Effects of Vitamin D and Dexamethasone on Lymphocyte Proportions and Their Associations With Serum Concentrations of 25-Hydroxyvitamin D$_3$ In Vitro in Patients With Multiple Sclerosis or Neuromyelitis Optica Spectrum Disorder

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Background: Clear associations have been found between vitamin D deficiency and several autoimmune diseases including multiple sclerosis (MS). However, the benefits of vitamin D supplementation on disease management remain a matter of debate.

Objective and Methods: Patients with MS ($N=12$) and neuromyelitis optica spectrum disorder (NMOSD; $N=12$) were enrolled along with 15 healthy controls. Changes in lymphocyte subset proportions during stimulation of their peripheral blood mononuclear cells (PBMCs) with the active form of vitamin D, 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$), and correlations with serum concentrations of the vitamin D precursor 25-hydroxyvitamin D$_3$ (serum 25(OH)D$_3$) were explored. The impact of 1,25(OH)$_2$D$_3$ stimulation on the expression of vitamin-D-responsive genes in immune cells was also investigated.

Results: In both MS and NMOSD, stimulation of PBMCs with 1,25(OH)$_2$D$_3$ followed by steroid suppressed the proliferation of total lymphocytes and T cells. The ratio of CD19$^+$CD27$^+$ memory B cells (Bmem) to all B cells after stimulation with 1,25(OH)$_2$D$_3$ was negatively correlated with serum 25(OH)D$_3$ in MS (Spearman’s $r=-0.594$, $p=0.042$), but positively correlated in NMOSD (Pearson’s $r=0.739$, $p=0.006$). However, there was no relationship between the ratio of Bmem to CD19$^+$CD24$^+$CD38$^+$ regulatory B cells and...
**INTRODUCTION**

1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃), the active form of vitamin D, is receiving increasing attention due to its role as a regulator of the immune system (1). Immune cells, such as macrophages, dendritic cells, and activated lymphocytes, express both the vitamin D receptor (VDR) and 1α-hydroxylase (CYP27B1), the key enzyme that catalyzes the bioactivation of 1,25(OH)₂D₃ from its precursor 25-hydroxyvitamin D₃ (25(OH)D₃) (2–4). Vitamin D suppresses the production of proinflammatory cytokines such as interferon-γ, interleukin (IL)-2, and IL-17, enhances the secretion of anti-inflammatory cytokines such as IL-4 and IL-10, and shifts the balance toward immune responses mediated by T helper (Th) type 2 cells and regulatory T cells (3, 5). In addition, vitamin D interferes with B-cell proliferation and differentiation into memory B cells (Bmem) and antibody-secreting plasma cells.

MS and NMOSD are autoimmune inflammatory demyelinating diseases that affect the central nervous system (CNS). Autoreactive T cells in the periphery and T cell–B cell collaboration contribute to the pathogenesis of CNS autoimmunity. CNS antigen-specific T cells, specifically CD4⁺ Th1 cells and Th17 cells, and antibodies against the aquaporin-4 water channel (AQP4) are believed to play key roles in the development of MS and NMOSD, respectively (14, 15). In the periphery, B cells, and especially Bmem, may serve as antigen-presenting cells, and activate and differentiate the autoreactive T cells into the Th17 lineage by producing cytokines such as IL-6 and IL-21 (14, 16). In addition, Th17 and T follicular helper cells produce the cytokines IL-17a and IL-6, which promote granulocyte activation, and B-cell differentiation and antibody production (17). Vitamin D may exert an immunomodulating effect by suppressing the inflammatory autoimmune response. However, the benefit of vitamin D in terms of therapeutic applications is not clear for either MS or NMOSD (11, 18, 19).

This study investigated the effects of vitamin D on immune cells through peripheral blood mononuclear cell (PBMC) stimulation in patients with MS or NMOSD, and in healthy controls (HCs). The associations between serum 25(OH)D₃ concentration (hereafter serum 25(OH)D₃) and the proportions of lymphocyte subsets following stimulation with 1,25(OH)₂D₃ were also evaluated, and 1,25(OH)₂D₃-induced changes in mRNA expression of the genes encoding VDR (VDR), the vitamin-D-activating and vitamin-D-degrading enzymes 1α-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24A1), respectively, and IL-10 (IL-10) were explored.

