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Influence of Vitamins A and D on the Expression of MicroRNA27-3p Isoforms and GATA3 in Experimental Autoimmune Encephalomyelitis

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ABSTRACT

Vitamins A, D, and microRNAs contribute to T cell differentiation into T_H2 phenotypes. We investigated the molecular mechanisms and effects of vitamin A and D on the expression of GATA3 and miR-27-3p isoforms in experimental autoimmune encephalomyelitis (EAE) animal model of multiple sclerosis.

EAE was induced in C57BL/6 mice by immunization with myelin oligodendrocyte glycoprotein, mixed with Complete Freund's Adjuvant, together with injection of pertussis toxin. Treatments began one day before immunization with (200 μg and 100 ng of vitamin A and vitamin D per mouse, respectively, and vitamin A+D (100 μg+50 ng) per mouse. Expression levels of GATA3 and miR-27-3p isoforms were measured in the CNS and splenocytes by real-time RT-PCR.

The expression level of GATA3 in the mice spinal cords and splenocytes was increased in the vitamin A and A+D-treated EAE mice at 24 h and 48 h after restimulation by 10 μg and 40 μg of myelin oligodendrocyte glycoprotein. Vitamins A and D and their combination upregulated the miR-27-3p isoforms compared with EAE mice with no treatments. We also demonstrated that miR-273p isoform expression was altered in splenocytes of vitamin-treated EAE mice. The results showed a positive correlation between splenocyte GATA3 levels and miR-27-3p isoform expression.

The protective impacts of vitamins A and D in EAE mice may be mediated by the upregulation of GATA3. However, it is not specified whether suppression of GATA3-targeting miRNAs of the miR-27-3p family is involved in this effect. These results do not rule out the possibility that miR-27-3p isoforms might have beneficial effects by targeting other transcripts, such as GluA2 and NR2B.

Keywords: Experimental autoimmune encephalomyelitis; Inflammation; MicroRNA-27; Mouse; Multiple sclerosis; Vitamin A; Vitamin D

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INTRODUCTION

Multiple sclerosis (MS) is a neurodegenerative disease characterized by inflammation in the central nervous system (CNS).[1] Studies have shown the involvement of various environmental factors, such as infections and nutrients, as well as genetic and epigenetic elements, such as HLA polymorphisms, DNA methylations, and microRNAs, in the development of MS.[2,3] MicroRNAs are small (18 to 22 nucleotides) noncoding RNAs that regulate gene expression by suppressing mRNA translation or inducing mRNA degradation by binding to 3′ UTR of their targets.[4] Studies have reported that in peripheral blood cells and CNS lesions of MS patients, the expression of various miRNAs is altered. Similar miRNA dysregulations have been observed in the animal model of MS, experimental autoimmune encephalomyelitis (EAE).[5] Members of the miR-27 family are among the dysregulated miRNAs in MS/EAE. These miRNAs are upregulated in active lesions as well as T cells of MS patients,[6] and they are known to inhibit Th2 differentiation through repression of BMI1, B lymphoma Moloney murine leukemia virus (Mo-MLV) insertion region 1 homolog, which in turn stabilizes GATA binding protein 3 (GATA3).[7] Studies on the immunopathogenesis of MS/EAE have shown that activated T cells in the periphery infiltrate the CNS and induce neurodegeneration.[8] Several studies have reported that vitamins A and D can exert immunomodulatory effects in autoimmune neuroinflammation, diminish clinical symptoms and improve disease outcomes in MS patients[9] and EAE mice.[10] It has been reported that helper T (T_H1) cells play an important role in MS pathogenesis and that the enhancement of Th2 responses can diminish disease severity in MS patients.[11] There is evidence that these vitamins can affect gene expression through epigenetic processes. Of note, Dicer expression can be controlled by vitamin D via regulating the vitamin D receptor elements that are located in the Dicer promoter, which in turn influence the processing and maturation of miRNAs.[12]

Few studies have shown a correlation between vitamin A and D and microRNA expression and their effects on microRNA expression in immune cells,[13-15] but the molecular and cellular mechanisms of vitamin A and D on microRNA expression in the EAE model are not fully understood. Analyzing microRNA expression during vitamin A and D therapy in EAE mice may be a useful method for understanding molecular mechanisms of the biological function of these vitamins. Therefore, for the first time, we investigated the effects of vitamin A and D on the expression of Th2 transcription factors (GATA3) and miR-27-3p isoforms in an EAE model. The aim of the present study was to investigate the effect of vitamin A, D or their combination treatment on the differentiation of T cells toward the protective Th2 phenotype. We also explored whether the expression of miR-27 isoforms is associated with T_H2 differentiation caused by vitamin treatment.

MATERIALS AND METHODS

Animals

In this study, inbred female 8-10 weeks old C57BL/6 mice were used. The mice were purchased from Pasteur Institute, Tehran, Iran. They were kept in standard controlled conditions, at 20±2°C and a 12-hour light/dark cycle. The Ethics Committee of Tehran University of Medical Sciences supervised and accepted all care methods and experiments on animals.

