-Jimenez W, Zapata W, Caruz A, Rugeles MT. High transcript levels of vitamin D receptor are correlated with higher mRNA expression of human beta defensins and IL-10 in mucosa of HIV-1-exposed seronegative individuals. PLoS One 2013;8:e82717.
[16] Zapata W, Aguilar-Jimenez W, Pineda-Trujillo N, Rojas W, Estrada H, Rugeles MT. Influence of CCR5 and CCR2 genetic variants in the resistance/susceptibility to HIV in serodiscordant couples from colombia. AIDS Res Hum Retroviruses 2013;29:1594–603.
[17] da Conceicao VN, Dyer WB, Gandhi K, Gupta P, Saksena NK. Genome-wide analysis of primary peripheral blood mononuclear cells from HIV + patients-pre-and post- HAART show immune activation and inflammation the main drivers of host gene expression. Mol Cell Ther 2014;2:11.
[18] Coussens AK, Naude CE, Goliath R, Chaplin G, Wilkinson RJ, Jablonski NG. High-dose vitamin D3 reduces deficiency caused by low UVB exposure and limits HIV-1 replication in urban Southern Africans. Proc Natl Acad Sci U S A 2015;112:8052–7.
[19] Taborda NA, Gonzalez SM, Alvarez CM, Correa LA, Montoya CJ, Rugeles MT. Higher frequency of NK and CD4+ T-cells in mucosa and potent cytotoxic response in HIV controllers. PLoS One 2015;10:e0136292.
[20] Kantele JM, Arvilommi H, Kontiainen S, Salmi M, Jalkanen S, Savilahti E, et al. Mucosally activated circulating human B cells in diarrhea express homing receptors directing them back to the gut. Gastroenterology 1996;110:1061–7.
[21] Herzberg MC, Weinberg A, Wahl SM. (C3) The oral epithelial cell and first encounters with HIV-1. Adv Dent Res 2006;19:158–66.
[22] Page-Shafer K, Shiboski CH, Osmond DH, Dilley J, McFarland W, Shiboski SC, et al. Risk of HIV infection attributable to oral sex among men who have sex with men and in the population of men who have sex with men. AIDS 2002;16:2350–2.
[23] Tugizov SM, Herrera R, Veluppillai P, Greenspan D, Soros V, Greene WC, et al. HIV is inactivated after transepithelial migration via adult oral epithelial cells but not fetal epithelial cells. Virology 2011;409:211–22.
[24] Agerberth B, Charo J, Werr J, Olsson B, Idali F, Lindbom L, et al. The human antimicrobial and chemotactic peptides LL-37 and alpha-defensins are expressed by specific lymphocyte and monocyte populations. Blood 2000;96:3086–93.
[25] Lehrer RI, Lichtenstein AK, Ganz T. Defensins: antimicrobial and cytotoxic peptides of mammalian cells. Annu Rev Immunol 1993;11:105–28.
[26] Shattock RJ, Moore JP. Inhibiting sexual transmission of HIV-1 infection. Nat Rev Microbiol 2003;1:25–34.
[27] Romas LM, Hasselrot K, Aboud LG, Birse KD, Ball TB, Broliden K, et al. A comparative proteomic analysis of the soluble immune factor environment of rectal and oral mucosa. PLoS One 2014;9:e100820.
[28] Goswami R, Mondal AM, Tomar N, Ray D, Chattopadhyay P, Gupta N, et al. Presence of 25(OH)D deficiency and its effect on vitamin D receptor mRNA expression. Eur J Clin Nutr 2009;63:446–9.
[29] Csardi G, Franks A, Choi DS, Airoldi EM, Drummond DA. Accounting for experimental noise reveals that mRNA levels, amplified by post-transcriptional processes, largely determine steady-state protein levels in yeast. PLoS Genet 2015;11:e1005206.
[30] Hirbod T, Kong X, Kigozi G, Ndyanabo A, Serwadda D, Prodger JL, et al. HIV acquisition is associated with increased antimicrobial peptides and reduced HIV neutralizing IgA in the foreskin prepuce of uncircumcised men. PLoS Pathog 2014;10:e1004416.
[31] Gal-Tanamy M, Bachmetov L, Ravid A, Koren R, Erman A, Tur-Kaspa R, et al. Vitamin D: an innate antiviral agent suppressing hepatitis C virus in human hepatocytes. Hepatology 2011;54:1570–9.