**METHODS**

**Subjects**

Patients with MS (20) (N=12) and AQP4-antibody-positive NMOSD (15) (N=12) were enrolled at Samsung Medical Center in Seoul, South Korea between November 2016 and August 2018. A total of 20 ml of peripheral venous blood samples were taken during remission in all patients. The same amount of venous blood was also obtained from 15 HCs who did not have a history of acute or chronic disease and had not been taking any medication during the previous 3 months. Total serum 25(OH)D₃ was measured using commercially available enzyme-linked immunosorbent assay kits (Eagle Biosciences, NH, USA) according to the manufacturer’s instructions. The study was approved by the Institutional Review Board of Samsung Medical Center and written informed consents were obtained from all subjects.

**PBMC Preparation and Culture**

Whole blood was collected into lithium heparin tubes and PBMCs were then separated using density-gradient centrifugation on Ficoll-Paque PLUS (GE Healthcare Biosciences, Pittsburgh, PA, USA). The isolated PBMCs were suspended in fetal bovine serum (FBS; Life Technologies, Grand Island, NY, USA) and 10% dimethyl sulfoxide (Sigma Aldrich, St. Louis, MO, USA) at a concentration of 1×10⁶ cells/ml, and then stored in liquid nitrogen until required for the stimulation experiments.

The PBMCs were cultured with RPMI (Roswell Park Memorial Institute) 1640 medium containing l-glutamine supplemented with 10% certified inactivated FBS and 50 units.
of penicillin/streptomycin (Life Technologies). They were then incubated with a nonspecific stimulation mixture of 10 ng/ml lipopolysaccharide (LPS; Sigma Aldrich), 100 ng/ml CD40 ligand (CD40L; Enzo Biochem, New York, NY, USA), and 5 nM cytosine-phosphate-guanosine-oligodeoxynucleotides (CpG-ODN 2006; Invivogen, USA), either alone (LPS+CD40L+CpG-ODN) or in combination with steroid (dexamethasone, 10 nM, ODN 2006; Invivogen, USA), either alone (LPS+CD40L+CpG-ODN alone or in combination with 1,25(OH)2D3, steroid, or both) for 24 hours prior to LPS+CD40L+CpG-ODN. The cells were incubated for 72 hours, after which they were analyzed with flow cytometry.

### Immunophenotyping by Flow Cytometry

The percentages of total lymphocytes and T cells, B cells, regulatory B cells (Breg), and Bmem in the PBMC samples were determined by flow cytometric analysis of the surface markers CD3, CD19, CD24, CD38, and CD27. In this study, CD19+CD24+CD38+ cells were defined as Breg and CD19+CD27+ cells as Bmem. PBMCs were incubated with the following surface marker antibodies (all from BD Biosciences, San Jose, CA, USA) for 30 min at 4°C in the dark: anti-CD19-APC-Cy7 (clone SJ25C1), anti-CD3-PerCPCy5.5 (clone SK7), anti-CD24-FITC (clone ML5), anti-CD38-PE (clone SK7), anti-CD27-PE (clone M-T271). They were then washed twice with phosphate-buffered saline (PBS), suspended in PBS, and analyzed using FACS Canto II flow cytometry with FACS DIVA software (version 6.1.3, BD Biosciences).

### Real-Time Polymerase Chain Reaction

Total RNA was isolated using the RNeasy Mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer’s instructions. cDNA was synthesized by reverse transcription at 37°C for 30 min, followed by reverse transcriptase (RT) inactivation at 95°C for 5 min using the Fast Advanced RT buffer and Enzyme mix (Thermo Fisher Scientific, Waltham, MA, USA). Each gene-expression assay consisted of a 6-carboxy-fluorescein (FAM)-dye-labeled TaqMan MGB (minor groove binder) probe and two polymerase chain reaction (PCR) primers (TaqMan human FAM assays). The target genes were VDR (Hs00167999_m1), CYP24A1 (Hs00168017_m1), IL-10 (Hs00961622_m1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Hs02786624_g1). All of these genes were obtained from Thermo Fisher Scientific. GAPDH, which is present in all genomes, is commonly used as an endogenous control (i.e., a housekeeping gene) for analyzing the relative levels of gene expression. Real-time PCR was carried out using the TaqMan Fast advanced Master Mix (Thermo Fisher Scientific). Uracil-N-glycosylase (UNG) was used to prevent the possible reamplification of carryover PCR products. The thermal cycle conditions were 50°C for 2 min (AMPerase UNG activation), 95°C for 20 sec (Taq activation), and then PCR for 40 cycles of 95°C for 1 sec and 60°C for 20 sec. Relative expression levels of DNA were calculated automatically using QuantStudio 6 Pro Real-Time PCR System software (Thermo Fisher Scientific). Cycle threshold (CT) values were defined as the thresholds required for reference amplification. The average CT was calculated for each target gene (CT, target gene) and for GAPDH (CT, GAPDH); the difference (ΔCT) was calculated for each target gene using the equation ΔCT = CT, target gene − CT, GAPDH.