EAE Induction and Mice Treatments

After two weeks of acclimation, EAE was induced by Hooke Kit (EK-2110, Hooke Kit™ MOG 35-55/CFA Emulsion PTX) (USA) as previously described.[10] Briefly, 100 μg myelin oligodendrocyte glycoprotein (MOG) 35–55 peptide emulsified in Complete Freund's Adjuvant were injected subcutaneously into the mice at the base of the tail. According to the Hooke kit instructions, two intraperitoneal injections of pertussis toxin (200 ng/100 μL PBS) were performed on the day of immunization and 24 h post-immunization, respectively. After MOG immunization (for up to 30 days), the daily clinical scores of all EAE mice were recorded using the 0-15 scoring.[16]

Group and Study Design

Female C57BL/6 mice were divided into 5 groups of seven or eight. Groups I to IV were EAE induced and group V were healthy mice. The groups were treated as follows: Group I (EAE mice) received only the vehicle. The solvent of vitamins A and D was used as a vehicle, which included disodium dihydrogen phosphate, monosodium phosphate monohydrate, sodium, ethylenediaminetetraacetic acid (EDTA), and sodium ascorbate. Group II (vitamin A-treated EAE mice) each

received 200 μg of all-trans retinoic acid (ATRA). Group III (vitamin D-treated EAE mice) each received 100 ng calcitriol. Group IV (vitamin A + D-treated EAE mice) received a combination of ATRA and calcitriol with a half-dose single therapy, 100 μg of ATRA and 50 ng of calcitriol. Group V (the healthy control group– no EAE induction) received equal amounts of the vehicle. Vitamin D and ATRA were obtained from Kern Pharma, Spain, and Sigma-Aldrich, St. Louis, MO, USA, respectively. All treatments were administered intraperitoneally every other day from 1 day before to 30 days after immunization.

Culture and Stimulation of Splenocytes

MOG-immunized mice splenocytes were isolated using Ficoll density gradient centrifugation. Then 2×10^6 cells were cultured and stimulated with MOG35-55 at concentrations of 0 (control), 10 and 40 μg/mL for 24 and 48 h in RPMI 1640 medium (Gibco, USA) supplemented with 5% Fetal Bovine Serum (FBS) (Gibco, USA). Cells were harvested after 24 and 48 h for the next experiment.

RNA Extraction and cDNA Synthesis

Total RNA, containing microRNAs, was extracted from stimulated splenocyte cultures and lumbar spinal cord tissues using miRNeasy Mini Kit (Qiagen)(Japan) which contains the real-time master mix, including a reverse transcriptase and a poly(A) polymerase, and the miScript RT Hispec Buffer, containing Mg^{2+}, dNTPs, oligo-dT primers, and random primers. Reverse transcription was conducted using a thermocycler (Applied Biosystems) with a program consisting of 37°C for 60 min and 95°C for 5 min. miRNA specific primers (miR-27a-3p, miR-27b-3p) were purchased from Qiagen. RNA concentrations were measured by Nanodrop (Thermo Scientific). First-strand cDNA synthesis was performed on 1 μg total RNA using the miScript II RT Kit (Qiagen)(Germany) for microRNAs and TAKARA kit (Japan) for gene expression analyses, according to the manufacturers' recommendations.

Real-time PCR

StepOnePlus Real-time PCR instrument (Applied Biosystems, Foster City, CA, USA) was utilized to evaluate the expression of GATA3, miR-27a-3p, and miR-27b-3p in cells and isolated spinal cord tissues. The sequences of the primers used in this study are shown in. Supplementary Table 1. The β-actin gene was used as a reference gene to normalize the target gene signals. To normalize the miRNA expression, SNORD-68 and SNORD-72 were used (purchased from Qiagen). The relative quantification method ($2^{-\Delta\Delta Ct}$) was applied to evaluate the relative expressions of miRNA and the target gene.

Bioinformatic Analyses

miRBase database was used to obtain microRNA sequences and annotations. miRTarBase and TargetScan 7.2 were used to extract miRNA and target interactions

Statistical Analysis

Statistical analyses were done using SPSS 20, and graphics were provided with GraphPad Prism 8 software. Statistical significance was determined by two-way ANOVA followed by appropriate post hoc testing for multiple comparisons and Student's t-test for two-group comparisons. Statistical significance was defined as p value of less than 0.05. Data are presented as mean±standard error of the mean (SEM).

RESULTS

Vitamins A, D and their Combination Impact the Weight of EAE Mice

The effects of vitamin A, D, and A+D treatments on the body weight of the mice are shown in (Figure 1). Weight loss was less severe in the vitamin D-treated EAE group compared with the vehicle-treated EAE animals ($p<0.05$). The effects of vitamin A treatment alone were more prominent; mice in this group had an initial weight loss but then began to gain weight about 20 days after immunization. The increase in their mean body weight was substantially higher compared with the vehicle-treated EAE mice ($p<0.0001$). Weight loss was also observed in vitamin A+D-treated EAE mice; however, the difference was not statistically significant compared to the vehicle-treated EAE group.

Vitamin A Increases GATA3 Gene Expression in the Spinal Cords of EAE Mice

Considering that T_H2 cells play a protective role in the context of MS/EAE, we examined the expression of GATA3 in the spinal cord tissues of the vehicle- and vitamins -treated EAE mice. Statistical analyses showed a significant increase in the expression of GATA3 only in vitamin A-treated EAE mice compared with the vehicle-treated EAE group ($p<0.05$) (Figure 2).