### Statistical Analysis

Lymphocyte subset percentages in PBMC samples were compared between the MS, NMOSD, and HC groups using one-way analysis of variance (ANOVA) or Kruskal–Wallis tests. Two-way ANOVA or Friedman’s test was used to compare the proportions of specific lymphocytes and ΔCT values for VDR, CYP24A1, CYP27B1, and IL-10 mRNA expression among the four stimulation groups (LPS+CD40L+CpG-ODN alone or in combination with 1,25(OH)2D3, steroid, or both) for each of the three study groups. Bonferroni’s correction for multiple comparisons was performed. Correlations between the study participants’ serum 25(OH)D3 and the percentage of each lymphocyte subset or ΔCT values for mRNA expression were evaluated using Pearson’s correlation or Spearman’s correlation. SPSS (version 20, SPSS, Chicago, IL, USA) and Prism (version 8.4.3, GraphPad, La Jolla, CA, USA) were used for statistical analysis and data presentation. The criterion for statistical significance was p < 0.05.

### RESULTS

The characteristics of the study participants are summarized by group in Table 1. Patients with NMOSD were significantly older than HCs (mean age, 44 vs. 32 years; p = 0.013). Vitamin D supplementation was reported by 25% (3/12) of the MS patients and 33% (4/12) of the NMOSD patients, but none of the HCs. Serum 25(OH)D3 was higher in the NMOSD group than in the MS group (p = 0.028).

In PBMC samples, the proportion of total lymphocytes was significantly lower in both the MS and NMOSD groups than in the HC group (p = 0.020 and 0.005, respectively). The frequency of Breg among B cells was lower and the Bmem/Breg ratio was significantly higher in the NMOSD group than in the MS group (p = 0.026 and 0.028, respectively); there were no significant differences in these parameters between the MS and HC groups or between the NMOSD and HC groups (Figure 1). There were no differences in the percentages of T cells, B cells, and Bmem between any of the study groups (HC vs. MS vs. NMOSD).

### Changes in the Proportion of Lymphocytes in Response to Stimulation With 1,25(OH)2D3

Nonspecific stimulation with LPS+CD40L+CpG-ODN resulted in a significant increase in total lymphocyte percentage (p = 0.014) and significant reductions in the Bmem percentage and Bmem/Breg ratio (p = 0.010 and 0.028, respectively) in the NMOSD group, while the T-cell percentage decreased in the MS group (p = 0.017) (Supplementary Figure 1).

Stimulation of HC PBMCs with 1,25(OH)2D3 significantly reduced the percentage of total lymphocytes (p = 0.027),
regardless of subsequent stimulation with steroid (Figure 2A). 1,25(OH)2D3 also caused a reduction in the percentage of total lymphocytes in PBMCs from the MS and NMOSD groups, but only when steroid was added after 1,25(OH)2D3 (p<0.05 vs. steroid or 1,25(OH)2D3 alone for both patient groups).

The proportion of T cells among total lymphocytes was also significantly reduced by stimulation of HC PBMCs with 1,25(OH)2D3 (p=0.012), but that proportion increased upon subsequent stimulation with steroid. In the MS and NMOSD groups, the percentage of T cells increased after PBMC stimulation with steroid (p=0.012 and 0.158, respectively), but not if they were first stimulated with 1,25(OH)2D3 (Figure 2B). 1,25(OH)2D3 stimulation did not alter the proportions of B cells or Breg, or the Bmem/Breg ratio in any of the three study groups (Figures 2C, D, F). However, stimulation with 1,25(OH)2D3 followed by steroid significantly increased the proportion of Bmem in PBMC samples compared with 1,25(OH)2D3 stimulation alone in the NMOSD group (Figure 2E).