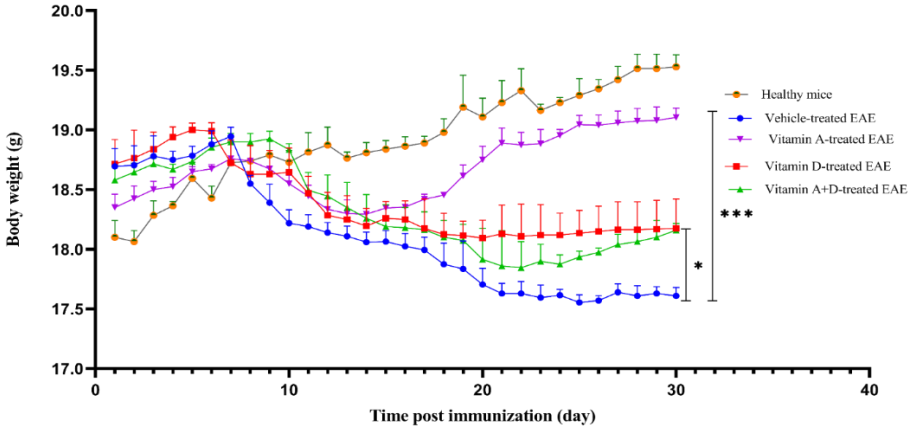


Figure 1. Body weights of vitamin-treated and vehicle-treated EAE mice. Treatment with vitamins increased body weight and the mean body weight in vitamin A-treated EAE mice compared with the vehicle-treated EAE group (p<0.0001). The values were presented as mean±SEM, eight mice in each group. *p<0.05, ***p<0.001.

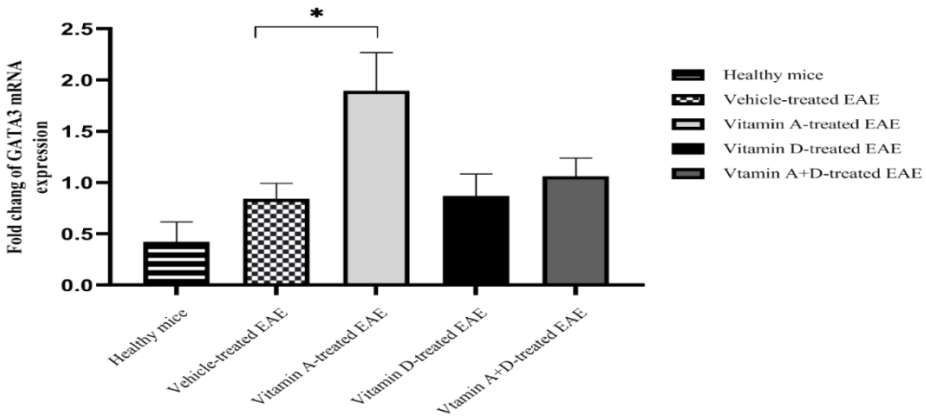


Figure 2. GATA3 mRNA expression in the spinal cord tissues in vehicle-treated and vitamin-treated EAE groups. Values are presented as mean±SEM; Eight mice per group, *p<0.05; one-way ANOVA, Tukey post hoc test was performed.

Effects of Vitamins A and D on the Expression of miRNAs which Target GATA3 in the CNS

GATA3 is targeted and regulated by several miRNAs, including miR-27a-3p and miR-27b-3p (Table 1A). miR-27 is highly conserved between humans and mice. Indeed, as shown in (Table 1B), the mature sequence of miR-27a-3p and miR-27b-3p is the same in humans and mice. Measuring the expression of miR-27-3p isoforms in the CNS of study groups showed an increase in miR-27a-3p and miR-27b-3p expression in the CNS tissues of vitamin-treated EAE mice compared with vehicle-treated animals; however, these differences were not statistically significant (Figure3A, B).

Treatment with Vitamins Decreases IL-2 Gene Expression in MOG-restimulated Splenocytes

Gene expression analyses showed that IL-2 gene expression was significantly higher in MOG-stimulated splenocytes of the vehicle-treated EAE group at 24 h and 48 h time points for 10 and 40 µg/mL concentrations compared with unstimulated cells (p<0.05, p<0.01) (Figure 4A). Significant IL-2 upregulation was also detected in splenocytes in the vitamin A-treated EAE group stimulated with 40 µg/mL of MOG after 24 h (Figure 4B). However, expression of IL-2 was significantly lower in vitamin A, D, and A+D groups after 24 h of stimulation with 10 and 40 µg/mL of MOG compared with vehicle-treated mice (Figure 4A).

Likewise, IL-2 expression was significantly lower in mice treated with vitamin A and D EAE mice after 48 h compared to vehicle-treated mice. The latter effect was only significant for 10 µg/mL concentration of MOG (Figure 4B). These findings indicate that treatment with vitamins A and D affects the production of the key T cell growth factor, IL-2.

Vitamin A Treatment Enhances GATA3 Gene Expression in MOG-stimulated Splenocytes

We next asked whether treatment with vitamins A and D might affect T cell differentiation, focusing on GATA3. The results of expression analyses demonstrated that GATA3 levels were significantly higher in splenocytes derived from vitamin A-treated EAE mice after 24 h stimulation with 40 µg/mL of MOG compared with the vehicle-treated EAE group (p<0.05) (Figure 5A). Likewise, GATA3 expression was significantly upregulated in vitamin A-treated EAE mice after 48 h of stimulation with 10 and 40 µg/mL of MOG peptide compared with the vehicle-treated EAE group (Figure 5B).

Table 1. GATA3, miR27a-3p and miR27b-3p sequences. Sequence of predicted binding sites for miR-27a-3p and miR-27b-3p on the 3' UTR of human and mouse GATA3 mRNA (A). Homology between human and mouse mature miRNA sequences (B).

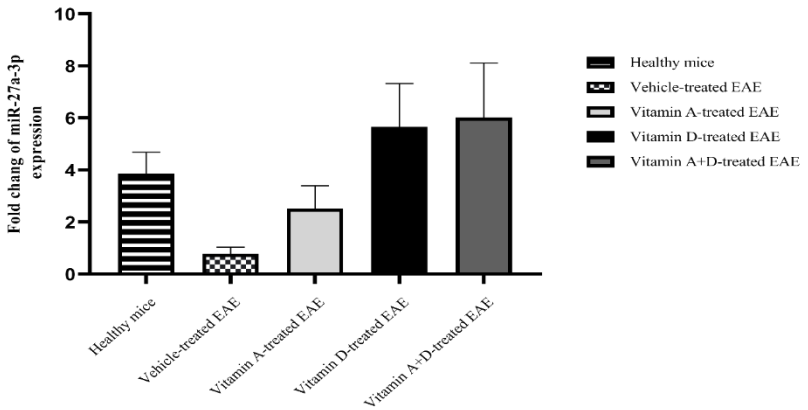
A

	Predicted consequential pairing of target region (top) and miRNA (bottom)
Position 251-257 of GATA3 3' UTR hsa-miR-27a-3p	5' ...ACAGGGUCUCUAGUGCUGUGAAA... 3' CGCCUUGAAUCGGUGACACUU
Position 251-257 of GATA3 3' UTR hsa-miR-27b-3p	5' ...ACAGGGUCUCUAGUGCUGUGAAA... 3' CGUCUUGAAUCGGUGACACUU
Position 705-711 of GATA3 3' UTR mmu-miR-27a-3p	5' ...CUAGGCCUACAUGCUCUGUGAAU... 3' CGCCUUGAAUCGGUGACACUU
Position 705-711 of GATA3 3' UTR mmu-miR-27b-3p	5' ...CUAGGCCUACAUGCUCUGUGAAU... 3' CGUCUUGAAUCGGUGACACUU

B

	sequence of MicroRNA
hsa-miR-27a-3p	UUCACAGUGGCUAAGUUCCGC
mmu-miR-27a-3p	UUCACAGUGGCUAAGUUCCGC
hsa-miR-27b-3p	UUCACAGUGGCUAAGUUCUGC
mmu-miR-27b-3p	UUCACAGUGGCUAAGUUCUGC

A



B

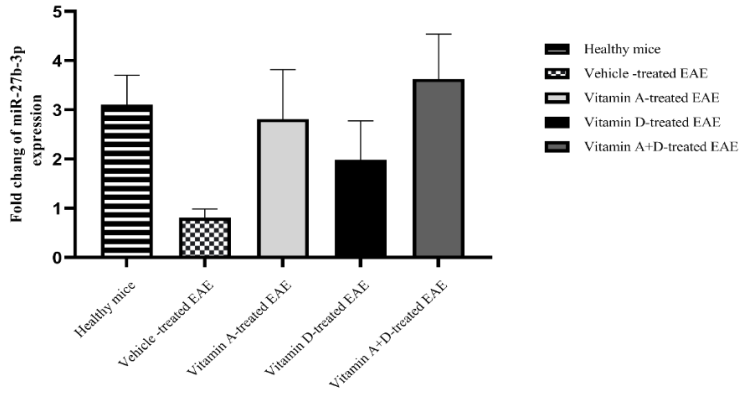
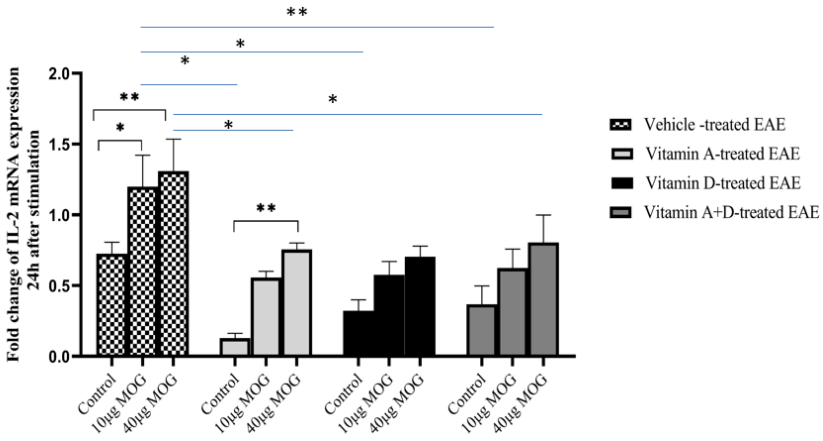


Figure 3. miR-27-3p isoform expression in the spinal cord tissues. The expression level of miR-27a-3p (A) and miR-27b-3p (B) in vitamin-treated EAE and healthy mice was higher than in the vehicle-treated EAE group; however, the differences were not significant. The values are presented as mean±SEM, eight mice in each group, sone-way ANOVA, Tukey post hoc.