**Associations Between Serum 25(OH)D3 and Percentage of Bmem**

Prior to PBMC stimulation, there were no correlations between the Bmem/B cell ratio and serum 25(OH)D3 in any of the study groups. However, after nonspecific PBMC stimulation with LPS+CD40L+CpG-ODN, there was a negative correlation between the Bmem/B cell ratio and serum 25(OH)D3 in the HC group (Spearman’s ρ = 0.529, p = 0.043), and a positive correlation (Pearson’s r = 0.731, p = 0.007) in the NMOSD group (Figures 3A, C). Additional stimulation with 1,25(OH)2D3 did not alter these correlations for either group. In the MS group,

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**TABLE 1 | Demographics and serum concentrations of 25(OH)D3 in study subjects.**

|                | HCs (N = 15) | MS (N = 12) | NMOSD (N = 12) | p value |
|----------------|--------------|-------------|----------------|---------|
| Age            | 32.3 ± 5.2   | 36.8 ± 9.8  | 44.1 ± 14.2    | 0.016*  |
| Female (%)     | 8 (53.3)     | 9 (75.0)    | 11 (91.7)      | 0.065   |
| Disease duration, months | N/A        | 27.5 (4.56–1.75) | 53.5 (21.75–115.75) | 0.236   |
| 25(OH)D3, mg/dl| 18.1 ± 5.8   | 20.9 ± 11.7 | 31.4 ± 18.5    | 0.028*  |
| On taking 1,25(OH)2D3, N (%) | 0         | 3 (25.0)    | 4 (33.3)       | 0.060   |
| ARR            | N/A          | 1.0 (0.625–2.875) | 3.0 (1.25–3.0)  | 0.078   |
| Use of drugs, N (%) | N/A       | 9 (75.0)    | 11 (91.7)      | 0.590   |

N, number; ARR, annualized relapse rate; EDSS, Expanded Disability Status Scale; HCs, healthy controls; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; Values are presented as either mean ± SD or median (IQR) unless otherwise indicated.

*Significant only between HCs and NMOSD.

**FIGURE 1 |** The proportions of lymphocytes in PBMC samples from HCs and patients with MS or NMOSD. The percentage of total lymphocytes was lower in MS and NMOSD than in HCs. The Breg/B cell ratio was higher and the Bmem/Breg ratio was lower in patients with MS than in those with NMOSD. The data are presented as scatter plots in which the horizontal line indicates the mean (A–D) or median (E, F). Only statistically significant p values (p<0.05) are presented. Bmem, CD19+CD27+ memory B cell; Breg, CD19+CD24+CD38+ regulatory B cell; HC, healthy control; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; PBMC, peripheral blood mononuclear cell.
an association between Bmem/B cell ratio and serum 25(OH)D3 was found only following stimulation with 1,25(OH)2D3, which yielded a negative correlation ($r = -0.594$, $p = 0.042$; Figure 3B). There was no correlation between the Breg/B cell ratio in PBMC samples and serum 25(OH)D3 in any of the groups, regardless of the stimulation conditions. A negative association was found between the Bmem/Breg ratio and serum 25(OH)D3 after stimulation of PBMCs with 1,25(OH)2D3 only in the HC group ($p = -0.583$, $p = 0.023$).

**Changes in Expression Levels of VDR, CYP27B1, CYP24A1, and IL-10 Genes in Response to 1,25(OH)2D3**

1,25(OH)2D3-induced changes in the PBMC mRNA expression levels of VDR, IL-10, CYP27B1, and CYP24A1 are shown in Figure 4. PBMCs from 15 HCs, 11 MS patients, and 13 NMOSD patients were included (Supplementary Table 1). PBMC stimulation with LPS+CD40L+CpG-ODN increased CYP27B1 mRNA expression, but the addition of 1,25(OH)2D3 suppressed
A negative correlation between the percentage of Bmem and serum 25(OH)D₃ was found (A) in HCs regardless of the type of stimulation (i.e., steroid or 1,25(OH)₂D₃), and (B) in patients with MS only after stimulation of PBMCs with 1,25(OH)₂D₃. (C) In NMOSD, significant positive correlations were found after PBMC stimulation, with or without 1,25(OH)₂D₃. Linear regression lines indicate significant correlations. 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; Bmem, CD19⁺CD27⁺ memory B cell; HCs, healthy controls; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; serum 25(OH)D₃, serum concentration of 1,25-dihydroxyvitamin D₃.
PBMC CYP24A1 expression was also induced by stimulation with 1,25(OH)2D3. These findings were observed in PBMCs from all study groups and were statistically significant. However, the expression levels of VDR and IL-10 mRNA were unaffected by stimulation of PBMCs, with or without 1,25(OH)2D3.