A



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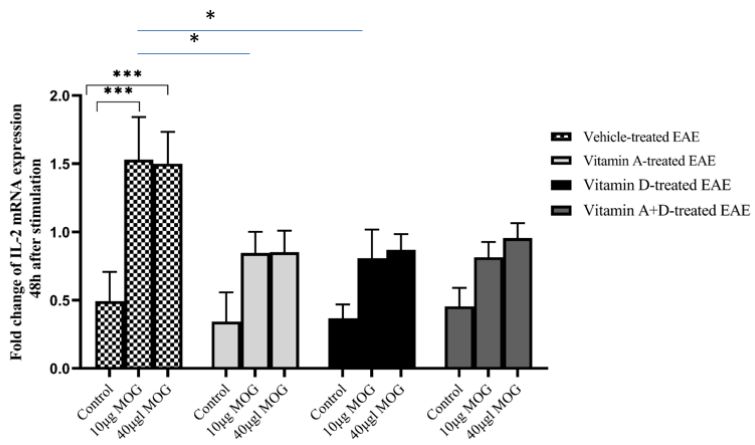

Figure 4. IL-2 expression levels in splenocytes. IL-2 expression levels in MOG-stimulated splenocytes obtained from different groups. Splenocytes were stimulated with 0 (control), 10, and 40 µg/ml of MOG peptide for 24 h (A) and 48 h (B). Values are presented as mean±SEM, eight mice in each group, *p<0.05, **p<0.01, ***p<0.001; two-way ANOVA, Tukey post hoc test was performed.

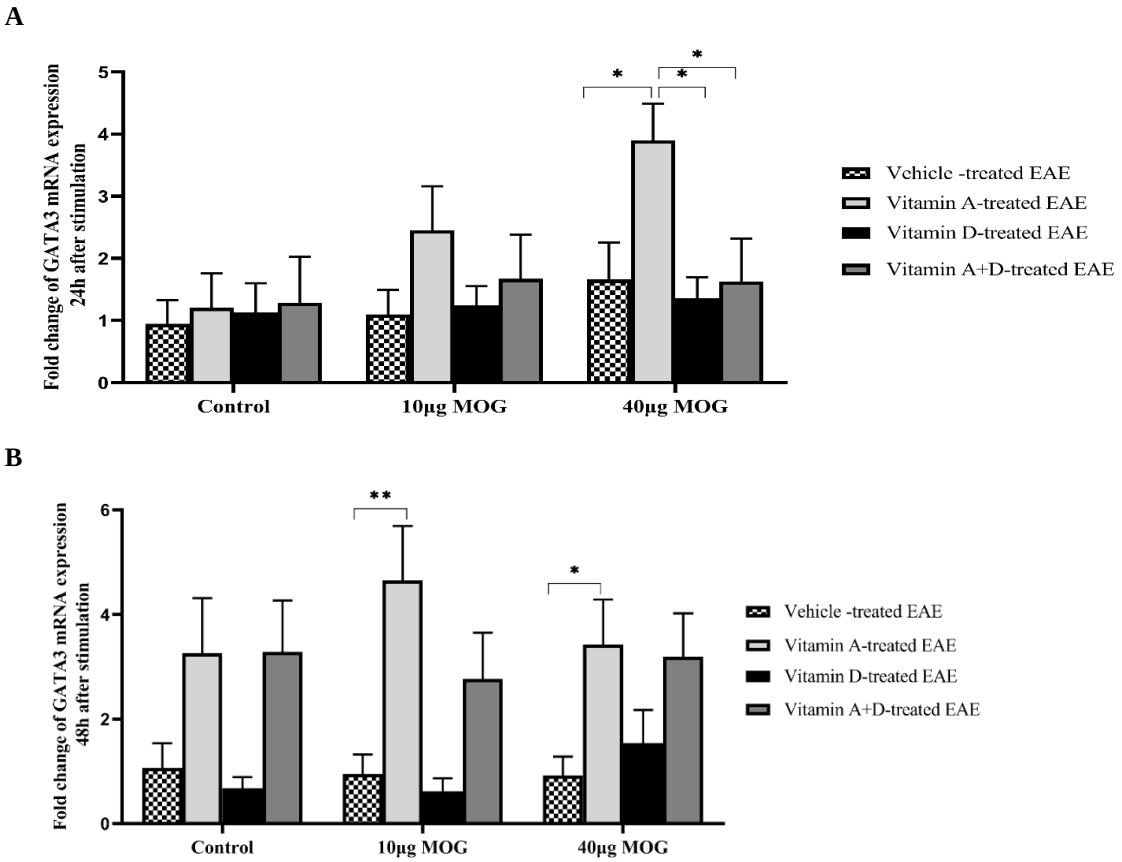

Figure 5. GATA3 expression levels in splenocytes. GATA3 expression levels in MOG-stimulated splenocytes obtained from different groups evaluated by real-time PCR. Splenocytes were stimulated with 0, 10, and 40 µg/ml of MOG peptide for 24 h (A) and 48 h (B). Values are presented as mean ± SEM, eight mice in each group, *p<0.05, **p<0.01; two-way ANOVA, Tukey post hoc test was performed.