In patients with MS, the level of 1,25(OH)2D3-induced CYP24A1 mRNA expression in PBMCs was significantly and negatively correlated with serum 25(OH)D3 (for ΔCt, r=0.744, p=0.014); however, no significant association was found for VDR, CYP27B1, and IL-10 mRNA expression (Figure 5). In HCs, the level of 1,25(OH)2D3-induced IL-10 mRNA expression was positively correlated with serum 25(OH)D3 (for ΔCt, r=0.590, p=0.026). In patients with NMOSD, there was no correlation between mRNA expression of VDR, CYP27B1, CYP24A1, or IL-10 and serum 25(OH)D3, irrespective of stimulation with 1,25(OH)2D3.

**DISCUSSION**

The findings of this study demonstrate that 1,25(OH)2D3 exerted an inhibitory effect on the proliferation of lymphocytes, and especially T cells. However, in patients with MS and NMOSD, this 1,25(OH)2D3-induced suppression of lymphocytes (total and T cells) was obvious upon subsequent stimulation with steroid. In addition, statistically significant associations in the opposite direction were found between serum 25(OH)D3 and the Bmem/Breg ratio and serum 25(OH)D3. Moreover, the expression of CYP24A1, the gene encoding a 1,25(OH)2D3-catabolizing enzyme, was less expressed in response to 1,25(OH)2D3 in MS patients with higher serum 25(OH)D3.

Stimulation with 1,25(OH)2D3 followed by steroid decreased the percentage of total lymphocytes and T cells in PBMCs from
patients with MS and NMOSD. This finding could be explained by the known immune regulatory role of vitamin D. The active form of vitamin D, 1,25(OH)2D3, induces monocyte proliferation, which contributes to the innate immune response and attenuates the cytotoxic activity and proliferation of CD4+ and CD8+ T cells by reducing proinflammatory cytokine production. 1,25(OH)2D3 could enhance the anti-inflammatory effects of corticosteroids on monocytes and T cells via the induction of glucocorticoid receptor binding of steroids both in vitro and in vivo. Combined treatment with steroid and 1,25(OH)2D3 may thus have therapeutic potential in patients with MS or NMOSD by decreasing the T-cell-mediated autoimmune processes. 1,25(OH)2D3 also exerts potent direct effects on B-cell responses, inhibiting their proliferation and differentiation into class-switched Bmem and plasma cells. However, there were no significant changes in the proportion of Breg and B cells in response to stimulation of PBMCs with 1,25(OH)2D3. Little is known about the effect of glucocorticoid on B cells. Dexamethasone could induce apoptosis of B cells at all stages, and especially immature B cells, and could stimulate T-cell-dependent immunoglobulin production by enhancing the differentiation of B cells into mature plasma cells without proliferation. In NMOSD, it was reported that the frequencies of mature Bmem increased after 2 weeks of high-dose steroid treatment. In the present study, the proportion of Bmem in PBMCs from NMOSD patients, which were initially reduced by stimulation with 1,25(OH)2D3, subsequently increased after stimulation with steroid, although the frequencies of Bmem did not differ between cells incubated with either 1,25(OH)2D3 or steroid alone. This may indicate that steroid-induced Bmem proliferation could be augmented by 1,25(OH)2D3 (22, 23). However, these findings were not observed in either HCs or MS patients; thus, further investigation is warranted.