Upregulation of miR-27a/b-3p Expression in Splenocytes of Vitamin A and D-treated EAE Mice

We examined the effect of in vivo treatment with vitamins A and D on the expression of miR-27a-3p and miR-27a-3p in MOG-stimulated splenocytes from different groups of mice. The expression of miR-27a-3p was significantly upregulated in vitamin A-treated EAE compared with vehicle-treated EAE mice at 10 µg/Ml MOG concentration after 24 h (p<0.05). Likewise, the expression of miR-27a-3p was significantly increased in vitamin A (p<0.05) and vitamin D (p<0.01)-treated EAE groups compared with the vehicle-treated EAE group at 40 µg/mL of MOG after 24 h (Figure 6A). miR-27a-3p expression was also induced in splenocytes treated with 40 µg/mL of MOG peptide for 48 h in vitamin A-treated EAE mice compared with vehicle-treated EAE mice (p<0.001) (Figure 6B). After 24 and 48 h of stimulation with 10 and 40 µg/mL of MOG peptide, the expression of miR-27b-3p in vitamin D and combined A+D-treated EAE mice was also increased compared with the vehicle-treated EAE group mice; however, this difference was not statistically significant (Figure 6C, D).

GATA3 Expression Levels are Positively Correlated with miR-27b-3p in Splenocytes

We then explored any correlation between miR-27-3P isoforms and GATA3 levels. Surprisingly, correlation analysis exhibited a positive correlation between miR-27a-3p and GATA3 expression levels in MOG-restimulated splenocytes after 24 h and 48 h at 10 and 40 µg/ml of concentration (Figures 7A, C). There were no significant correlations between miR-27b-3p and GATA3 levels (Figures 7B, D).