It was particularly interesting that significant associations were observed between serum 25(OH)D3 and Bmem frequency in PBMCs stimulated with LPS+CD40L+ CpG-ODN, with the correlation being negative in HCs and positive in NMOSD. In MS patients, the PBMC Bmem frequency was negatively correlated with serum 25(OH)D3 after preincubation with 1,25(OH)2D3. Clinical data regarding the relationship between Bmem and serum 25(OH)D3 in autoimmune diseases are scarce. In SLE, a significant negative association was identified between serum 25(OH)D3 and Bmem, but not total B cells or plasmablasts. MS patients with a low vitamin D status exhibited decreased cerebrospinal fluid levels of vitamin D and greater intrathecal accumulation of class-switched Bmem and antibody-secreting plasma cells. Since Bmem are considered to be a source of proinflammatory cytokines, which are responsible for pathogenic effects during autoimmune processes, it seems to be contradictory that NMOSD patients with higher serum 25(OH)D3 would have higher Bmem frequencies. However, the immune balance represented by the Bmem/Breg ratio was not correlated with the vitamin D level in NMOSD. After PBMC stimulation, the proportion of Bmem was significantly decreased in NMOSD, unlike in MS. The significance of a lower proliferation of non-Bmem in NMOSD patients with higher serum 25(OH)D3 remains to be established. The present findings also suggest that the suppressive effect of vitamin D on Bmem is stronger in MS patients with higher serum 25(OH)D3. A recent meta-analysis suggested that vitamin D supplementation has a therapeutic role in the treatment of MS; however, there is uncertainty about the most appropriate dose and factors influencing the immune regulatory roles of 1,25(OH)2D3. The therapeutic potential of vitamin D intake in patients with MS and NMOSD needs to be investigated further.

The biological activity of vitamin D is determined by the combination of levels of VDR expression and the activities of the metabolizing enzymes 1α-hydroxylase (CYP27B1) and 24-hydroxylase (CYP24A1). 1,25(OH)2D3 acts mainly via VDR-mediated regulation of gene expression, and VDR transcription is induced by 1,25(OH)2D3 itself. IL-10 expression in activated B cells is enhanced by 1,25(OH)2D3 more than threefold, primarily through the recruitment of VDR to the promoter of IL-10. However, the mRNA expression levels of VDR and IL-10 in PBMCs were not significantly influenced by the presence of 1,25(OH)2D3 in the present study. This may be attributable to upregulated VDR expression by inflammatory signals offsetting the effects of 1,25(OH)2D3 on immune cells, or mRNA measurement times that were inappropriate for allowing the detection of any effect. In stimulated PBMCs, feedback regulation of vitamin D metabolism by 1,25(OH)2D3 was observed in HCs, and in patients with MS and NMOSD. The presence of CYP27B1 has been demonstrated in immune cells, enabling them to produce locally active 1,25(OH)2D3 from 25(OH)D3. CYP24A1 expression is also induced by 1,25(OH)2D3, creating a self-regulatory feedback loop and enabling 1,25(OH)2D3 to fulfill its role in maintaining immune balance. It is noteworthy that in response to stimulation with 1,25(OH)2D3, CYP24A1 mRNA expression was lower in MS patients with higher serum 25(OH)D3. This suggests that serum 25(OH)D3 is associated with impaired regulation of CYP24A1 activity. There are few previous reports on the associations between genetic polymorphisms in vitamin-D-regulated genes such as CYP24A1, vitamin D status, and MS risk. Further studies with larger samples are needed to reveal the mechanism underlying the impaired regulation of vitamin D hydroxylation in MS.

This study had several limitations. The small number of samples in each study group must be considered when interpreting these data, which reduced the statistical power and may have introduced unintended bias. In addition, the enrolled patients were all in remission with heterogeneous disease duration and had been taking immunosuppressive or disease-modifying drugs, which could affect immune cell function. Moreover, we did not obtain age-matched HCs compared with the NMOSD group, which could affect the composition and quality of the lymphocyte pool.

In conclusion, the findings of this study suggest that vitamin D plus steroid has a therapeutic benefit on T cells in MS and NMOSD, and that differential transcriptional activities of the CYP24A1 gene could exist that affect serum 25(OH)D3 in MS. In addition, vitamin D may have different inhibitory effects on
Bmem that are dependent upon serum 25(OH)D3 in MS and NMOSD. The positive association between CD19+CD27+ B-cell frequency and serum 25(OH)D3 in NMOSD after immune-cell stimulation must also be further explored to establish whether vitamin D does have beneficial effects in this autoimmune disease, or if this was simply an accidental correlation. Further large-scale studies could help to elucidate the immunoregulatory mechanism of vitamin D supplementation in patients with MS and NMOSD.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**ETHICS STATEMENT**

The study was approved by the Institutional Review Board of Samsung Medical Center and written informed consents were obtained from all subjects.

**AUTHOR CONTRIBUTIONS**

Study concept and design: EBC and J-HM. Acquisition of data: EBC, JHS, SK, JK, JMS, BK, and J-HM. Analysis and interpretation of the data: EBC, BK, and J-HM. Draft and revision of the manuscript for content: EBC and J-HM. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.677041/full#supplementary-material

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