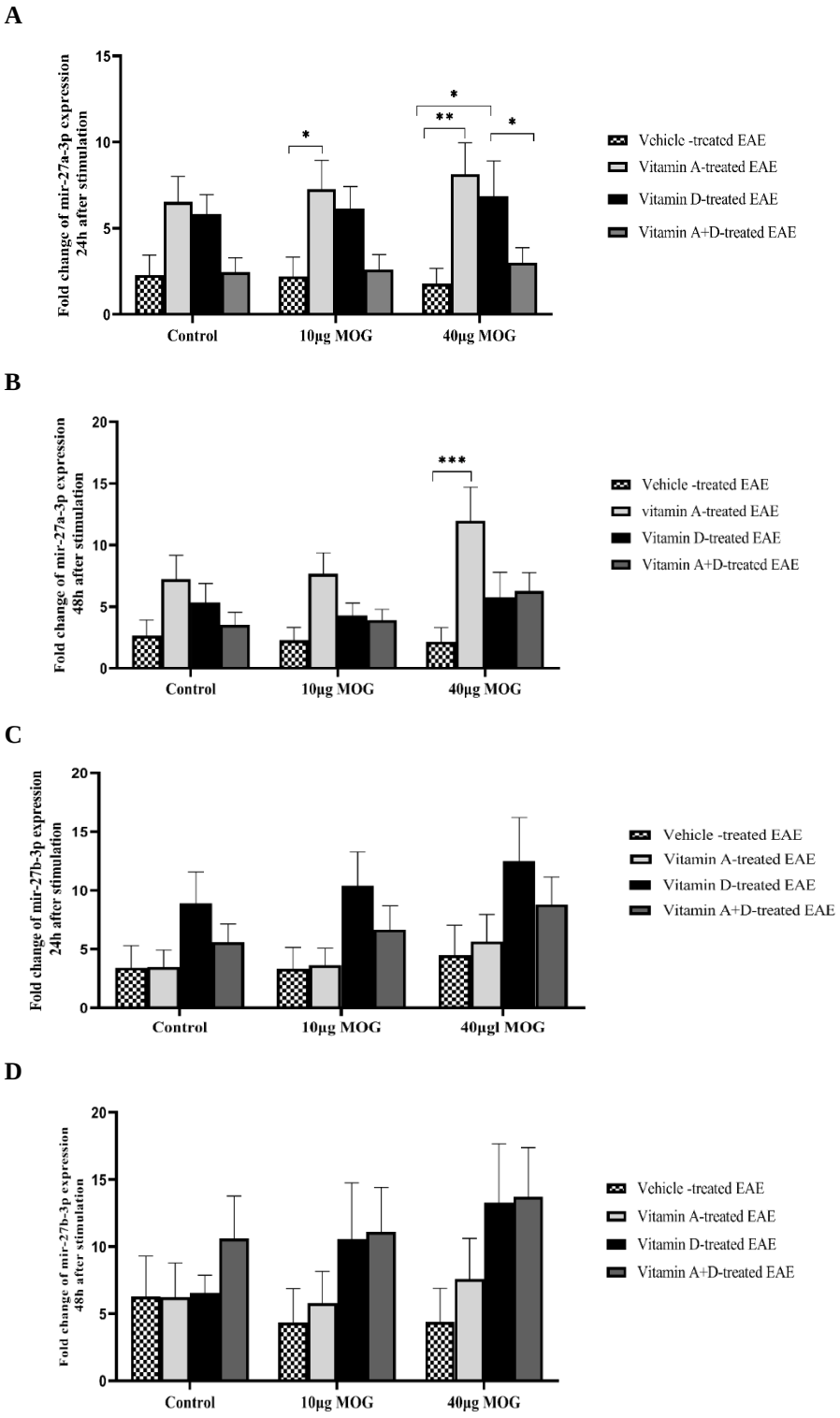

Figure 6. Expression levels of miR-27-3p isoforms in splenocytes. Splenocytes were stimulated with 0 (control), 10, and 40 µ/mL MOG peptide at 24 h (A and C) and 48 h (B and D). Data for miR-27a-3p are shown in panels and b. Data for miR-27b-3p are shown in panels c and d. The values were presented as mean±SEM, eight mice in each group, *p<0.05, **p<0.01, ***p<0.001; two-way ANOVA, Tukey post hoc test was performed.

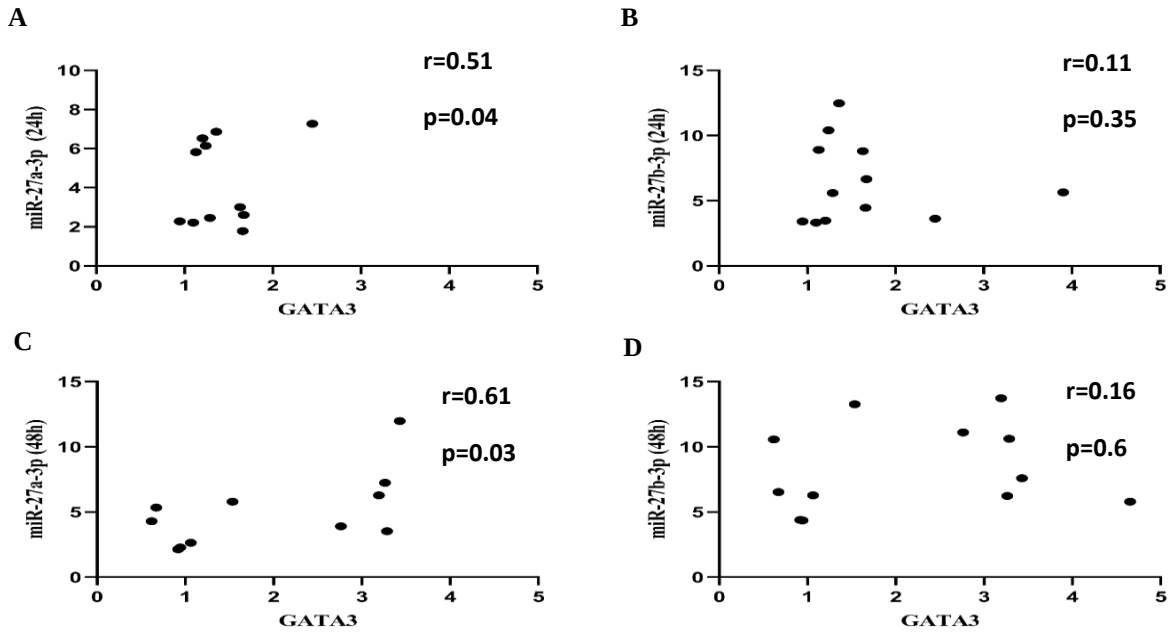

Figure 7. Correlation analysis between levels of GATA3 mRNA and isoforms of miR-27-3p. The dot plot demonstrates the correlation between GATA3 mRNA and miR-27a-3p/miR-27b-3p in splenocytes 24 h (A and B) and 48 h (C and D) after stimulation with MOG peptide. (Pearson correlation; *p<0.05).

DISCUSSION

We have previously reported the protective effects of vitamin A and D treatment in EAE mice.[17] In this study, we explored whether the beneficial effects of vitamins A and D might be associated with any change in the differentiation of T cells towards the protective T_H2 phenotype. We also investigated the role of two T_H2-related miRNAs in the process. Our results showed that while treatment with vitamin A and D could enhance the expression of T_H2-specific transcript factor GATA3, this was not associated with a suppression of GATA3-targeting miRNAs, miR-27a-3p and miR-27b-3p species.

The effects of vitamins A and D, alterations in the function of innate immune cells, including the production of inflammatory cytokines and antigen presentation by monocytoid B cells (MBCs), differentiation of macrophages, and activity of NK cells have been reported.[18] That said, a large body of evidence also points to the effects of these vitamins on lymphocyte activity and differentiation.[19] Effects on differentiation will, in turn, lead to alterations in the profile of cytokines produced by T cells with ensuing pathogenic and protective effects. Indeed, Dawson et al. have reported that the in vitro treatment of human peripheral blood mononuclear cells (PBMCs) with vitamin A can downregulate IFNγ, IL-2, IL-12p70, and TNF-α levels and enhance the expression and IL-4, IL-5, and IL-13, all associated with elevated T_H2 transcription factor expression GATA3.[20] These findings are consistent with the current study in which we performed in vivo treatments in EAE mice. Although we focused on GATA3, other T_H2-related genes, including c-Maf and STAT6, could also be influenced by these vitamins, as reported previously.[20]

The question of what mechanisms interconnect vitamin treatment with GATA3 expression remains to be answered. From a molecular biology point of view, regulation of a transcription factor's expression could happen at either transcriptional or post-transcriptional levels. Post-transcriptional mechanisms of gene expression regulation are quite diverse, and miRNAs are one possible way that an environmental factor could control the expression of a protein–GATA3 in our case. To explore this, and as alluded to in the Methods section, we searched miRTarBase, the miRNA target database of experimentally validated miRNA-transcript interactions, for GATA3-targeting miRNAs. For human GATA3, miR-27a-3p and miR-29b-3p were the only reported miRNAs whose interactions with GATA3 were confirmed by reporter assays.[21] Our decision was then

influenced by findings showing that miR-27-3p isoforms were altered in MS patients' blood. Notably, recent studies have reported increased expression of miR-27a-3p and miR-27b-3p in the relapsing phase compared with the remission phase in MS patients. Moreover, miR-27a could target SMAD4, SMAD3, SMAD2 and SP1 genes that are involved in regulating FOXP3 expression and inhibiting TGF-β signaling.[22] MS patients have higher levels of miR-27b expression in their naïve CD4 T lymphocytes and miR-340 expression in CD4 T memory lymphocytes. These microRNAs increase/suppress T_H1/T_H2 responses by targeting the IL-4 and BMI1 genes and decreasing the levels of GATA3, respectively. In addition, when miR-27b mimic sequences were transfected to naïve CD4 T lymphocytes in EAE mice, IFN-γ secretion was increased; while treating with miR-27b antagomir decreased the level of IL-17 expression because of T_H2 pathway inhibition which induce T_H17 differentiation and IL-17 cytokine secretion, suggest that cytokine secretion is shifting from T_H2 to T_H17. T_H2 immune response is reactivated when miR-27b antagomirs are transfected into PBMC of MS patients.[7] The remyelination process requires the expression of six genes (Atp10b, Kcna1, Itga8, C1ql3, Capn6, and Gjc3) for oligodendrocyte cell differentiation and maturation from their precursors. It has been reported that miR-27a regulates the expression of these genes during the demyelination process. Indeed, the expression of miR-27a is increased in oligodendrocyte precursors. This increase is positively correlated with demyelination in the brain of MS patients.[23] Based on these findings, miR-27-3P can be considered a proinflammatory as well as a myelination-inhibiting microRNA.

In the current study, we found that miR-27a-3p and miR-27b-3p levels were lower in the spinal cords of EAE mice compared with healthy animals and that treatment with vitamin A (or its combination with vitamin D) increased their expression. Likewise, when we studied the expression of these miRNAs in MOG-stimulated splenocytes, we noticed that vitamin A treatment increased miR-27a-3p levels in these cells. These findings raise two possibilities. One possibility is the ever-present discordance between human diseases and their animal models. Another possibility is discordance between the molecular findings of PBMC and CNS. While a miRNA might be increased in peripheral blood leukocytes, its expression might differ in the CNS, where a collection of parenchymal cells, i.e., neurons and glia, together with a number of infiltrating leukocytes, do contribute to the miRNA/mRNA pool under study. This issue can also explain the lack of negative correlation between miR-27a-3p and miR-27b-3p with GATA3 in the spinal cord tissues of mice in our study. We did not analyze the expression of miRNAs in healthy mice about splenocytes. However, treatment of cells with MOG (which in a way recapitulates in vivo stimulation of cells in peripheral lymphoid tissues) did not lead to any change in miR-27-3P levels. However, the effect of treatment with vitamin A was an increase in miR27a-3p levels, which, together with enhanced GATA3 expression in these cells, leading to a positive (rather than a negative) correlation between miR-27a-3p, miR-27b-3p, and GATA3. Two issues are worth mentioning here. First, despite the fact that miRNAs generally downregulate their targets' levels, positive correlations have been reported between miRNA and transcripts. In a global correlation analysis between miRNAs and mRNAs in human PBMCs by Wang et al, researchers have shown that roughly 30% of miRNA-mRNA interactions display positive correlations.[24] Interestingly, Nunez et al. have reported the existence of positively correlated miRNA-mRNA regulatory networks in mouse brains during alcohol insult.[25] Another issue is that miRNAs do not always control their targets through transcript degradation. One key mechanism that does not affect the stability of the mRNA is translation inhibition. This can also lead to the lack of a negative correlation between miRNA and mRNA levels.

Finally, it should be noted that miR-27a-3p and miR-27b-3p have other targets that might be relevant to MS/EAE pathogenies. Morquette et al, have found that miR-27a-3p is upregulated in neurons in EAE mice and that glutamate receptor signaling pathway mRNAs are likely targets of miR-27a-3p. They also reported that enhanced levels of miR-27a-3p could antagonize N-methy- D-aspartate (NMDA) and A-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in turn, lead to a neuroprotective effect.[26]

Overall, the results of this study highlight the potential of vitamin A and D treatment in the differentiation of T cells toward the protective T_H2 phenotypes in EAE through upregulating the GATA3 transcription factor. Our findings, however, do not support a role for miR-27-3P isoforms in this process while not ruling out the possibility of other miR-27-3P targets playing roles in the pathogenic/protective process.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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